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Title: **Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility**

Running title: Sirtuins and reproductive functions

Key words: Sirtuin, SIRT1, SIRT3, oocyte, sperm, embryo, ovary, testis

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Abstract

Background: Sirtuins (SIRT1-7) are a family of NAD+-dependent deacetylases that catalyze post-translational modifications of proteins. Together, they respond to metabolic challenges, inflammatory signals, or hypoxic/oxidative stress, and are associated with aging and longevity. The role of Sirtuins in the regulation of fertility has emerged in 2003 when a defective reproductive phenotype was observed in Sirt1-null mice. Since studies on Sirtuins in reproductive biology have been increasing in the last years, a recent comprehensive update on this issue is still lacking.

Objective and rationale: This review is aimed to give an update on the rapid development of Sirtuin research in female and male reproduction and in embryogenesis under physiological and pathological conditions. An overview of Sirtuin modulation and involvement in signalling networks is provided along with needs for further research.

Search methods: MEDLINE database was examined for peer-reviewed original articles. The following keywords were searched: 'Sirtuin', 'ovary', 'oocyte', 'ovarian follicle', 'embryo', 'endometrium', 'sperm' and 'testis'. These keywords were combined with other search phrases relevant to the topic.

Outcomes: Our knowledge of Sirtuins in reproductive functions has grown exponentially over the last few years. The majority of the work carried out so far has focused on SIRT1 with a prevalence of studies on female reproduction. Numerous studies have provided evidence that down-regulation of SIRT1 is associated with physiological or pathological reduction of ovarian reserve. SIRT1 has also been shown to regulate proliferation and apoptosis in granulosa cells whereas SIRT3 was found to promote luteinization. Biochemical modulation of Sirtuin activity has led to discover the role of SIRT1, SIRT3 or SIRT6 in improving the competence of oocytes grown or matured in vitro in humans and animal models. Recently, SIRT1, SIRT2 or SIRT3 have emerged as protectors of oocyte against postovulatory aging. Transgenic models provide strong evidence that SIRT1 is involved in spermatogenesis by influencing specific functions of male germ cell, Sertoli cells and Leydig cells. When our attention moves to post-fertilization events, maternally derived SIRT3 appears crucial in

71 the protecting early embryos against stress conditions. Finally, increasing SIRT1 activity may have
72 the potential to ameliorate fertility in PCOS, diabetes, endometriosis, xenobiotic stress and aging.
73 Overall, these effects have been ascribed to Sirtuin-mediated regulation of energy homeostasis,
74 mitochondrial biogenesis, chromatin remodelling and protection against oxidative stress.
75 Wider implications: The present review provides challenges and opportunities to stimulate research
76 and exploit Sirtuin-based signalling as a potential target for therapeutic applications in reproductive
77 health care.
78
79

Introduction

Sirtuins are a family of NAD+-dependent deacetylases recently emerged as key metabolic sensors for body homeostasis. Together, they respond to metabolic challenges, inflammatory signals, or hypoxic/oxidative stress, and are associated with aging and longevity (Grabowska et al., 2017; Vachharajani et al., 2016). The role of Sirtuins in the regulation of fertility has arisen in 2003 when a defective reproductive phenotype was observed in male and female Sirt1-null mice (McBurney et al., 2003). Since then, different genetically transgenic models have been developed (Table I) and our knowledge of Sirtuins in reproductive functions has grown exponentially along with the awareness that Sirtuin-regulated processes are key issues in gonadal functions.

Fertility is very sensitive to redox perturbations related to aging and metabolic dysfunctions, which disrupt the link between energy metabolism and reproduction (Seli et al., 2014). Reproductive cells and organs are constantly challenged by stresses and privations and require adaptive responses for their survival. In addition to redox perturbations related to aging or diseases, reproductive cells have to face stress conditions during their manipulation during assisted reproductive procedure (Agarwal and Majzoub, 2017). In this scenario, Sirtuins have emerged as critical players in the regulation of key processes in oogenesis and spermatogenesis and cellular stress response. The current lifestyle of "Western societies" characterized by excessive consumption of high-energy diets, physical inactivity and delayed family planning has pressured reproductive system towards subfertility. In this context, the present study aims to review knowledge about Sirtuins as critical players in the control of gametogenesis and post-fertilization events under physiological and pathological conditions.

2. Mechanisms of Sirtuin activation

In 1999, Kaeberlein and co-workers first identified the evolutionarily conserved gene silent information regulator 2 (Sir2), whose overexpression increased lifespan by 30% in yeast S. cerevisiae (Kaeberlein et al., 1999). Since then, mammal homologs of yeast Sir2 (Sirtuins) have been found and classified in a family of seven members of class III histone deacetylases (SIRT1-SIRT7), with SIRT1 being the most phylogenetically similar to yeast Sir2, and the most frequently studied (Wątroba and Szukiewicz, 2016). In addition to marked differences found in subcellular distribution, mammalian Sirtuins catalyse a range of different enzymatic reactions beyond the deacetylase function that was initially described for these proteins (Osborne et al., 2016). An increasing number of studies have determined that Sirtuin ability to influence metabolism and longevity relies on their capacity to function as protein deacetylases in a NAD+ dependent manner. The absolute requirement of the oxidized form of nicotinamide adenine dinucleotide (NAD^+) in the reaction catalysed by Sirtuins (Figure 1) suggests that these enzymes may represent a sort of energy sensor within the cells, with the ability to detect modifications of the redox status of the cellular environment (Michan and Sinclair, 2007). Protein deacetylation as a post-translational modification was initially identified in the modification of histones to silence gene transcription. It is now recognized that lysine-residue deacetylation is an efficient means through which cells respond to intra- and extra-cellular stimuli, and are used in cell signaling cascades, which in turn control protein functions through different mechanisms (Kupis et al., 2016). Hence, unexpected activities of Sirtuins towards several transcription factors and cytoplasmic protein substrates, well beyond simple histone deacetylation, have drawn attention of researchers over the years on the role of Sirtuin family members in the regulation of cellular homeostasis, with particular emphasis on oxidative stress (Santos et al., 2016), flogosis (de Mingo et al., 2016), metabolism (Houtkooper et al., 2012), and senescence (Sack and Finkel , 2012) (Figure 2).

Activity and expression of several Sirtuins can be regulated at both transcriptional and post-transcriptional levels (Revollo and Li, 2013). Numerous transcription factors can modulate SIRT1

expression including CREB (cyclic AMP response-element-binding protein), FOXOs (Forkhead box) and PPARs (Peroxisome proliferator-activated receptors). The abundance of SIRT1 transcripts is controlled by the RNA binding protein HuR (Hu antigen R) and miRNAs. Interaction with HuR results in prolonged half-life of mRNAs with subsequent increase of SIRT1 expression and activity stability. By contrast, a growing list of miRNAs appears to target Sirt1 mRNA and suppress its translation or reduce its stability in numerous tissue and organs (Yamakuchi , 2012).

Many researchers have investigated the possibility to influence the state of activation of Sirtuins through pharmacological and non-pharmacological interventions, with the aim of controlling SIRT-dependent downstream pathways. One of the first compounds that was recognized as activator of Sirtuins is resveratrol (3,5,4'-hydroxystilbene), a natural polyphenolic compound commonly found in grapes, berries and red wine, that was shown to activate SIRT1 in several experimental paradigms. Resveratrol was found to be an extender of lifespan in Saccharomyces cerevisiae, Caenorhabditis elegans and Drosophila melanogaster (Howitz et al., 2003; Wood et al., 2004) to increase energy metabolism and mitochondrial oxidative capacity (Lagouge et al., 2006; Hui et al., 2017). Based on its ability to reduce glucose production and efflux in diabetic rats, Yonamine and colleagues (2016) suggested the use of resveratrol as an adjunctive agent to insulin therapy.

In search of more potent and specific Sirtuin activating compounds (STACs), high throughput screening led to identification of SRT1720, SRT1460 and SRT2183. Imidazoquinoxalines and pyrroloquinoxalines, along with 1,4-dihydropyridines, which are all structurally unrelated to resveratrol, were found to act as SIRT1 activators (Nayagam et al., 2006; Valente et al., 2016). The most studied synthetic STAC is SRT1720, that has been shown to enhance mitochondrial biogenesis or function (Rowlands et al., 2015) and degradation of fatty acids (Yamazaki et al., 2009), to prevent oxidative stress-related organ injury (Hansen et al., 2016) and inflammatory status (Ichikawa et al., 2013; Jia et al., 2015), as well as to extend lifespan (Mitchell et al., 2014). While activators that exploit the unique structural N-terminus feature of SIRT1 are available, small molecules capable of activating the other mammal Sirtuins are lacking.

153 Non-pharmacological interventions promoting SIRT1 activation include caloric restriction (CR)
154 (Cohen et al., 2004; Boily et al., 2008; Anderson et al., 2009). When calories are restricted, the flux
155 of carbons through the mitochondrial oxidation shifts the NAD^+/NADH balance towards the oxidized
156 state, thus establishing a redox environment that promotes Sirtuin activity (Wang, 2014). The CR-
157 induced enhancement of SIRT1 activity have been shown to be set through the activation of adenosine
158 monophosphate (AMP)-activated kinase (AMPK), a sensor of falling energy status that promotes fast
159 ATP production (Cohen et al., 2004; Suchankova et al., 2009; Sebastián et al., 2012).
160 Sirtuin inhibitors available so far lack adequate physicochemical characteristics, selectivity for Sirtuin
161 isotype, or potency (Schiedel et al., 2017). The best-known endogenous inhibitor of all Sirtuin
162 isotypes is the product of the catalytic reaction of both deacylation and ADP-ribosylation (i.e.,
163 nicotinamide (NAM), along with its structural analogues). Among other low molecular weight
164 inhibitors, Sirtinol, a cell-permeable hydroxynaphthaldehyde derivative, exhibits low-potency against
165 SIRT1 and SIRT2 (Grozinger et al., 2001; Kumari et al., 2015), whereas the indole-based EX-527 is
166 among the most potent SIRT1-selective inhibitors known so far (Napper et al., 2005; Schiedel et al.,
167 2017). Other chemical classes of molecules that have been studied as SIRT inhibitors include
168 thiobarbiturates and kinase inhibitors even though several issues were experienced with regard to
169 both low selectivity among the different Sirtuin isotypes and unspecific effects caused also by indirect
170 effects via kinase inhibition (Schiedel et al., 2017).
171

3. Sirtuin activity and cell functions

a. Bioenergetics

Sirtuins are verified to exert various impacts on gluconeogenesis, glycolysis, insulin secretion and sensitivity, and also occupies a special place in lipid metabolism via enhancing lipid oxidation.

SIRT1 activity affects degradation of fatty acids, mitochondrial function, and regulates the homeostasis of bile acid and cholesterol (Kupis et al., 2016). Accordingly, among the most widely studied targets of SIRT1 is peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC1α), which is known to act as a master regulator of mitochondrial biogenesis and functions (Li, 2013; Chang and Guarente, 2014). SIRT1-dependent deacetylase action induces cytoplasmic activation of LKB1, whose signalling leads to increased fatty acid oxidation in the liver (Lee et al., 2012). In addition, SIRT1 down-regulates gluconeogenesis by inhibiting CREB-regulated transcription coactivator 2 (CRTC2), as well as it regulates cholesterol homeostasis by modulating the activity of farnesoid X receptor (FXR) (Li, 2013; Chang and Guarente, 2014).

SIRT2 has a major role in regulating metabolism of carbohydrates, by inhibiting the proteasomal degradation of phosphoenolpyruvate carboxykinase 1 (PEPCK1), the rate-limiting enzyme of gluconeogenesis (Jiang et al., 2011). SIRT3, the most studied mitochondrial Sirtuins, affects critical mitochondrial functions, such as ATP production, reactive oxygen species (ROS) regulation, β-oxidation, cell death, and ketogenesis (Lombard and Zwaans, 2014). Indeed, SIRT3 deacetylates a range of mitochondrial target enzymes, such as ATP-synthase (Law et al., 2009), hydroxymethylglutaryl-CoA-synthase 2 (HMGCS2) (Shimazu et al., 2010), electron transport chain complexes (Finley and Haigis, 2012), and isocitrate dehydrogenase 2 (IDH2) (Schlicker et al., 2008). SIRT4, the second mitochondrial Sirtuin protein, mediates ADP-ribosylation of glutamate dehydrogenase, thus decreasing carbon flux in the Krebs cycle (Haigis et al., 2006; Jeong et al., 2013). The importance of SIRT4 in energy metabolism has been recently confirmed by the novel lipoamidase function, through which SIRT4 is thought to regulate the activity of pyruvate dehydrogenase complex (PDH), thus controlling the production rate of acetyl-CoA (Mathias et al.,

198 2014). The third and final mitochondrial Sirtuin is SIRT5, which shows also desuccinylase,
199 demalonylase, and deglutarylase activities (Du et al., 2011; Nakamura et al., 2012; Hirschey and
200 Zhao, 2015; Parihar et al., 2015). SIRT5 was shown to up-regulate ammonia disposal, through the
201 deacetylation of carbamoylophosphate synthetase (CPS1), which catalyses the first rate-limiting step
202 of the urea cycle (Yu et al., 2013). SIRT6, a nuclear protein with both mono ADP-ribosylase and
203 NAD^+-dependent deac(et)ylase activities, was found to suppress the expression of glucose metabolic
204 genes and reduce glucose uptake (Kugel and Mostoslavsky, 2014). SIRT7 has a distinctive role in
205 regulating fatty acids uptake and triglyceride synthesis/storage (Tsai et al., 2014; Yoshizawa et al.,
206 2014) and plays also a critical role in the regulation of mitochondrial homeostasis, by deacetylating
207 distinct lysine residues on GABPβ1 (GA binding protein β1),), which is a master regulator of nuclear-
208 encoded mitochondrial genes (Ryu et al., 2014).

209 **b. Redox signalling**

210 An overwhelming generation of reactive species or a disruption of the redox cellular environment
211 modify the Sirtuin-dependent signalling by either regulating the expression of Sirtuin genes, or
212 altering the post-translational modification patterns of Sirtuin proteins and changing NAD levels
213 (Santos et al., 2016). It has been observed that mild oxidative stress conditions are able to up-regulate
214 the SIRT1 expression, and this in turn induces the response to changes in the cell redox status,
215 whereas harsh oxidative stress increases degradation of SIRT1 leading to apoptosis. Among the main
216 redox-related targets of SIRT1 are p53, FOXOs, and nuclear factor NF-kappa-B (NF-κB), which all
217 play distinct roles in mediating SIRT1 response to changes of the redox status (Li, 2013; Chang and
218 Guarente, 2014). In particular, p53 is a transcription factor that activates genes involved in antioxidant
219 defence, like SOD2 (superoxide dismutase 2) and GPx1 (glutathione peroxidase) (Sablina et al.,
220 2005). FOXO3a induces antioxidant responses via up-regulation of SOD2 and catalase (Hasegawa et
221 al., 2008; Pardo et al., 2011; Hori et al., 2013; Di Emidio et al., 2014). Some authors provided
222 evidence that SIRT1 counteract pro-oxidant conditions through its deacetylating action on nuclear
223 factor E2-related factor 2 (NRF2), one of the most important master regulators of enzymatic and non-

enzymatic antioxidant defences (Ding et al., 2016). FOXO3a can be also deacetylated by SIRT2, thus promoting the activation of antioxidant defences (e.g., SOD2) and the up-regulation of pro-apoptotic molecules under cellular stress (Wang et al., 2007). Recent reports also indicate that SIRT2 may utilize also p53 as a substrate (Jin et al., 2008). In addition, SIRT2 has been recently reported to modify the acetylation state of glucose-6-phosphate dehydrogenase (G6PD) in response to extracellular oxidative challenges, thus regulating the availability of NADPH, one of the major intracellular reductants (Wang et al., 2014). SIRT2 seems to affect also the NF-κB-dependent signaling, by deacetylating the p65 subunit at lysine 310, thus repressing cell proliferation and regulating immune and inflammatory response (Rothgiesser et al., 2010; Li et al., 2013).

Tao and colleagues (2010) demonstrated that SIRT3 activates SOD2 through deacetylation at Lys 122, thus improving the efficacy of ROS removal from the mitochondrial compartment. Accordingly, mice overexpressing SIRT3 exhibit enhanced protection against ROS, as well as delayed onset of age-related pathologies (Sundaresan et al., 2009; Sundaresan et al., 2015). Conversely, $SIRT3^{-/-}$ mouse embryonic fibroblasts show ROS overproduction and increased chromosomal instability in response to exogenous stress, as well as decreased oxidative phosphorylation (Kim et al., 2010). Additional physiological SIRT3 substrates are FOXO3a, a transcriptional activator involved in oxidative stress-triggered cell response, (Tseng et al., 2013) and Ku70, which participates to non-homologous end joining (NHEJ) DNA double strand break repair and telomere maintenance (Sundaresan et al., 2008; Tseng et al., 2013). Accordingly, some authors have argued that SIRT3 is also present in the nucleus and cytoplasm (Scher et al., 2007; Iwahara et al., 2012). Others found that SIRT5 is able to activate SOD1, thus helping to scavenge efficiently superoxide anions within the cell (Lin et al., 2013).

SIRT6 stimulates DNA double-strand breaks (DSBs) repair in paraquat-treated cells, thus representing a key protective enzyme against oxidative stress-induced DNA damage (Mao et al., 2011). Not surprisingly, $SIRT6^{-/-}$ mouse embryonic fibroblasts stem cells both exhibit increased sensitivity to γ irradiation and elevated chromosomal aberrations, with respect to wild-type

counterparts, thus implying that SIRT6 plays a pivotal role in maintaining genome integrity (Mostoslavsky et al., 2006).

c. Histone modifications and chromatin remodelling

Sirtuins mediate some of their functions through mechanisms that remodel chromatin structure and accessibility (Martinez-Redondo and Vaquero, 2013). By definition, chromatin remodelling is an enzyme-based process by which the DNA wrapped around histones becomes accessible to the regulatory transcription machinery proteins, thus controlling gene expression. It is mainly carried out through post-translational modification of histone tails that comprise methylation, acetylation, phosphorylation, ubiquitination, sumoylation, citrullination, and ADP-ribosylation (Bannister and Kouzarides, 2011). In somatic cells, acetylation of histone tails is commonly associated with chromatin decondensation (Toth et al., 2004) and gene transcription (reviewed in Clayton et al., 2006; Turner, 2014; Zentner and Henikoff, 2013). Several classes of histone acetyltransferases (HATs) and histone deacetylases (HDACs) are responsible for the acetylation status at specific histone residues (Lombardi et al., 2011; Marmorstein and Zhou, 2014). In mammals, eighteen HDACs, bearing a common well-conserved catalytic deacetylase domain, have been identified and classified into four classes: I, II, III, and IV (Konsoula and Barile, 2012). Among the four classes, the class III HDACs (Sirtuins) are those that have the least ambiguity for histone substrate specificity (Table II). H4K16 and H3K9 acetylation are the two major, well-characterized and evolutionary well-conserved modifications that specifically regulate chromatin structure. Previous biochemical studies indicated that SIRT1 deacetylates histones H4K16 and H3K9 and mediates heterochromatin formation through the recruitment and the deacetylation of histone H1K26 (Vaquero et al., 2004). Moreover, SIRT1, together with the lysine-specific histone demethylase 1 (LSD1), play a concerted role in deacetylating histone H4K16 and demethylating H3K4 to ultimately repress gene expression (Mulligan et al., 2011). During the G2/M transition phase, SIRT2 shuttles to the nucleus and deacetylates H4K16 thus contributing to chromatin compaction (Contrepois et al., 2012). H4K16 is also deacetylated by SIRT3 when transported to the nucleus under certain conditions and determines the formation of

higher order of chromatin compaction (Scher et al., 2007). In human fibroblasts SIRT6 and SIRT7 are found in the nucleus associated with heterochromatic regions and nucleoli, respectively (Michishita et al., 2005). Deacetylation of H3K9 by SIRT6 modulates telomeric chromatin function (Michishita et al., 2008). SIRT7 is a highly selective H3K18 deacetylase that plays a crucial role in maintaining the transformed phenotype in cancer cells (Barber et al., 2012).

The coordinated process of chromatin remodelling is based also on the interplay of Sirtuins with other chromatin-associated mechanisms, such as histone-modifying enzymes and transcription factors (Martinez-Redondo and Vaquero, 2013; Garcia-Cao et al., 2004; Vaquero et al., 2004).

d. Cell proliferation and aging

Despite the fact that transgenic mice overexpressing Sirt1 do not live longer that the wild-type counterpart, the overexpression of SIRT1 has been shown to counteract the onset of important age-related phenotypes, whereas down-regulation of SIRT1 accelerates aging (Grabowska and Sikora, 2017). It is likely that SIRT1 participates in the mechanisms for sustaining the balance between DNA repair, senescence and apoptosis. Deacetylation of p53 by SIRT1 leads to suppression of its apoptotic effects and p53 hyperacetylation is thought to mediate anticancer effects of Sirtuin inhibitors (Lain et al., 2008). Along with other nuclear Sirtuins, SIRT1 regulate genomic stability throughout diverse DNA repair pathways. In particular, SIRT1 promotes deacetylation of WRN, a DNA helicase and Ku70 (Grabowska and Sikora, 2017).

An important role in cell division is played by SIRT2 whose levels have been seen to fluctuate markedly during cell cycle (Dryden et al., 2003). SIRT2 was found to deacetylate α-tubulin and the mitotic checkpoint BubR1, which ensures accurate chromosome segregation, as well as correct microtubule–kinetochore interaction during mitosis (de Oliveira et al., 2012; North et al., 2014).

SIRT4-mediated deacetylation participates in the cell cycle control by regulating glutamine metabolism (Rauh et al., 2013) and responding primarily to DNA damage promoting nucleotide synthesis (Jeong et al., 2013).

SIRT6 transgenic mice have been reported as long-lived rodents (Kanfi et al., 2012) and are protected against age-related metabolic dysfunction and inflammation (Roichman et al., 2017).

SIRT6 modulates DNA repair pathways in cooperation with a Poly (ADP-ribose) polymerase 1 (PARP1)-dependent manner and DNA-PK (DNA-dependent protein kinase).

In actively proliferating cells, SIRT7 protein is mostly found in the nucleolar region, is involved in the transcriptional regulation of ribosomal DNA, (Ford et al., 2006; Kim and Kim, 2013; Tsai et al., 2014). Reduced rDNA transcription resulting from translocation of SIRT7 from the nucleolar regions to the cytosol is linked to replicative senescence (Grob et al., 2009).

4. Sirtuins in female reproductive functions

a. Ovarian reserve and folliculogenesis

The ovarian reserve represents the stockpile of dormant primordial follicles, which supports ovulations throughout the reproductive lifespan. The primordial follicles remain in a dormant state until activation to primary follicles, which occurs after the onset of puberty. The size of ovarian reserve is controlled by complex dynamic events involving degeneration and progression through folliculogenesis until exhausted (Zhang and Liu, 2015). How to maintain the ovarian reserve is a key issue by considering the negative effects of menopause and the high risk of exposure to gonadotoxic insults during life (Buckler, 2005; Tatone et al., 2008). Genetically modified mice have provided evidence that among factors regulating the dynamics of the primordial follicle pool there are genes interacting with Sirtuin signalling. This is the case of FOXO3a, a SIRT1 and SIRT3 target, whose suppression results in accelerated primordial follicle loss, and the mTOR signalling, known to be under SIRT1 and SIRT2 influence, whose suppression promotes follicle dormancy (Adhikari and Liu, 2009; Adhikari et al., 2013; Chen et al., 2015). In this context, a key role of mTOR signaling in the regulating the primordial follicle reserve has been proposed in relation to its ability to sense nutrient status (Seli et al., 2014; Wang et al., 2014; Li et al., 2015).

A possible role of Sirtuins in folliculogenesis has emerged since 2007 when it was discovered that transgenic mice overexpressing SIRT1, beyond displaying a CR-like phenotype, showed delayed sexual maturity (Bordone et al., 2007). Subsequent studies in animal models subjected to different CR regimens have provided insights into this reproductive phenotype by discovering that the reduced ovarian activity, as an adaptive response to low energy supply, was associated to beneficial effects on the size of the primordial follicle pool. Selesniemi et al. (2011) showed that CR initiated in adult life significantly counteracted ovarian aging in terms of yield, maturational status, and post-fertilization developmental competency of oocytes obtained from 12-month old mice. Ever since, a growing number of studies have established strategies based on CR or administration of Rapamycin, the mTOR inhibitor, in an effort to preserve primordial follicle size with minor effects on ovarian

cycles. As expected, these effects were found to be associated with attenuation of mTOR signalling and increased levels of ovarian SIRT1 and SIRT6 and their substrates NRF1 and FOXO3A (Zhang et al. 2016; Liu et al. 2015; Zhou et al., 2014; Wang et al., 2014; Xiang et al., 2012; Luo et al., 2012; Dou et al., 2017). Moreover, studies based on the administration of the SIRT1 activators SIRT1720 or Resveratrol significantly have achieved similar results supporting the view that targeting SIRT1, as an essential factor in the regulation of follicle dynamics, may be a promising strategy for preventing ovarian ageing (Liu et al., 2013; Zhou et al., 2014).

Cinco et al. (2016) proposed a role of SIRT1 in relation to changes in follicle NAD metabolism during primordial to primary oocyte transition. According to this study, oocytes nuclear expression of SIRT1 increases during primordial follicle awakening when a significant decrease in nuclear ratio NADH/NAD+ occurs in preparation to the shift from glycolytic metabolism to oxidative phosphorylation for supplying energy during growth. Indeed, this view would be consistent with SIRT1-mediated activation of PGC1-α, which promotes mitochondrial biogenesis and oxidative phosphorylation. Based on the above observations, it can be speculated that Sirtuins, by acting as energy sensors, may be considered both as intraovarian paracrine factors and intrinsic oocyte regulators deciding the fates of primordial follicles.

b. Oocyte differentiation and meiotic maturation

Oocyte chromatin remodelling

In oocytes, the involvement of Sirtuins has been considered during the final oocyte growth phase leading to the formation of a competent oocyte. Through this period, the chromatin enclosed within the germinal vesicle (GV) is subjected to profound morphological, structural and functional remodelling (De La Fuente, 2006; Luciano and Lodde, 2013). Thus, GV stage oocytes can be further subdivided according to the level of chromatin compaction, and this is biologically relevant since chromatin configuration is now considered a marker of oocyte differentiation and developmental competence in all the species studied (De La Fuente, 2006; Luciano and Lodde, 2013). In cow, four

362 GV stages oocytes have been characterized (from GV0 to GV3), with increasing level of chromatin
363 compaction from early antral follicles to medium antral follicle >2 mm in diameter (Lodde et al.,
364 2007). In mice, oocytes with uncondensed chromatin are referred to as 'Non Surrounded Nucleolus'
365 (NSN) oocytes, while oocytes with compacted chromatin are referred to as 'Surrounded Nucleolus'
366 (SN) oocytes (De La Fuente, 2006; Luciano and Lodde, 2013). During the transition from
367 uncondensed to more compacted configuration, a major transcriptional silencing take place in mouse
368 and bovine oocytes (Bouniol-Baly et al., 1999; Lodde et al., 2008; Luciano et al., 2011). In mammals,
369 histone acetylation increases in the later stages of chromatin compaction while during oocyte
370 maturation is observed a complete histones de-acetylation (reviewed in (Lodde et al., 2017). An
371 increase in acetylation during the chromatin compaction, when the major transcriptional silencing
372 occurs, seems to be apparently in contradiction with the common rules of the 'Histone Code' that
373 applies to somatic cells (Jenuwein and Allis, 2001).
374 In mouse, defective SIRT1 protein expression was observed in aging GV stage oocyte and this
375 suggested that a decrease in H3K9 deacetylation was causative of the reduction in H3K9 methylation
376 (Manosalva and Gonzales, 2010). This is consistent with previous reported activity of Sirt1 in
377 promoting H3K9me3 through the histone methyltransferase Suv39h1 (Vaquero et al., 2007).
378 However, in the same study, a correlation between SIRT1 expression and changes in chromatin
379 configuration was not finally determined.
380 In bovine, the transcriptomic profile of oocytes characterized by different degree of chromatin
381 compaction from GV0 to GV3 configuration revealed that amount of SIRT1 and SIRT6 RNA changes
382 significantly during chromatin compaction (GSE48376; Labrecque et al., 2015). An increase was
383 observed in the transition between GV0 and GV1, when chromatin compaction begins. The list of
384 genes in the original microarray dataset that is accessible through an interactive web interface (Khan
385 et al., 2016) includes data from oocytes and cumulus cells (Lodde et al., 2017). In agreement with
386 previous studies, the increased expression of SIRT1 and SIRT6 could be responsible for an increase
387 of H3K9 deacetylation that is required for the subsequent methylation in the same residue, which

induce chromatin compaction (Krauss, 2008). However, this hypothesis should be confirmed by determining the levels of SIRT1 and SIRT6 protein expression in the oocyte, also by evaluating the progression of the acetylation state and the concurrent methylation state of H3K9. Moreover, the increase of the transcripts during the growth phase of the oocyte could be functional to subsequent use, starting from maturation and in the subsequent stages of development of the zygote.

In addition to issues related to the limited availability of material (oocytes) and the appropriate tools to analyse the expression of specific enzymes and the peculiar posttranslational modification of histones, deciphering the changes in the levels of specific Sirtuins mRNAs involved in chromatin remodelling is overall limited by the lack of an "Histone Code". The interpretation is not an easy task as the meaning of acetylation and deacetylation in certain residues is not unique. Functional significance should be sought in the overall value of histone modifications and that resulting from the combination of different histone modifications during chromatin remodelling.

Oocyte meiotic maturation

As described in the previous paragraph, during the final oocyte growth phase, a significant increase of Sirt1 and Sirt6 transcripts was observed at the beginning of chromatin compaction in bovine oocytes (GSE48376; Labrecque et al., 2015; Lodde et al., 2017). Interestingly, during mouse oocyte maturation genome-wide analysis of polysome-bound mRNAs pool in GV and MII stage oocytes indicate that the same transcripts (Sirt1 and Sirt 6) are significantly translated during maturation (GSE35106; Chen et al., 2011,). Indeed, several Sirtuins are actively involved in the meiotic resumption and progression up to MII stage. In several studies, the modulation of SIRT1, SIRT2, SIRT3 and SIRT6 activity has revealed their role in the developmental competence acquisition of oocytes grown in vivo or matured in vitro in humans and animal models. For example, the administration of NAM, a non-competitive pan-Sirtuin inhibitor, during in vitro maturation impairs entry into meiosis I and the establishment of MII arrest in mouse (Riepsamen et al., 2015) and pig (Zhang et al., 2015). Conversely, activation of SIRT1 by means of resveratrol supplementation during in vitro maturation improved oocyte quality and embryo development in mouse (Liu et al., 2013), pig

(Itami et al., 2015; Li et al., 2016) and cow (Khan et al., 2017; Takeo et al., 2014; Wang et al., 2014). The increase of SIRT1 expression induced by resveratrol was correlated to enhanced mitochondria biosynthesis and degradation in oocytes, thereby improving mitochondrial function and developmental capability of the female gamete (Sato et al., 2014). Moreover, Ex527, a specific inhibition of SIRT1, caused an increase of reactive oxygen species (ROS) production and abnormal metaphase II plate in mouse oocyte suggesting that SIRT1 might be involved in oocyte maturation by regulating the redox state and ensuring normal spindle assembly (Di Emidio et al., 2014).

In mouse, the use of a specific SIRT2 inhibitor during oocyte in vitro maturation blocked the progression behind GVBD (Riepsamen et al., 2015). This was confirmed by loss-of-function experiments where Sirt2 knockdown affected spindle organization and chromosome alignment during meiosis (Zhang et al., 2014). In a mouse obesity model, high-fat diet increased ROS content and reduced SIRT3 expression was detected in oocytes (Zhang et al., 2015). Subsequent experiments ascertained that the specific depletion of SIRT3 in control oocytes elevates ROS levels while the overexpression SIRT3 attenuates ROS production in oocytes mouse obesity model, with a significant reduction of spindle defects and chromosome misalignment (Zhang et al., 2015). Consistently, Liu et al. (2017) demonstrated that SIRT3 exerts antioxidant effects in oocytes from diabetic mice by deacetylating SOD2. The role of SIRT3 was investigated also in human oocyte (Zhao et al., 2016). Using loss- and gain-of-function experiments, Zhao and collaborators demonstrated that a defective Sirt3 expression provoked a decrease of mitochondrial biogenesis (DNA copy number) thus impairing the developmental competence of in vitro matured oocytes in both human and mouse (Zhao et al., 2016). Most importantly, in the same study a retrospective analysis revealed a higher spontaneous abortion rate and a decreased embryo quality in patients undergoing IVM versus controlled ovarian stimulation cycles (Zhao et al., 2016).

Also SIRT6 has been proven to have a key role in controlling meiotic progression and its depletion after injection of Sirt6-targeting morpholino resulted in disruption of spindle morphology and chromosome alignment in oocytes (Han et al., 2015). In this study, Han and collaborators indicated

a role of Sirt6 in oocyte deacetylation since the depletion of Sirt6 resulted in the hyperacetylation of histone H4K16 that was hypothesized to cause chromatin perturbation thus contributing to spindle defects and chromosome misalignment (Han et al., 2015). This agrees with previous studies demonstrating that inadequate histone deacetylation causes chromosome missegregation and aneuploidy in eggs, which is afterwards responsible for embryonic developmental defects (Ma and Schultz, 2008). Precisely, H4K16 hyperacetylation in mouse oocyte affected kinetochore function generating a defective meiotic apparatus (Choy et al., 2011; Zhang et al., 2014; Ma and Schultz, 2013).

However, the involvement of Sirtuins in chromatin acetylation state during oocyte maturation remains substantially unexplored and the information is still fragmentary. Recent studies demonstrated also that in vitro maturation condition induced alteration of H4K16 acetylation that in turn affected chromosomal segregation during horse oocyte meiosis I (Franciosi et al., 2012; 2017). Compared to in vivo matured counterpart, in vitro maturation induced a massive H4K16 deacetylation, which was associated with an increased frequency of aneuploidy and spindle morphology anomalies (Franciosi et al., 2017). However, any difference in the relative mRNA abundance of transcripts encoding for SIRT1 was found between in vivo and in vitro matured oocytes, although translational or post-translational mechanisms cannot be ruled out. This hypothesis remains to be confirmed as well as the involvement of SIRT6 or different HAC/HDAC system in altering the histone acetylation status during culture conditions.

Overall, during oocyte development Sirtuins appear to sustain the process of chromatin remodelling by selectively deacetylating histone substrates, such as H3K9, to promote methylation and chromatin condensation, while during meiotic resumption Sirtuins activity is mainly focused in controlling oxidative stress response in order to ensure faithful chromosome segregation through meiotic division.

c. Oocyte aging

The developmental competence of the mature oocyte results from negative and positive signals targeting the germ cell during folliculogenesis and following ovulation. Negative signals promote oocyte aging processes in the ovary, in the oviduct, or during in vitro culture prior to IVF. The oocyte ages as the ovary ages. This process underlies the female age-dependent process known as "reproductive aging". It may derive from the prolonged stay of the oocyte in a resting phase as well as to its exposure to the aged ovarian microenvironment during growth and final maturation (Tatone et al., 2008). Mammalian oocytes have limited time for fertilization after ovulation (mouse 8-12 h; humans <24 h). Thus, extended presence of the oocyte in the oviduct before fertilization and IVF in vitro culture prior to insemination induces a time-dependent aging process, known as "postovulatory aging" (Lord and Aitken, 2013). These two aging phenomena are characterized by similar oocyte phenotype such as metaphase II spindle aberrations, cellular fragmentation, associated with an impaired control of cell cycle and decay of survival factors (Tatone et al., 2006). Moreover, both reproductive aging and postovulatory aging have been shown to lead to faulty spindle checkpoint predisposing oocytes to premature chromosome separation and aneuploidy. Further alterations shared by the two aging processes involve decline of mitochondrial functions and changes in the redox state, crucial determinants of oocyte competence (Tatone, 2008; Eichenlaub-Ritter, 2012; Lord and Aitken, 2013). Changes in oocyte SIRT1 expression have been associated with both reproductive aging and postovulatory aging

Reproductive ageing

Recently, changes involving Sirtuins have been included in the complex phenotype underlying the low developmental competence of the aged oocyte, as a part of the age-dependent decline of antioxidant defences. SIRT1 has been recently found to orchestrate the adaptive response to oxidative stress in the mouse oocyte probably by promoting the activities of FoxO3a and Sod2. Changes in SIRT1 localization confirm its involvement in oxidative stress suggesting that both nuclear and cytoplasmic targets participate in SIRT1 signalling (Di Emidio et al., 2014). Indeed, inhibition of

SIRT1 suppresses the oocyte ability to up-regulate Sod2 gene and counteract ROS increase under oxidative stress. Moreover, SIRT1-dependent antioxidant response is disrupted in aged oocytes where a lower ability to regulate the SIRT1 expression is detected. A novel aspect of the involvement of SIRT1 in oocyte antioxidant response and aging is the role of miR-132 in the modulation of mRNA levels of Sirt1, a validated target of this microRNA (Strum et al., 2009), a finding which opens new horizons in the knowledge of upstream signalling targeting oocyte Sirtuins. The potential role of SIRT1 as a countermeasure against oocytes aging also arises from studies on aged cows where supplementation of maturation medium with N-acetyl-cysteine (NAC) reduced the levels of ROS while SIRT1 inhibition increased the rate of abnormal fertilization (Takeo et al., 2013). Finally, observations in aged mice provide significant evidence that a contributing factor to oocyte age-dependent spindle defects and chromosome disorganization is the decreased level of SIRT2 with subsequent effects on the acetylation status of H4K16, α-tubulin and BubR1. Accordingly, Sirt2 overexpression in aged oocytes is able to lower acetylated H4K16, α-tubulin and BubR1 so reducing the penetrance of maternal age-associated meiotic defects. (Zhang et al., 2014; Qiu et al., 2017).

Postovulatory ageing

Zhang et al. (2016) reported that postovulatory aging is associated with the precocious decrease of Sirt1 transcripts followed by that of Sirt2 and Sirt3, suggesting that these Sirtuins have an important role in the maintenance of oocyte competence and that manipulating Sirtuin activity may reverse the aging phenotype. A possible role of Sirtuins as sensors of the oxidative stress occurring during postovulatory aging has been hypothesized. Indeed, SIRT1 stimulation by resveratrol delays oocyte aging in pig and mouse oocytes (Ma et al., 2015; Zhang et al., 2016) as demonstrated by decreased levels of abnormal MII spindles and mitochondrial distribution, whereas Sirtuin inhibition by NAM, a pan-inhibitor of Sirtuins, during in vitro aging results in a marked increase of ROS levels. Moreover, exposure of mouse oocytes to quercetin, a natural antioxidant, attenuates the decrease in maturation-promoting factor (MPF) activity, the increase of ROS, the onset of apoptosis, prevents histone modifications by preventing Sirtuin decay during postovulatory aging (Wang et al., 2017). Finally it

could be hypothesized that reduced levels of SIRT2 during post ovulatory aging may contribute to spindle stability after ovulation as an effect of altered tubulin acetylation (REF? o da quali evidenze?).

d. Granulosa cells

The expression of SIRT1, SIRT3 and SIRT5 in mural granulosa cells (GCs) and cumulus cells (CC) of the Graafian follicle is well documented (Morita et al., 2012; Zhao et al., 2014; Pacella-Ince et al., 2014a; Pacella-Ince et al., 2014b).

A main role of SIRT1 in the regulation of GC proliferation is suggested by the observation that porcine GCs transfected with Sirt1 cDNA showed enhanced expression of proliferation markers along with a significant decrease of NF-κB, a SIRT1 substrate, in response to in vitro FSH stimulation (Pavlova et al. 2013; Sirotkin et al., 2014). On the other hand, stimulation of SIRT1 with resveratrol concentration higher than 50 μM decreases GC proliferation and promotes the expression of key steroidogenic enzymes (STAR, LH-R and P450Aromatase) and progesterone secretion (Morita et al., 2012). On this basis, the authors have proposed a role for SIRT1 in terminal differentiation and luteinisation of GCs, which has been supported by further studies (Pavlova et al. 2013; Sirotkin et al., 2014).

It has been recently reported that in response to the anti-proliferative alkylating agent cyclophosphamide (CPM), human GC line COV434 activate the transcription of Sirt1 and HuR, its mRNA binding protein. Sirt1 and HuR increase with the same kinetics demonstrating the involvement of SIRT1 in the early steps of cell response to CPM damage in these cells (Di Emidio et al., 2017).

A role for SIRT1 as energy sensor in GCs has arisen by the finding that SIRT1 signalling is involved in the response of human GCs and KGN cells to the insulin sensitizer metformin (MetF). MetF is found to increase NAD^+/NADH ratio and SIRT1 activity in a dose-dependent manner (Cantó et al., 2009; Caton et al., 2010). According to the authors, beneficial effects of MetF on progesterone and estradiol secretion are mediated by SIRT1 throughout visfatin, a cytokine hormone and rate-limiting enzyme in NAD biosynthesis (Reverchon et al., 2016). Finally, there are data suggesting that SIRT1 orchestrates cell stress response to oxidative stress in human GC lines by targeting FOXL2, a

544 transcription factor essential for ovarian functions and maintenance (Benayoun et al., 2009; 2011).
545 By focusing on mitochondrial Sirtuins, SIRT3 seems to cooperate with SIRT1 in the regulation of
546 steroidogenic genes, progesterone secretion and ROS detoxification in human GCs (Fu et al., 2014).
547 Moreover, SIRT3 has been revealed to be an important sensor of metabolic state in human GCs and
548 CCs by targeting mitochondrial enzymes, such as glutamate dehydrogenase (GDH). A decrease of
549 SIRT3 activity along with an increase of inactive acetylated GDH form characterizes the process of
550 GCs and CCs cell aging, whereas SIRT5 expression and activity decreased with aging along with
551 alteration of activity of the SIRT5 target carbamoyl phosphate synthase I (CPS1) in GCs and CCs.
552 Therefore, alterations of mitochondrial Sirtuins may affect post-translation modifications of
553 mitochondrial proteins therefore producing metabolic alterations in the aged follicle (Pacella-Ince et
554 al., 2014a; 2014b).
555

5. Sirtuins in male reproductive functions

The first evidence on a possible role for Sirtuins in male fertility control came from mice carrying a null allele of the SIRT1 gene (McBurney et al., 2003). Although many of these whole body SIRT1 knockout mice died before reaching maturity, due to a variety of disorders in heart, pancreas, liver, lung, and skeleton, some of them did survive, allowing the evaluation of the impact of SIRT1 loss on fertility in the adulthood, when mutant mice displayed infertility with decreased testes size (McBurney et al., 2003; Coussens et al., 2008). The rare sperms recovered from their cauda epididymis were immotile, exhibited abnormal morphology (McBurney et al., 2003) and were not as efficient in *in vitro* fertilization (IVF) experiments as wild-type sperm (Coussens et al., 2008). Subsequently, a similar phenotype was also observed in a mouse strain homozygous for a point mutation (H355Y) that ablates the catalytic activity but does not affect the whole amount of the SIRT1 protein (Seifert et al., 2012), thus strengthening the notion that an active SIRT1 would be required for normal male reproductive function.

Nevertheless, whether SIRT1 regulates spermatogenesis at postnatal stages by controlling hypothalamic-pituitary-gonadotropin (HPG) axis signalling rather than having an intrinsic direct effect on male germ cells per se, still remains a matter of debate.

a. The spermatogenetic process

As Sirtuins are highly expressed in mammalian testicular tissue (Michishita et al., 2005), where, in particular, SIRT1 has been detected in the nuclei of spermatogonia, spermatocytes and round spermatids (McBurney et al., 2003), an intrinsic direct activity of SIRT1 in developing male germ cell during spermatogenesis has been claimed.

Histological analyses of testes from $Sirt1^{-/-}$ mice revealed dramatically reduced numbers, or even a total absence, of spermatids (McBurney et al., 2003; Kolthur-Seetharam et al., 2009), due to a spermatogenesis arrest in late-meiotic prophase with degenerating or dying spermatocytes (Kolthur-Seetharam et al., 2009). This was associated to severe morphological abnormalities, apoptotic

581 features and increased DNA damage within the seminiferous epithelium (McBurney et al., 2003;
582 Coussens et al., 2008; Kolthur-Seetharam et al., 2009; Bell et al., 2014).
583 Molecular mechanisms by which SIRT1 deficiency could lead to this spermatogenesis derangement
584 are still largely unknown and most of explanatory attempts remain speculative.
585 As testicular apoptosis is dependent from the activity of p53 (Beumer et al., 1998; Yin et al., 1998;
586 Allemand et al., 1999), which, in turn, is triggered by acetylation, a role of uncontrolled p53 activity
587 has been proposed (McBurney et al., 2003). Under physiological conditions, in several cell types,
588 SIRT1 binds and deacetylayes p53, thus repressing its pro-apoptotic signaling (Luo et al., 2001;
589 Vaziri et al., 2001; Langley et al., 2002). Therefore, germ cell apoptosis in testes from SIRT1
590 knockout mice could reflect an up-regulation of p53 activity (McBurney et al., 2003).
591 Sperm defects observed in $Sirt1^{-/-}$ mice could also result from oxidative stress, since SIRT1 co-
592 operates with SIRT3 and PGC-1α in triggering antioxidant defense systems (Rato et al., 2016). The
593 transcriptional co-activator PGC-1α promotes the activity of enzymes involved in mitochondrial
594 functions (Rodgers et al., 2005; Scarpulla et a., 2012). In particular, when deacetylated and activated
595 by SIRT1, PGC-1α induces the transcription of gene encoding SIRT3 (Kong et al., 2010), a
596 mitochondrial Sirtuin, which is also expressed in mammalian testicular tissue (Michishita et al.,
597 2005). SIRT3 is required for proper mitochondrial activities and mediates the induction of several
598 components of the ROS detoxifying system (Kong et al., 2010): in this scenario, a derangement of
599 the SIRT1/PGC-1α/SIRT3 system could lead to an imbalance between ROS and antioxidant defenses,
600 as demonstrated in testes from a high-energy-diet induced pre-diabetic rat model (Rato et al., 2014).
601 Since the activity of Sirtuins is dependent on NAD^+ concentrations and changes in NAD^+/NADH
602 ratio reflect the cellular energy levels, all members of this family are regarded as key metabolic
603 sensors (Yang et al., 2007; Houtkooper et al., 2010; Cantó et al., 2012) and a decreased expression
604 of SIRT1 and SIRT3 is associated with a higher glycolytic activity in different tissues (Ye et al.,
605 2017). In particular, the high-energy-diet induced pre-diabetes significantly decreased SIRT3 levels
606 (Rato et al., 2014), while promoting glycolysis in rat testis (Rato et al., 2013; Rato et al., 2014).

607 Although glucose metabolism and resultant lactate production are crucial for normal spermatogenetic
608 process (Boussouar and Benahmed, 2004; Oliveira et al., 2015), an increased glycolytic activity may
609 promote a mitochondrial overproduction of ROS (Rato et al., 2013). This would be further favoured
610 by the fact that the loss of SIRT1 and SIRT3 also leads to a dysfunctional electron transport chain,
611 while lowering antioxidant defenses (Rato et al., 2016). Mammalian spermatozoa, indeed, are
612 uniquely sensitive to oxidative stress, mainly due to the high polyunsaturated fatty acid (PUFA)
613 content of their membranes (Tremellen, 2008). PUFAs play a major role in maintaining membrane
614 fluidity and fusogenicity, which are required for sperm acrosomal exocytosis and sperm-oolemma
615 interaction. Unfortunately, they are also particularly vulnerable to lipid peroxidation (Aitken et al.,
616 1993; Wagner et al., 1994; Barbonetti et al., 2011).
617 Oxidative stress could also mediate the association between SIRT1 silencing and gem cell apoptosis.
618 Reactive oxygen species, indeed, can potentially impact mitochondrial pathway of apoptosis in many
619 ways (Wu and Bratton, 2013). They can disrupt the activity of anti-apoptotic factors, such as the
620 BCL-2 proteins, which hinder mitochondrial membrane permeabilization, a key step in the intrinsic
621 pathway of apoptosis (D'Alessio et al., 2005). Moreover, ROS can facilitate the cytochrome *c* release
622 from the inter-mitochondrial membrane space into the cytoplasm through the oxidative inactivation
623 of mitochondrial cytochrome *c*-binding factors, such as cardiolipin (Petrosillo et al., 2001; Shidoji et
624 al., 1999). The cytochrome *c* release, in turn, also disrupts the electron transport chain and stimulates
625 mitochondrial ROS generation, thus triggering a vicious cycle (Cai and Jones, 1998). Both oxidative
626 stress and apoptosis could be responsible for the DNA damage, which has been revealed in germ cells
627 from Sirt1$^{-/-}$ mice by TUNEL (Kolthur-Seetharam et al., 2009; Bell et al., 2014) and comet assay
628 (Coussens et al., 2008; Bell et al., 2014). Notably, mutant animals also exhibit a defect in the histone
629 to protamine transition and altered chromatin condensation (Bell et al., 2014), which increases the
630 susceptibility of sperm DNA to apoptotic/oxidative damage (reviewed in Zini and Libman, 2006).
631 SIRT1 could cooperate with SIRT6 in driving chromatin condensation, as a poor sperm protamination
632 has been also reported in high-fat diet-fed obese mice, which exhibited a significant decrease of

633 SIRT6 expression in the nucleus of spermatids (Palmer et al., 2011). Very recently, a role of SIRT1
634 in spermiogenesis has been confirmed in a germ cell-specific SIRT1 knockout mouse model, where
635 Liu and collaborators (2017) demonstrated an accumulation of acetylated LC3 in the spermatid
636 nucleus, which affected the recruitment of several acrosome biogenesis-related proteins to the
637 acrosomal vesicles: these findings pointed to a novel function for SIRT1 during acrosome biogenesis.
638 To better clarify the role of SIRT1 in the spermatogenesis, Coussens and collaborators (2008) carried
639 out a microarray analysis of global gene expression in the testis from SIRT1 deficient mice, revealing
640 aberrant expression of several genes involved in spermatogenesis, and sumoylation of proteins.
641 Sumoylation, indeed, can be modulated by the SIRT1 deacetylase activity (Bouras et al., 2005;
642 Stankovic-Valentin et al., 2007) and represents a post-translational protein modification which, in
643 somatic cells, has been implicated in a number of processes, such as transcription, nuclear transport,
644 DNA repair, mitochondrial activity, plasma membrane ion transport, cell cycle and chromatin
645 structure (reviewed in Andreou and Tavernarakis, 2009). Intriguingly, it has been reported that
646 sumoylation also play multiple roles in testicular function and spermatogenesis, such as
647 spermatogonia proliferation, meiotic sex chromosome inactivation, centromeric heterochromatin
648 organization, and reshaping the spermatid nucleus (Rogers et al. 2004; Vigodner and Morris 2005;
649 Vigodner et al. 2006; Brown et al. 2008; Metzler-Guillemain et al. 2008; Vigodner 2009).
650 Most of the data about the possible role of Sirtuins in male fertility have been produced in the
651 knockout mouse with a whole-body defective SIRT1 activity. Obviously, this model suffers from a
652 major limitation: it does not discriminate whether the spermatogenesis damage results from the loss
653 of spermatogenetic cell-specific SIRT1 activities rather than the lack of an extrinsic positive control
654 exerted by SIRT1 on the spermatogenetic process. In this view, the role of SIRT1 in controlling the
655 HPG axis activity has been well documented.

656 **b. Hypothalamic-pituitary-gonadal axis**

657 The biological plausibility of an involvement of Sirtuins in the hypothalamic-pituitary-gonadal
658 (HPG) axis function arises from the evidence that SIRT1 is highly expressed in the hypothalamus

659 (Cakir et al., 2009) and, in particular in gonadotropin releasing hormone (GnRH) neurons (Di Sante
660 et al., 2015). Kolthur-Seetharam and collaborators (2009) observed first that in testes from SIRT1
661 knockout mouse, spermatogenesis arrest and apoptosis of germ cells were associated to abnormal
662 Leydig and Sertoli cell maturation, and strongly reduced intratesticular testosterone levels. They
663 demonstrated that this phenotype is the consequence of reduced hypothalamic GnRH secretion,
664 resulting in a significant reduction of FSH and LH levels (Kolthur-Seetharam et al., 2009). A closer
665 analysis showed that several Leydig cell-expressed genes involved in steroidogenesis, such as StAR,
666 were also down-regulated (Kolthur-Seetharam et al., 2009). Under physiological conditions,
667 gonadotropins cooperate to drive normal spermatogenesis: FSH binds to its receptor (FSHR)
668 expressed by Sertoli cells and regulates their development and function, meanwhile, LH binding to
669 its cognate receptor (LHR) on Leydig cells promotes testosterone biosynthesis (Ramaswamy and
670 Weinbauer, 2014). In particular, intratesticular testosterone has a pivotal role for initiation and
671 maintenance of spermatogenesis by acting on androgen receptors expressed in Sertoli cells (Walker,
672 2011). Interestingly, the blockage of testosterone biosynthesis by inactivating genes encoding LH or
673 LHR, leads to a phenotype similar to that of $Sirt1^{-/-}$ mice (Lei et al., 2001; Zhang et al., 2001; Ma et
674 al., 2004; Zhang et al., 2004). Recently, a hypogonadotropic hypogonadism has been confirmed in
675 SIRT1 knockout mice and attributed to a failure in the GnRH neuronal migration from the
676 vomeronasal organ towards the hypothalamus (Di Sante et al., 2015). Authors demonstrated that in
677 GnRH neuronal cell lines, SIRT1 binds and deacetylates cortactin (Di Sante et al., 2015). As
678 previously demonstrated in different cell lines, cortactin, upon deacetylation by SIRT1, promotes cell
679 migration by modulating F-actin polymerization (Zhang et al., 2009; Nakane et al., 2012; Byles et
680 al., 2012). Interestingly, in the study by Di Sante and collaborators (2015), $Sirt1^{-/-}$ mice were also
681 affected by anosmia, thus exhibiting a phenotype overlapping the Kallmann's Syndrome, the
682 congenital hypogonadotropic hypogonadism resulting from failed GnRH neuronal migration in
683 humans.
684

6. Sirtuins in post-fertilization events

The role of Sirtuins in post-fertilization is known since 1994 when the pan Sirtuin inhibitor NAM was found to inhibit mouse embryo development in vitro (Tsai and Gardner, 1994). According to this insight, further studies have shown that other Sirtuin inhibitors (i.e. sirtinol, BML-210) induced embryo developmental arrest in murine and porcine IVF embryos (Kawamura et al., 2010; Kwak et al., 2012).

All Sirtuins are expressed in the MII oocyte and their expression gradually decreased following the first cleavage indicating that Sirtuins mRNAs are stored during oogenesis. Sirtuin inhibition decreases the formation of blastocysts, the total cell number in blastocysts and results in the down-regulation of essential genes including Sirt2 and Sirt3 (Kawamura et al., 2010; Kwak et al., 2012).

Overall, Sirt1 deficient mice displayed a compromised fetal development, increased postnatal mortality rates and smaller size with developmental defects including reduced development of mammary gland (McBurney et al., 2003; Li et al., 2007; Coussens et al.; 2008). In search for Sirtuin-mediated mechanisms, a recent paper provides evidence that the negative effect of Sirtinol on embryo development may be ascribed to defective modulation of genes involved in autophagy and apoptosis (Kim et al., 2017).

In the zygote, where the level of Sirtuin expression is very low, oxidative stress conditions promote the up-regulation of some Sirtuin genes including Sirt3 (Kawamura et al., 2010). Further experiments based on knockdown and knockout models have clearly demonstrated that SIRT3 protects embryos against stress conditions during in vitro fertilization and embryo culture by maintenance of mitochondrial functionality. Pronuclei and blastocysts rates significantly decreased when Sirt3 null oocytes are subjected to IVF regardless of sperm genotype supporting the conclusion that storage of Sirt3 during oogenesis is essential for fertilization and preimplantion development. Indeed, in the absence of SIRT3 in vitro embryos undergo developmental arrest when exposed to oxidative stress via a mitochondrial ROS-p53-mediated mechanism as demonstrated by elegant experimental approaches at multiple levels (Kawamura et al., 2010).

All Sirtuins are expressed in the human endometrium (Bartosch et al., 2016). Preliminary evidence would suggest a possible role of Sirtuin in embryo implantation and uterine receptivity. By using an *in vitro* implantation assay, Shirane et al. (2012) proposed a role for SIRT1 in regulating the expression of E-cadherin, the cytoskeletal protein present in luminal epithelium and trophectoderm, and involved in the initial attachment of embryos. Therefore, the possible role of Sirtuins as novel targets for improvement of uterine receptivity deserves attention in future investigations.

7. Manipulating Sirtuins to ameliorate fertility

In addition to extending lifespan in numerous invertebrates and exerting beneficial effects on several diseases in mammals (i.e., cancer, inflammation, cardiovascular diseases, and neurodegeneration), preclinical and clinical studies have provided significant evidence that strategies aimed to improve Sirtuins expression or activity counteract deleterious effects on fertility of polycystic ovary syndrome PCOS, endometriosis, diabetes, xenobiotic stress and aging.

PCOS is a metabolic disorder affecting about 6-10% of women of reproductive age with heterogeneous clinical manifestations. In addition to changes in reproductive functions (infertility, anovulation, polycystic ovaries and hyperandrogenism), PCOS is associated with insulin resistance and hyperinsulinemia (Balen et al., 2016). The hypothesis that SIRT1 may be involved in the development and progression of PCOS has been investigated, and evidence that resveratrol exert beneficial effects on PCOS has been provided (Ortega and Duleba, 2015). Recently, a randomized controlled trial revealed that resveratrol administration significantly reduced ovarian and adrenal androgens in PCOS patients in association with an improvement of insulin sensitivity and a decline of insulin level (Banaszewska et al., 2016). Based on the results on a preclinical model, Ergenoglu et al. (2015) concluded that beneficial effects of resveratrol on PCOS phenotype can be ascribed to its antioxidant properties. In PCOS rat models, SIRT1 expression in the ovary was found to be lower than in normal rats and this effect was reversed by treatments with MetF or exenatide employed to reduce ovarian insulin resistance (Reverchon et al., 2013; Tao et al., 2015). Moreover, in human granulosa cells MetF effects on Visfatin were shown to be dependent on SIRT1 activity (Reverchon et al., 2013). This is consistent with data based on the use of SIRT1 inhibitors showing that SIRT1 mediates the positive effects of Visfatin on steroid production in bovine granulosa cells.

The Sirtuin role in the regulation of inflammatory pathways underlying endometriosis has been recently proposed. Endometriosis, a disorder in which endometrial cells grow outside of the uterus, represents a common gynecological condition frequently associated with pelvic pain and infertility. Recently, SIRT1 has been suggested as a key driver of the progesterone resistance contributing to

744 pathophysiology and survival of ectopic lesion and proposed as a valuable endometrial marker in
745 women with endometriosis (Yoo et al., 2017). Pro-inflammatory mediators are involved in the
746 progression of endometriosis and inhibiting inflammation is important in the control of the disease.
747 Experiments carried out in primary endometriotic stromal cells and peritoneal immune cells exposed
748 to resveratrol or sirtinol, have revealed an important role of SIRT1 in regulating the expression and
749 production of inflammatory cytokines (Takuchi et al., 2014; Mvunta et al., 2016). Overall, these data
750 represent a strong starting point for further investigations about the role of Sirtuin activators as
751 negative regulators of the inflammatory response in endometriosis, thereby validating their potential
752 as novel agents in endometriosis therapy.
753 Significant evidence from experiments on high-fat diet induced obese mice and rats support the
754 potential of SIRT1 as a therapeutic target for preventing the negative effects of obesity on ovarian
755 lifespan. Consistent with reduced levels of ovarian SIRT1 and SIRT6 expression, oral administration
756 of resveratrol or SRT1720, a 1000 times more potent SIRT1 activator, preserves follicle reserve and
757 suppress follicle atresia via activating SIRT1, suppressing mTOR signaling, and modulating NFκB
758 signalling (Luo et al., 2012; Wang et al., 2014; Zhou et al., 2014; Liu et al., 2015). Therefore, a better
759 understanding of the relationship between SIRT1 and mTOR signaling could promote the
760 development of new pharmacological approach to treat metabolic diseases associated with obesity.
761 Recently, gonadotoxicity exerted by chemotherapy and radiotherapy treatments has been associated
762 with reduced levels of ovarian Sirtuins. Accordingly, administration of Sirtuin stimulators provides
763 protection against ovarian toxicity induced by anticancer therapies in animal models. It is well known
764 that alkylating agents, i.e. cyclophosphamide, and /or radiations pose high risk of POF in humans and
765 animal models (Roness et al., 2016). In rats, the ovarian damage induced by these treatments results
766 in the decline of SIRT1, SIRT3 and SIRT6 expression and can be counteracted by Sirtuin activating
767 strategies, i.e. resveratrol administration or caloric restriction (Xiang et al., 2012; Zhang et al., 2016;
768 Said et al., 2016). In particular, resveratrol-activated SIRT1 expression was associated with
769 increasing AMH levels and down-regulation of pro-inflammation signalling in rats exposed to a

single dose of γ-radiation, and with reduced p53 levels in cyclophosphamide treated rats. One of the mechanisms through which SIRT1 signalling promotes a fertoprotective effect is the interrelationship with mTOR signalling (Liu et al., 2016). Indeed, numerous mTOR inhibitors, including rapamycin, were recently found to preserve ovarian reserve from genotoxic chemotherapy in mice (Goldman et al., 2017).

By focusing on the early phase of ovarian response to CPM insult, Di Emidio et al. (2017) have recently discovered that ovarian SIRT1 increases in CPM mouse ovaries probably as a result of increased levels of its mRNA binding protein HuR. Similarly, the mitochondrial sirtuin SIRT3 rises, whilst SOD2 and the mitochondrial biogenesis activator PGC1-α decrease, providing the first evidence of CPM induced mitochondrial damage in the ovay. Therefore when a single dose of CPM provoking 60% loss of primordial follicles is applied, engagement of SIRT1 and SIRT3 seems to be ineffective to orchestrate repair but could be considered as an early marker of ovarian damage. In line with this hypothesis, in vivo administration of the natural antioxidant crocetin or AS101 prevents both Sirtuin up-expression and loss of ovarian follicles (REF).

As reported in the paragraph 4.a, improved expression of ovarian Sirtuin can be achieved by means of CR, rapamycin, resveratrol and SRT1720. All these strategies have the potential to prolong reproductive lifespan in aging female mice. Anti-aging dietary strategies associated with increased Sirt1 expression include NAC supplementation for 2 months. Indeed, increased rate of fertilization and early embryo development in association with higher expression level of Sirt1 and Sirt2 and increased telomerase activity length (Liu et al., 2013). All these data need to be extended in future studies in order to prove safety and potential side effects in the long term of these strategies, before being taken into account to delay the occurrence of menopause.

8. Concluding remarks and future perspectives

In the present study, we have provided an overview and update of the function of Sirtuins in the reproductive system also suggesting that manipulation of Sirtuin activity may have a great therapeutic potential in the reproductive field. Furthermore, Sirtuin-mediated regulation of energy homeostasis, mitochondrial biogenesis, chromatin remodelling and protection against oxidative stress makes these molecules relevant to improve our understanding of reproductive cells and organs.

Being evolutionary conservative NAD+ dependent histone deacetylases, it is not surprising that Sirtuins are involved in a plethora of functions underlying the development of gametes competent for fertilization and embryo development. More specifically, Sirtuins participation in the regulation of ovarian reserve, follicle development and luteinisation has clearly emerged (Table III). Although deserving more attention and specific investigation, Sirtuins have been found to regulate specific functions of male germ cell, Sertoli cells and Leydig cells (Table IV). The role of Sirtuins in oocyte function seems to be twofold and blurred between two main functions: during oocytes development Sirtuins appear to sustain the process of chromatin remodelling while during meiotic resumption Sirtuins activity is mainly focused in controlling mitochondrial activity and biogenesis to face the oxidative stress response. Also, oocyte Sirtuins appear to be crucial in preimplantation embryo development. Finally, evidence in preclinical models supports the potential of Sirtuin modulators for ameliorating fertility in PCOS, diabetes, endometriosis, xenobiotic stress and aging (Figure 3).

Although many aspects need to be clarified, the most relevant concept arisen from the current scenario is that Sirtuins, by accomplishing the role of guardians of the energy status and genome integrity, are essential to maintain the strict link between energy metabolism and reproductive physiology underlying fertility.

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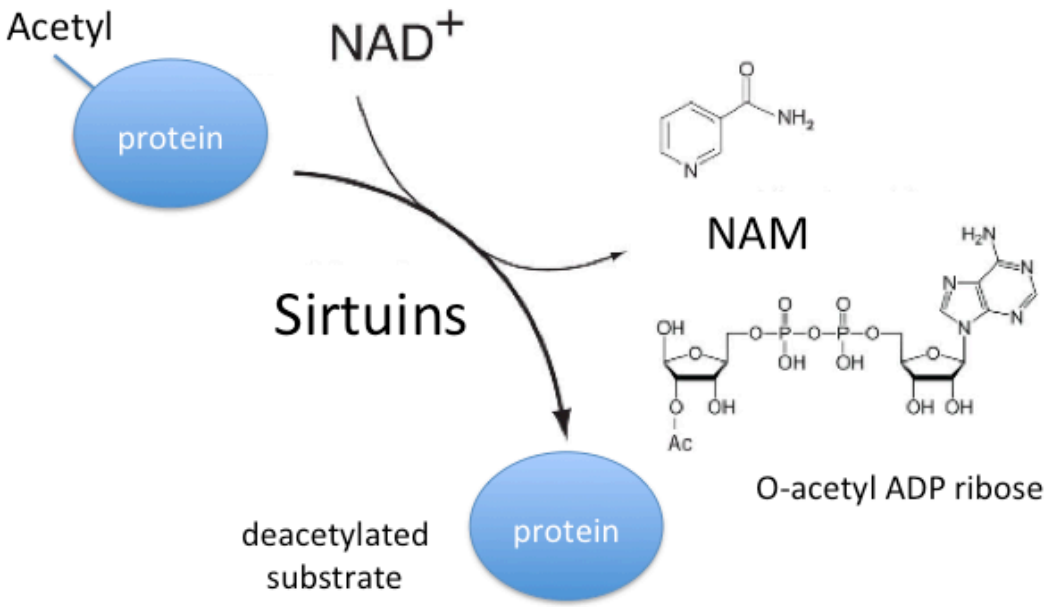
Figure Legend

Figure1. Sirtuins reverse acetyl modifications of lysine residue on histones and other proteins in a reaction that consumes NAD+, releasing nicotinamide (NAM), a Sirtuin competitive inhibitor, and the de-acetylated substrate.

Figure 2. Main cellular functions regulated by mammalian Sirtuins, and major targets of Sirtuin-dependent enzymatic activities. Ac, acetyl group; AOX, antioxidants; Succ, succinyl group; BubR1, beta homolg of S. cerevisiae BUB1; CPS1, carbamoyl phosphate synthetase 1; FOXOs, forkhead box transcription factors; G6PD, glucose-6-phosphate dehydrogenase; H3, histone H3; H4, histone H4; NRF2, nuclear factor erythroid 2-related factor 2; OS, oxidative stress; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; p53, pumor protein p53; SOD1, Cu-Zn superoxide dismutase.

Figure 3. Main Sirtuin functions during different stages of folliculogenesis and embryo development and positive effects of Sirtuin stimulation in aging, endometriosis, PCOS (Polycystic Ovarian Syndrome) and metabolic diseases, chemo- and radiotherapy.

Figure 1


Acetyl
protein
NAD+
Sirtuins
NAM
O-acetyl ADP ribose
deacetylated
substrate
protein

1558 Figure 2
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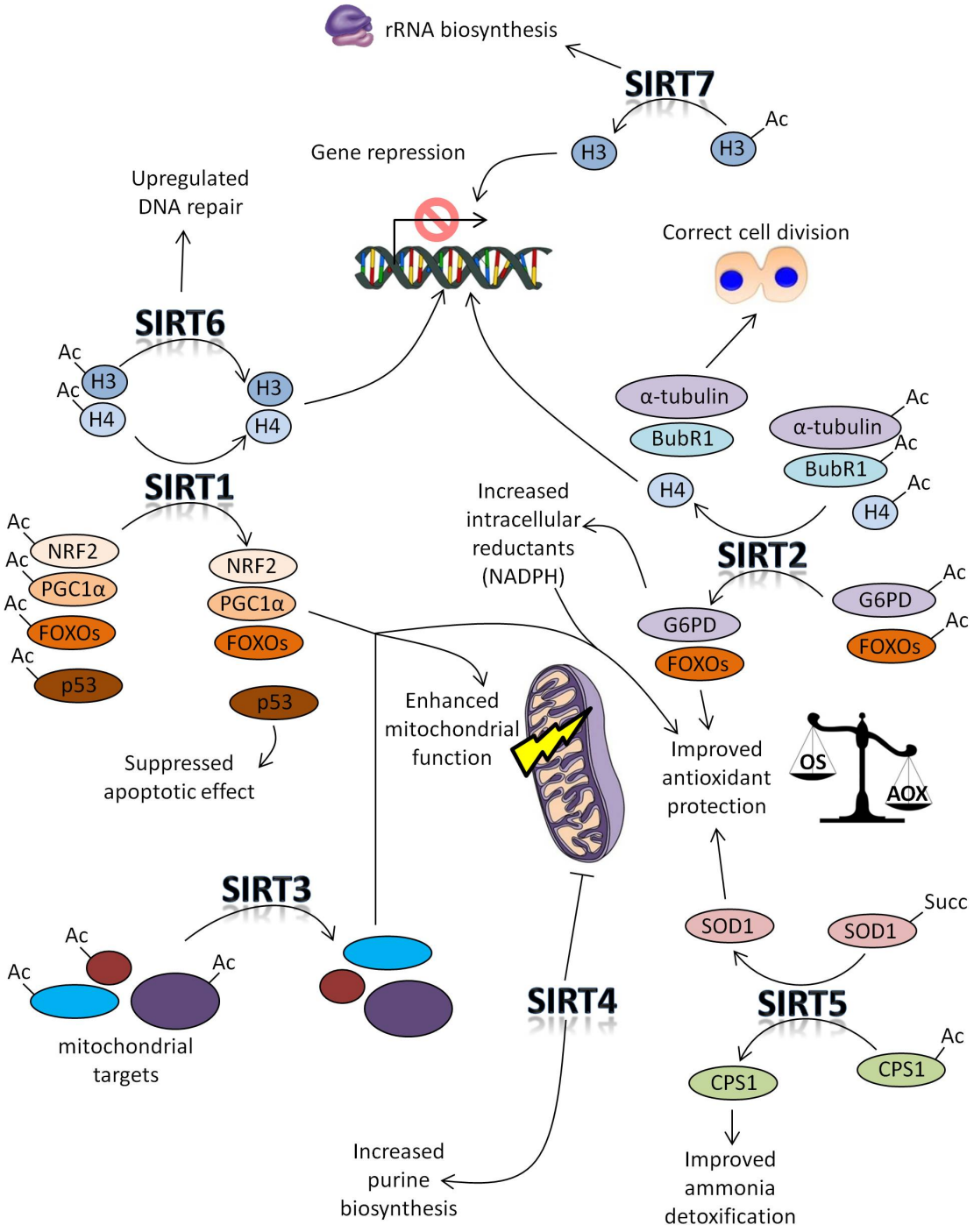

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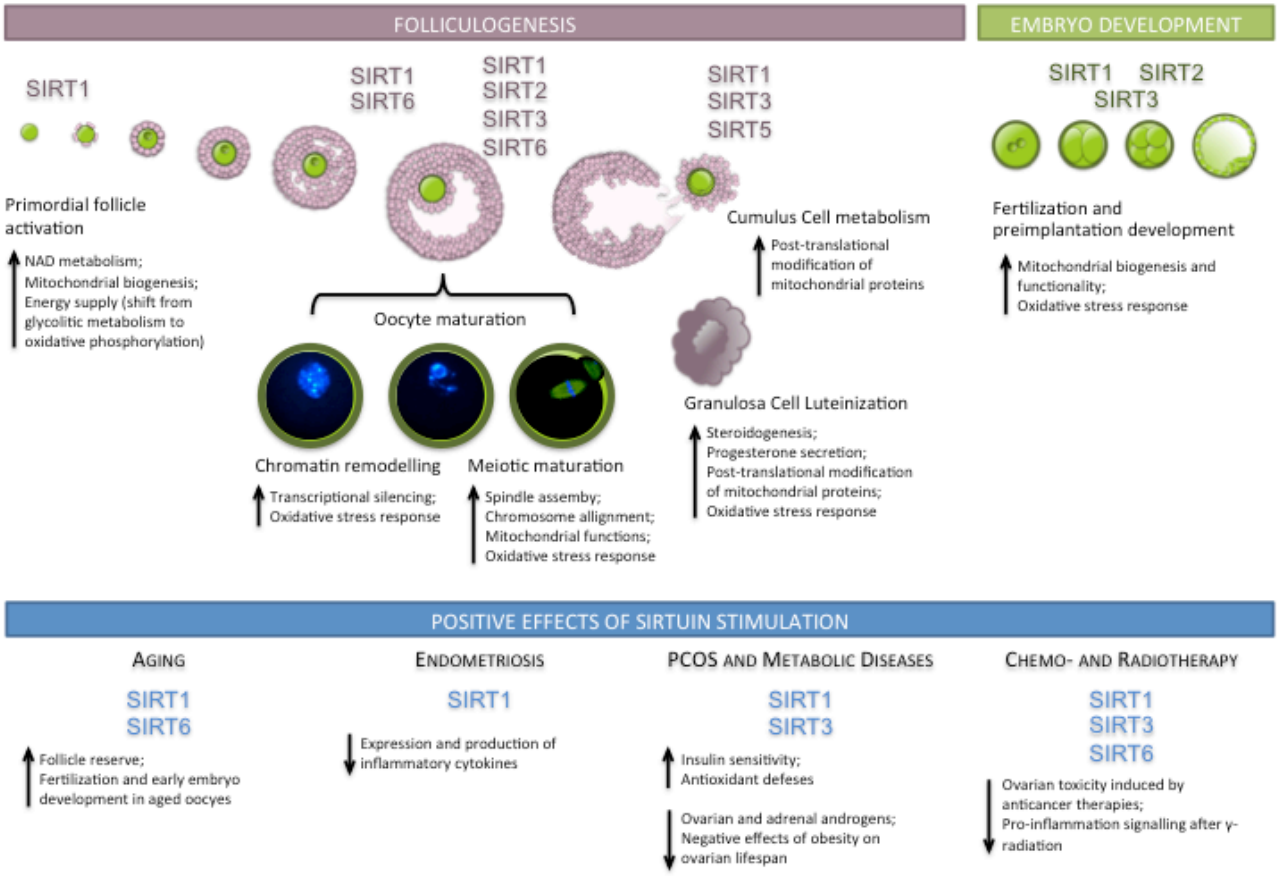
1562 Figure 3



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1567 Table I Fertility in Sirtuin transgenic models
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Mouse strain	Modification induced		Fertility	Appearance of reproductive organs and germ cells	Proposed target	Proposed mechanism	References
129/Sv	Sirt1 knockout	female	very low; only one female out of seven caged with male for 7 months was fertile but unable to suckle	small ovaries; thin walled uterus; presence of all follicles classes but no corpora lutea; normal oocytes; lack of ovulation and permanent diestrus	SIRT1 is part of IGF signalling	defective IGF signaling in granulosa cells may be responsible for hormonal defects leading to abnormal ovulation	McBourney et al., 2003
				Small organs; presence of all stages of spermatogenesis but abnormal spematids; abnormal immotile sperm	p53 is deacetylated and downregulated by SIRT1	Apoptosis in the testis is mediated by p53, elevated frequency of apoptotic cells may be a consequence of lack of SIRT1	McBourney et al., 2003;
		male	no	Abnormal Leydig and Sertoli cell maturation; prepubertal reduction or loss of spermatids and spermatocytes degeneration; reduced expression of steroidogenic genes resulting in low intratesticular testosterone	SIRT1 regulates spermatogenesis at postnatal stages by controlling HPG signalling	Reduced GNRH expression; reduced FSH levels and undetectable LH	Kolthur-Seethram et al., 2009
129SvJ/C57B6	Sirt1 knockout	female	low	Impeded ductal morphogenesis	SIRT1 is part of IGF signalling	Reduced levels of IGF	Li et al., 2007
		male	low	unknown	unknown	unknown	Li et al., 2007
C57BL/6	Sirt1 knockout	female	no; oocytes can be fertilised by IVF but present a reduced efficiency in generating 2-cell embryos and live offspring	unknown	unknown	unknown	Coussens et al., 2008
		male	no; sperm can fertilise oocytes by IVF but present a reduced efficiency in generating 2-cell embryos and live offspring	Abnormal seminiferous tubules; reduced sperm number; sperm with DNA single or double strand breaks; aberrant expression of genes involved in spermatogenesis	Sirt1 promotes the transcriptional silencing of specific genes	SIRT1 controls spermatogenesis	Coussens et al., 2008
129SvJ and 129/CD1	Sirt1 H355Y (point mutation at catalytic domain)	female	yes	unknown	unknown	This phenotype is less debilitating than Sirt1 null/null supporting the hypothesis that SIRT1 has functions not dependent on its deacetylase activity	Seifert et al., 2012
		male	no	Presence of all stages of spermatogenesis; reduced number of mature sperm that failed to acquire motility	unknown		
Sirt1 flox/flox; FVB/N-Tg(Stra8-cre)1Reb (Stra8-cre)	Male germ line specific Sirt1 knockout	male	no	Small testes; delay in differentiation of premeiotic germ cells; decreased sperm number but increased proportion of aberrant morphology and DNA damage; aging like phenotype	SIRT1 acetylates histone H4 at residues K5, K8 and K12 which are important for histone to protamine transition	SIRT1 controls chromatin packaging	Bell et al., 2014
Sirt1 flox/flox; Tnap-Cre	Male germ line specific Sirt1 knockout	male	very low	Round head sperm and abnormal acrosome biogenesis	SIRT1 deactetylases LC3 which is essential for autophagosome and ATG7, which promotes acrosome biogenesis	Acrosome biogenensis	Liu et al., 2017

SIRT1 knock in Cre mice	SIRT1 was knocked into the ubiquitously expressed β -actin locus	yes	Reproductive organs not analysed; Delayed sexual maturity	unknown	unknown	Bordone et al., 2007
SirBACO	Sirt1 knockin	yes	No anatomical or reproductive defects	unknown	unknown	Banks et al., 2008
C57BL6	Sirt3 knockout	yes	Reproductive organs not analysed; No evident phenotypic abnormalities	unknown	unknown	Kawamura et al., 2010
129/Sv	Sirt4 knockout	yes	Reproductive organs not analysed; No evident phenotypic abnormalities	unknown	unknown	Haigis et al., 2006

Table I. Specific Sirtuin histone substrates

Sirtuin	Histone substrate	Main biological function
SIRT1	H3K9 H3K14 H3K56 H4K16 H1K26	Chromatin organization, DNA repair/genome stability, cancer
SIRT2	H4K16 H3K56	Chromatin condensation/ mitosis, DNA repair, cancer

SIRT3	H4K16	Chromatin silencing, DNA repair, cellular stress
SIRT4	None	
SIRT5	None	
SIRT6	H3K9 H3K56 H4K16	Telomeric chromatin/senescence, DNA repair/genome stability
SIRT7	H3K18	Cellular transformation, RNA pol I transcription

(From Seto et al, 2014, modified)

Table II. Role of Sirtuins in female reproductive cells and organs

Sirtuin	Intracellular localization	Cell type	Species	Proposed function	Proposed mediator	References
SIRT1	Cytoplasmic Nuclear	Ovary	*Rat*	Folliculogenesis; ovarian aging	mTOR; FOXO3a; NRF-1; SIRT6	Luo et al., 2012; Zhang et al., 2013; Wang et al., 2014
			Mouse	Folliculogenesis; ovarian aging; glycolytic/oxidative metabolic shift during primordial to primary oocyte transition; stress response	HuR	Zhang et al., 2016; Cinco et al., 2016; Di Emidio et al., 2017
			Porcine	Follicle atresia	unknown	Zhao et al., 2014
		Granulosa Cells	*Human*	Proliferation; activation of steroidogenesis; response to insulin sensitizers	visfatin	Reverchon et al., 2013; 2016
			Porcine	Proliferation; secretory activity;	p53; NF-κB; MAPK; ERK1-2	Pavolova et al., 2013; Sirotkin et al., 2014
			Rat	Mediation of FSH action; activation of steroidogenesis	StAR	Morita et al., 2012

			Bovine	Steroidogenesis	StAR, IGF1R	Reverchon et al., 2016
		KGN		Cell homeostasis; response to metformin; activation of steroidogenesis; proliferation; response to insulin sensitizers	FOXL2; visfatin	Benayoun et al., 2011; Cantò et al., 2009; Caton et al., 2010; Reverchon et al., 2013; 2016
		COV434		Cell homeostasis; stress response	FOXL2; HuR	Benayoun et al., 2011; Di Emidio et al., 2017
		Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	Chromatin configuration; Maturation; oxidative stress response; reproductive and post-ovulatory aging	FOXO3a; miR-132; SOD2	Kawamura et al., 2010; Manosalva and Gonzalez, 2010; Di Emidio et al., 2014; Zhang et al., 2016;
			Porcine	Maturation	unknown	Wang et al., 2014
			Bovine	Chromatin configuration	unknown	Labrecque et al., 2015
			Equine	Oocyte maturation	unknown	Franciosi et al., 2017
		Embryo	*Porcine*	Embryo development; regulation of apoptosis	unknown	Kwak et al., 2012; Kim et al., 2017
			Bovine	Embryo development	unknown	Khan et al., 2017
		Endometrium	*Human*	Embryo implantation; Carcinogenesis	E-cadherin	Shirane et al., 2012; Bartosch et al., 2015
SIRT2	Cytoplasmic Nuclear	Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	Metaphase II spindle assembly and chromosome alignment; reproductive and post-ovulatory aging	Histone H4K16 and α-tubulin	Kawamura et al., 2010; Zhang et al., 2014; Zhang et al., 2016
		Embryo	*Porcine*	Embryo development; regulation of apoptosis; autophagy	unknown	Kwak et al., 2012; Kim et al., 2017
		Endometrium	*Human*	unknown	unknown	Bartosch et al., 2015

SIRT3	Mitochondrial	Ovary	*Mouse*	Folliculogenesis; ovarian aging; stress response	unknown	Zhang et al., 2016; Di Emidio et al., 2017
		Granulosa cells	*Human*	Follicle metabolism; aging process; folliculogenesis; luteinization; progesterone secretion; oxidative stress response;	GDH; SOD1; CAT; 17βHSD1; StAR; P450arom	Pacella-Ince et al., 2014a; Fu et al., 2014
		COV434		Folliculogenesis; luteinization; progesterone secretion	SOD1; CAT; 17βHSD1; StAR; P450arom	Fu et al., 2014
		Cumulus cells	*Human*	Follicle metabolism; aging process	GDH	Pacella-Ince et al., 2014a
		Oocyte	*Human*	Oxidative stress response; mitochondrial biogenesis	PGC1-α	Zhao et al., 2016
			Mouse	Oxidative stress response; maintenance of mitochondrial functionality; post-ovulatory aging; mitochondrial biogenesis	SOD2	Kawamura et al., 2010; Zhang et al., 2016; Zhao et al., 2016; Liu et al., 2017
		Embryos	*Human*	Oxidative stress response; mitochondrial biogenesis	unknown	Zhao et al., 2016
			Mouse	Embryo development; oxidative stress response; maintenance of mitochondrial functionality; mitochondrial biogenesis	p53	Kawamura et al., 2010; Zhao et al., 2016
			Porcine	Embryo development; regulation of apoptosis; marker of embryo potential	unknown	Kwak et al., 2012; Kim et al., 2017
		Endometrium	*Human*	unknown	unknown	Bartosch et al., 2015
SIRT4	Mitochondrial	Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	unknown	unknown	Kawamura et al., 2010
		Endometrium	*Human*	unknown	unknown	Bartosch et al., 2015
SIRT5	Mitochondrial	Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	unknown	unknown	Kawamura et al., 2010

		Cumulus cells	*Human*	Follicle metabolism; aging process	CPS1	Pacella-Ince et al., 2014b
		Endometrium	*Human*	unknown	unknown	Bartosch et al., 2015
SIRT6	Nuclear	Ovary	*Rat*	Folliculogenesis	mTOR; FOXO3a; NRF-1; SIRT6	Luo et al., 2012; Zhang et al., 2013; Wang et al., 2014;
			Mouse	Folliculogenesis; ovarian aging	unknown	Zhang et al., 2016
		Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	Follicle development	unknown	Kawamura et al 2010; Wang et al., 2014
			Bovine	Chromatin configuration	unknown	Labrecque et al., 2015
		Endometrium	*Human*	unknown	unknown	Bartosch et al., 2015
SIRT7	Nuclear	Oocyte	*Human*	unknown	unknown	Zhao et al., 2016
			Mouse	unknown	unknown	Kawamura et al., 2010
		Endometrium	*Human*	Carcinogenesis	unknown	Bartosch et al., 2015

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1591 Table III. Role of Sirtuins in male reproductive cells and organs

Sirtuin	Intracellular localization	Cell type	Species	Possible function	Proposed mediators	References
SIRT1	Nucleus and cytoplasm	Spermatogonia, spermatocytes, spermatids	*Mouse*	Regulation of apoptosis	p53	McBurney et al., 2003
				Anti-oxidant protection	PGC-1α and SIRT3	Rato et al., 2014 Rato et al., 2016
				Chromatin condensation	BRDT-H4 binding	Bell et al., 2014
				Sumoylation	SUMO1 and SUMO2	Coussens et al., 2008
				Acrosome biogenesis	Map1lc3a	Liu et al., 2017
		Hypothalamic GnRH neurons	*Mouse*	GnRH neuronal migration	Cortactin	Cakir et al., 2009 Di Sante et al., 2015
SIRT3	Mitochondria	Sertoli cells?	*Rat*			
				Glycolysis control	HIF-1	Rato et al., 2014 Rato et al., 2016
				Regulation of OXPHOS and anti-oxidant protection	ETC complexes	Rato et al., 2016

SIRT6	Nucleus and acrosome	Spermatids	*Mouse*	Chromatin condensation	Palmer et al., 2011

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1593 BRDT, testis-specific bromodomain protein; ETC, electron transport chain; GnRH, gonadotropin-
1594 releasing hormone; H4, histone 4; HIF-1, hypoxia-inducible factor-1; Map1lc3a, Microtubule-associated protein light chain (LC3); OXPHOS, oxidative
1595 phosphorylation; PGC-1α, peroxisome proliferator activated receptor γ co-activator 1 α;
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