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

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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 emerged in 2003 when a defective reproductive phenotype was observed in *SIRT1*-null mice. Although 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 provide knowledge on the activation mechanism and cellular role of Sirtuins and to give an update of the rapid development of Sirtuin research in female and male reproduction under physiological and pathological conditions. The final goal is to assess whether strategies aimed to improve Sirtuin expression or activity could have therapeutic potential for infertility associated with polycystic ovarian syndrome (PCOS), endometriosis, diabetes, xenobiotic stress and aging.

SEARCH METHODS: The 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 luteinisation. Biochemical modulation of Sirtuin activity has led to discoveries of the roles of SIRT1, SIRT2, SIRT3 and SIRT6 in improving the competence of oocytes grown or matured *in vitro* in humans and animal models. Recently, SIRT1, SIRT2 and 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 the protecting early embryos against stress conditions. Finally, increasing SIRT1 activity may have the potential to ameliorate fertility in PCOS, diabetes, endometriosis, xenobiotic stress and aging. Overall, these effects have been ascribed to Sirtuin-mediated regulation of energy homoeostasis, mitochondrial biogenesis, chromatin remodelling and protection against oxidative stress.

WIDER IMPLICATIONS: The present review provides challenges and opportunities to stimulate research and exploit Sirtuin-based signalling as diagnostic tools and potential targets for therapeutic applications in reproductive medicine.

Key words: Sirtuins / oocyte / sperm / ovary / testis / polycystic ovarian syndrome (PCOS) / endometriosis / diabetes / gonadotoxicity / reproductive aging

Introduction

Sirtuins are a family of NAD^+-dependent deacetylases which have recently emerged as key metabolic sensors for body homoeostasis. Together, they respond to metabolic challenges, inflammatory signals or hypoxic/oxidative stress, and are associated with aging and longevity (Vachharajani *et al.*, 2016; Grabowska *et al.*, 2017). The roles of Sirtuins in the regulation of fertility have arisen since 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 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 (Table I).

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 if they undergo manipulation during assisted reproductive procedure (Agarwal and Majzoub, 2017). Sirtuins have emerged as critical players in the regulation of key processes in oogenesis and spermatogenesis and cellular stress response (Tatone *et al.*, 2015; Rato *et al.*, 2016). 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. To this end, we provide knowledge on their mechanism of activation and their role in somatic cells and organs as an essential supporting structure for understanding the potential of Sirtuins in the female and male reproductive cells and organs.

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 *Saccharomyces cerevisiae* (Kaeberlein *et al.*, 1999). Since then, mammal homologues 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 catalyze 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 ability of Sirtuin to influence metabolism and longevity relies on their capacity to function as protein deacetylases in a manner dependent on the oxidized form of nicotinamide adenine dinucleotide (NAD^+; Fig. 1). The absolute requirement of NAD^+ in the reaction catalyzed by Sirtuins 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

Table I Fertility in Sirtuin transgenic models.

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 diestrous	SIRT1 is part of IGF signalling	Defective IGF signalling in granulosa cells may be responsible for hormonal defects leading to abnormal ovulation	McBurney *et al.*, (2003)
		Male	No	Small organs; presence of all stages of spermatogenesis but abnormal spematids; abnormal immotile sperm	p53 is deacetylated and down-regulated by SIRT1	Apoptosis in the testis is mediated by p53, elevated frequency of apoptotic cells may be a consequence of lack of SIRT1	McBurney *et al.* (2003)
				Abnormal c 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-Seetharam *et al.* (2009)
129SvJ/C57B6	*SIRT1* knockout	Female	Low	Impeded mammary 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 fertilized 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 fertilize 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 $SIRT1^{-/-}$ supporting the hypothesis that SIRT1 has functions not dependent on its deacetylase activity	Seifert *et al.* (2012)
		Male	No	The 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 *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 *SIRT1* knockout	Male	Very low	Round head sperm and abnormal acrosome biogenesis	SIRT1 deacetylases LC3 which is essential for autophagosome and ATG7, which promotes acrosome biogenesis	Acrosome biogenesis	Liu *et al.* (2017)

Continued

Table I *Continued*

Mouse strain	Modification induced	Fertility		Appearance of reproductive organs and germ cells	Proposed target	Proposed mechanism	References
C57BL6/129Sv	*SIRT1* knockin	Male and female	Yes	Reproductive organs not analysed; delayed sexual maturity	Unknown	Unknown	Bordone *et al.* (2007)
SirBACO	*SIRT1* knockin	Male and female	Yes	No anatomical or reproductive defects	Unknown	Unknown	Banks *et al.* (2008)
C57BL6	*SIRT3* knockout	Male and female	Yes	Reproductive organs not analysed; no evident phenotypic abnormalities	Unknown	Unknown	Kawamura *et al.* (2010)
129/Sv	*SIRT4* knockout	Male and female	Yes	Reproductive organs not analysed; no evident phenotypic abnormalities	Unknown	Unknown	Haigis *et al.* (2006)

respond to intra- and extra-cellular stimuli, and are used in cell signalling 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 homoeostasis, with particular emphasis on oxidative stress (Santos *et al.*, 2016), inflammation (de Mingo *et al.*, 2016), metabolism (Houtkooper *et al.*, 2012), and senescence (Sack and Finkel, 2012) (Fig. 2).

The 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 numerous miRNAs. Interaction with HuR results in a prolonged half-life of *SIRT1* mRNAs with a subsequent increase of SIRT1 expression, stability and activity (Revollo and Li, 2013). 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 of influencing the state of activation of Sirtuins through pharmacological and non-pharmacological interventions, with the aim of controlling Sirtuin-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 *S. cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* (Howitz *et al.*, 2003; Wood *et al.*, 2004) and to increase energy metabolism and mitochondrial oxidative capacity (Lagouge *et al.*, 2006; Hui *et al.*, 2017). 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, which 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), and 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 mammalian Sirtuins are lacking. Non-pharmacological interventions promoting SIRT1 activation include caloric restriction (CR) (Cohen *et al.*, 2004; Boily *et al.*, 2008; Anderson *et al.*, 2009). When calories are restricted, the flux of carbons through the mitochondrial oxidation shifts the NAD^+/NADH balance towards the oxidized state, thus establishing a redox environment that promotes Sirtuin activity (Wang, 2014). The CR-induced enhancement of SIRT1 activity has been shown to occur through the activation of adenosine monophosphate-activated kinase (AMPK), a sensor of falling energy status that promotes fast ATP production (Cohen *et al.*, 2004; Suchankova *et al.*, 2009; Sebastián *et al.*, 2012).

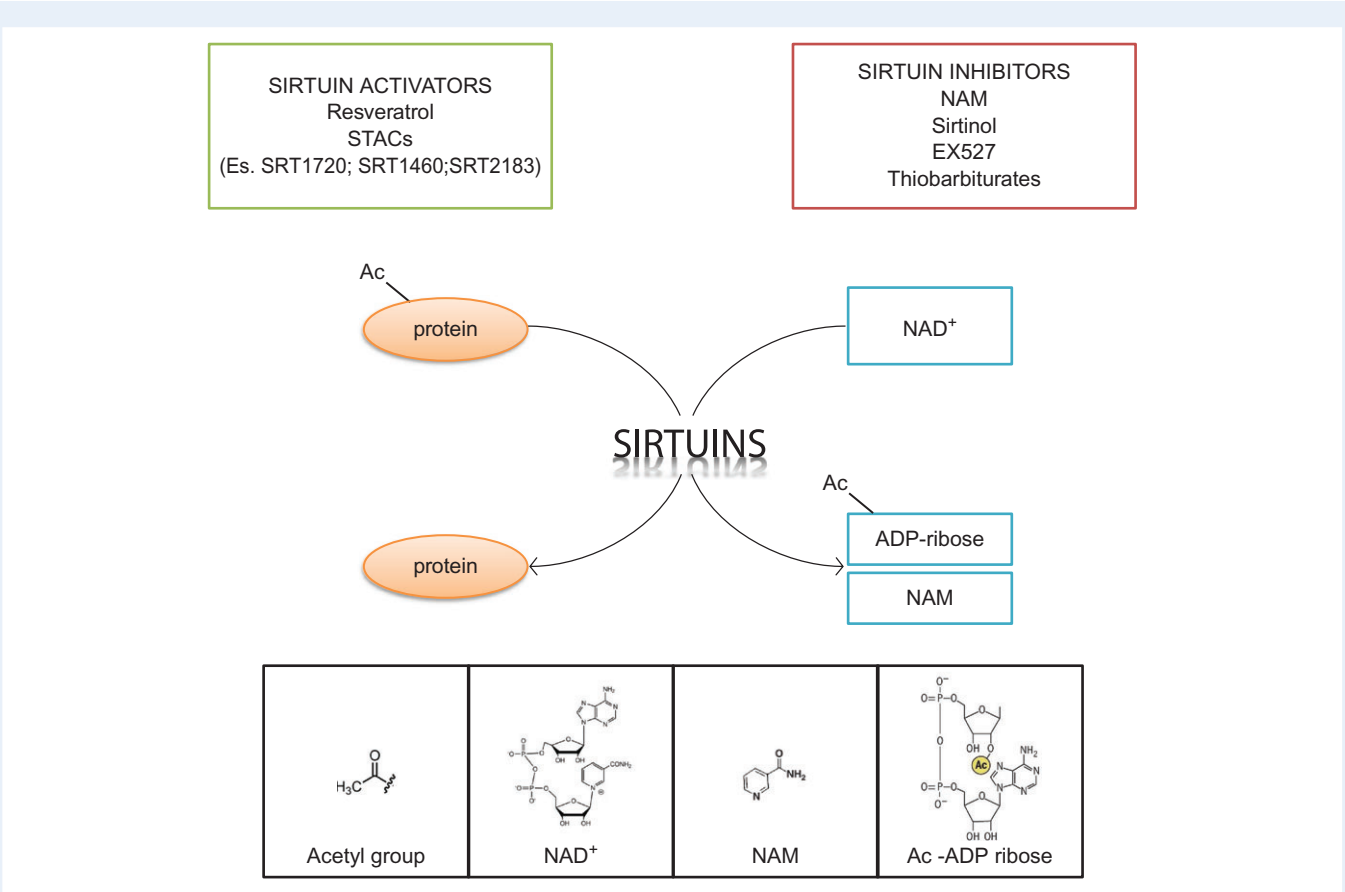

Figure 1 The deacetylase reaction catalyzed by Sirtuins. Sirtuins reverse acetyl modifications of lysine residue in a reaction that consumes NAD^+ (nicotinamide adenine dinucleotide) releasing NAM (nicotinamide), Acetyl-ADP (adenosine diphosphate) ribose and the deacetylated protein. Sirtuins are activated by resveratrol and STACs (Sirtuin activating compounds) and inhibited by NAM, sirtinol, EX527 and thiobarbiturates.

In contrast with the activators, the Sirtuin inhibitors available so far lack adequate physicochemical characteristics, selectivity for Sirtuin isotype or potency (Schiedel *et al.*, 2017). The best-known endogenous inhibitor of all Sirtuin isotypes is the product of the catalytic reaction of both deacetylation and ADP-ribosylation (i.e. nicotinamide (NAM), along with its structural analogues). Among other low-molecular weight inhibitors, sirtinol, a cell-permeable hydroxynaphthaldehyde derivative, exhibits low-potency against SIRT1 and SIRT2 (Grozinger *et al.*, 2001; Kumari *et al.*, 2015), whereas the indole-based EX527 is among the most potent SIRT1-selective inhibitors known so far (Napper *et al.*, 2005; Schiedel *et al.*, 2017).

Sirtuin activity cell functions

Bioenergetics

Sirtuins are known to have various effects on gluconeogenesis, glycolysis, and insulin secretion and sensitivity, and they also have a special role in lipid metabolism by enhancing lipid oxidation.

SIRT1 activity affects degradation of fatty acids, influences mitochondrial function and regulates the homoeostasis of bile acid and cholesterol (Kupis *et al.*, 2016). Accordingly, among the most widely studied targets of SIRT1 is peroxisome proliferator-activated receptor gamma coactivator 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), and regulates cholesterol homoeostasis 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 Sirtuin, 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

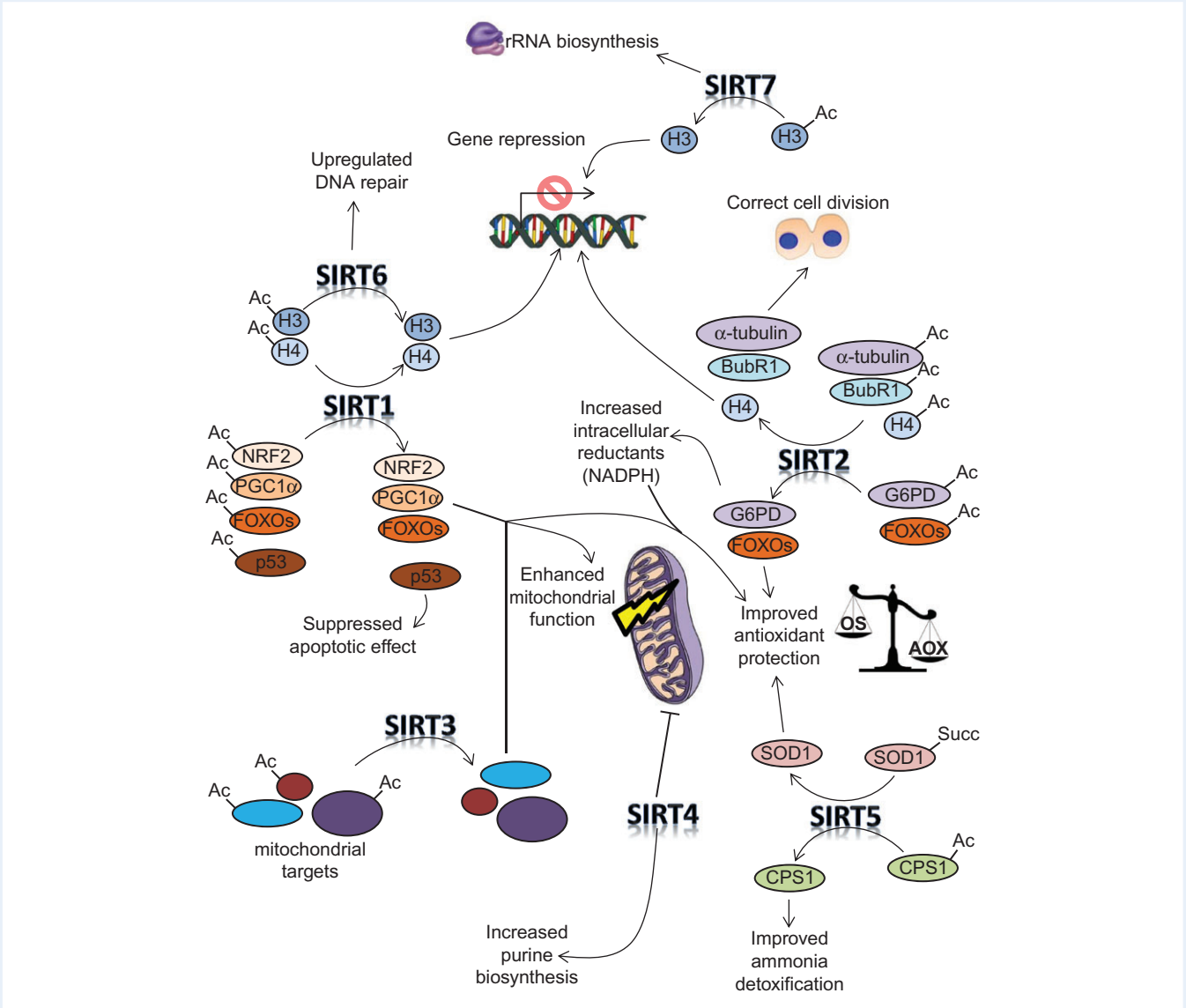

Figure 2 Main cellular functions regulated by mammalian Sirtuins, and major targets of Sirtuin-dependent enzymatic activities. Ac, acetyl group; AOX, antioxidants; Succ, succinyl group; BubR1, budding uninhibited by benzimidazole-related 1; CPS1, carbamoyl phosphate synthetase 1; FOXOs, forkhead box 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, tumour protein p53; SOD1, Cu-Zn superoxide dismutase.

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

Redox signalling

An overwhelming generation of reactive species or a disruption of the redox cellular environment modifies Sirtuin-dependent signalling by either regulating the expression of Sirtuin genes or altering the post-translational modification patterns of Sirtuin proteins and changing NAD levels (Santos *et al.*, 2016). It has been observed that mild oxidative stress conditions are able to up-regulate SIRT1 expression, and this in turn induces the response to changes in the cell redox status, whereas harsh oxidative stress increases degradation of SIRT1,

leading to apoptosis. Among the main redox-related targets of SIRT1 are p53, FOXOs and nuclear factor NF-kappa-B (NF-κB), which all play distinct roles in mediating SIRT1 response to changes of the redox status (Li, 2013; Chang and Guarente, 2014). In particular, p53 is a transcription factor that activates genes involved in antioxidant defence, like superoxide dismutase 2 (SOD2) and glutathione peroxidase (GPx1) (Sablina *et al.*, 2005). FOXO3a induces antioxidant responses via up-regulation of SOD2 and catalase (Hasegawa *et al.*, 2008; Pardo *et al.*, 2011; Hori *et al.*, 2013; Di Emidio *et al.*, 2014). Some authors have provided evidence that SIRT1 counteracts pro-oxidant conditions also through its deacetylating action on nuclear 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 further indicate that SIRT2 may also utilize 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 extra-cellular oxidative challenges, thus regulating the availability of NADPH, one of the major intracellular reductants (Wang *et al.*, 2014c). SIRT2 seems to affect also the NF-κB-dependent signalling, by deacetylating the p65 subunit at lysine 310, thus repressing cell proliferation and regulating immune and inflammatory responses (Rothgiesser *et al.*, 2010; Li *et al.*, 2013).

Tao *et al.* (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, 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 (DSB) 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 have 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 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).

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; Zentner and Henikoff, 2013; Turner, 2014). 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, 18 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), plays a role in deacetylating histone H4K16 and demethylating H3K4, to ultimately repress gene expression (Mulligan *et al.*, 2011). SIRT2 shuttles to the nucleus during the G2/M transition phase, and also deacetylates H4K16, thus contributing to chromatin compaction (Contrepois *et al.*, 2012). H4K16 is also deacetylated by SIRT3 when it is transported to the nucleus under certain conditions and this 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) while SIRT7

Table II 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	H3K4	Unknown
SIRT6	H3K9 H3K56 H4K16	Telomeric chromatin/senescence, DNA repair/genome stability
SIRT7	H3K18	Cellular transformation, RNA pol I transcription

From Seto and Yoshida (2014), modified.

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 (Garcia-Cao et al., 2004; Vaquero et al., 2004; Martinez-Redondo and Vaquero, 2013).

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 et al., 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 the mediate anticancer effects of Sirtuin inhibitors (Lain et al., 2008). Along with other nuclear Sirtuins, SIRT1 regulates genomic stability throughout diverse DNA repair pathways. In particular, SIRT1 promotes deacetylation of WRN (Werner protein), 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 protein BubR1 (budding uninhibited by benzimidazole-related 1), 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 by 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 and 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).

Sirtuins in female reproductive functions

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 the primordial follicles are exhausted (Zhang and Liu, 2015). How to maintain the ovarian reserve is a key issue, considering the negative effects of early menopause and the high risk of exposure to gonadotoxic insults during the reproductive lifespan (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 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 signalling in regulating the primordial follicle reserve has been proposed in relation to its ability to sense nutrient status (Seli et al., 2014; Wang et al., 2014b; 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 with 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 (Luo et al., 2012; Xiang et al., 2012; Wang et al., 2014b; Zhou et al., 2014; Liu et al. 2015; Zhang et al. 2016a; 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 the primordial to primary oocyte transition. According to this study, nuclear expression of SIRT1 in oocytes increases during primordial follicle awakening when a significant decrease in the nuclear ratio of 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 PGC1α, 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 as intraovarian paracrine factors and intrinsic oocyte regulators, deciding the fate of primordial follicles.

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 the cow, four GV stages oocytes have been characterized (from GV0 to GV3), with increasing level of chromatin compaction from early antral follicles to medium antral follicle >2 mm in diameter (Lodde *et al.*, 2007). In mice, oocytes with uncondensed chromatin are referred to as 'Non Surrounded Nucleolus' (NSN) oocytes, while oocytes with compacted chromatin are referred to as 'Surrounded Nucleolus' (SN) oocytes (De La Fuente, 2006; Luciano and Lodde, 2013). During the transition from uncondensed to more compacted configuration, a major transcriptional silencing take place in mouse and bovine oocytes (Bouniol-Baly *et al.*, 1999; Lodde *et al.*, 2008; Luciano *et al.*, 2011). In mammals, histone acetylation increases in the later stages of chromatin compaction while a complete histones deacetylation is observed during oocyte maturation (reviewed in Lodde *et al.*, 2017). An increase in acetylation during the chromatin compaction, when the major transcriptional silencing occurs, seems to be apparently in contradiction with the common rules of the 'Histone Code' that applies to somatic cells (Jenuwein and Allis, 2001).

In the mouse, defective SIRT1 protein expression was observed in aging GV stage oocyte and this suggested that a decrease in H3K9 deacetylation was causative of the reduction in H3K9 methylation (Manosalva and González, 2010). This is consistent with previous reported activity of SIRT1 in promoting H3K9me3 through the histone methyltransferase Suv39h1 (Vaquero *et al.*, 2007). However, in the same study, a correlation between SIRT1 expression and changes in chromatin configuration was not finally determined.

In bovine oocytes, their transcriptomic profile characterized by different degree of chromatin compaction from GV0 to GV3 configuration revealed that the amount of *SIRT1* and *SIRT6* mRNA changes significantly during chromatin compaction (GEO accession number GSE48376; Labrecque *et al.*, 2015). An increase was observed in the transition between GV0 and GV1, when chromatin compaction begins. The list of genes in the original microarray dataset (that is accessible through an interactive web interface; Khan *et al.*, 2016) includes data from oocytes and cumulus cells (Lodde *et al.*, 2017). In agreement with previous studies, the increased expression of SIRT1 and SIRT6 could be responsible for an increase of H3K9 deacetylation that is required for the subsequent methylation in the same residue, which induces chromatin compaction (Krauss, 2008). However, this hypothesis should be confirmed by determining the levels of SIRT1 and SIRT6 protein expression in the oocyte, and also by evaluating the progression of the acetylation state and the concurrent methylation state of H3K9. Moreover, the increase in 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 post-translational modification of histones, deciphering the changes in the levels of specific Sirtuins mRNAs involved in chromatin remodelling is overall limited by the lack of a '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 (GEO accession number 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 *SIRT6*) are significantly translated during maturation (GEO accession number 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 the mouse (Riepsamen *et al.*, 2015) and pig (Zhang *et al.*, 2015b). Conversely, activation of SIRT1 by means of resveratrol supplementation during *in vitro* maturation improved oocyte quality and embryo development in the mouse (Liu *et al.*, 2013), pig (Itami *et al.*, 2015; Li *et al.*, 2016) and cow (Takeo *et al.*, 2014; Wang *et al.*, 2014a; Khan *et al.*, 2017). The increase of SIRT1 expression induced by resveratrol was correlated with enhanced mitochondria biosynthesis and degradation in oocytes, thereby improving mitochondrial function and the developmental capability of the female gamete (Sato *et al.*, 2014). Moreover, EX527, a specific inhibitor of SIRT1, caused an increase in ROS production and abnormal metaphase II plates in mouse oocytes, 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 the mouse, the use of a specific SIRT2 inhibitor during oocyte *in vitro* maturation blocked the progression behind the GV breakdown (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, a high-fat diet increased ROS content and reduced SIRT3 expression in oocytes (Zhang *et al.*, 2015a). Subsequent experiments ascertained that the specific depletion of SIRT3 in control oocytes elevates ROS levels while the overexpression of *SIRT3* attenuates ROS production in oocytes from a mouse obesity model, with a significant reduction of spindle defects and chromosome misalignment (Zhang *et al.*, 2015a). Consistently, Liu *et al.* (2017b) demonstrated that SIRT3 exerts antioxidant effects in oocytes from diabetic mice by deacetylating SOD2. The role of SIRT3 was investigated also in human oocytes (Zhao *et al.*, 2016). Using loss- and gain-of-function experiments, Zhao and collaborators demonstrated that a defective SIRT3 expression provoked a decrease in 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 *in vitro* maturation (IVM) versus 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 mis-segregation and aneuploidy in oocytes, 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; Ma and Schultz, 2013; Zhang *et al.*, 2014).

However, the involvement of Sirtuins in the chromatin acetylation state during oocyte maturation remains substantially unexplored and the information is still fragmentary. Recent studies also demonstrated that *in vitro* maturation conditions 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 counterparts, *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.

Recent findings by Qiu *et al.* (2017) have pointed to a key role of SIRT2 in oocyte spindle assembly checkpoint (SAC). This Sirtuin deacetylases the lysine 243 on BubR1, a core SAC component, so alleviating meiotic defects and aneuploidy generation.

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. The activity of Sirtuins is mainly focused in controlling oxidative stress response and spindle assembly in order to ensure faithful chromosome segregation through meiotic division.

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 the resting phase as well as from its exposure to the ageing ovarian microenvironment during oocyte 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 and 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 result in faulty spindle checkpoints, which predisposes oocytes to premature chromosome separation and aneuploidy. These two aging processes also lead to a decline in mitochondrial function and changes in the redox state, which are 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 ability of the oocyte to up-regulate SOD2 and counteract increase in ROS under oxidative stress. Moreover, the 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 responses and aging is the role of miR132 in the modulation of mRNA levels of *SIRT1*, a validated target of this microRNA (Strum *et al.*, 2009; Di Emidio *et al.*, 2014), a finding which opens new horizons in the knowledge of upstream signalling targeting oocyte Sirtuins. The potential role of SIRT1 as a countermeasure against oocyte 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.* (2016b) reported that postovulatory aging is associated with the precocious decrease in *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.*, 2015b), 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 and prevents histone modifications by preventing Sirtuin decay during postovulatory aging (Wang *et al.*, 2017). Given the role of SIRT2 in spindle stability (Zhang *et al.*, 2014; Qiu *et al.*, 2017), it could be hypothesized that reduced levels of SIRT2 may contribute to oocyte postovulatory aging as an effect of altered tubulin acetylation.

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; Pacella-Ince *et al.*, 2014a,b; Zhao *et al.*, 2014).

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 (Pavlová *et al.*, 2013; Sirotkin *et al.*, 2014). On the other hand, stimulation of SIRT1 with resveratrol concentrations 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 (Pavlová *et al.*, 2013; Sirotkin *et al.*, 2014).

It has been recently reported that in response to the antiproliferative alkylating agent cyclophosphamide (CPM), the human GC line COV434 activates 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 an 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 the cell stress response to oxidative stress in human GC lines by targeting FOXL2, a transcription factor essential for ovarian functions and maintenance (Benayoun *et al.*, 2009, 2011).

By focusing on mitochondrial Sirtuins, SIRT3 seems to cooperate with SIRT1 in the regulation of steroidogenic genes, progesterone secretion and ROS detoxification in human GCs (Fu *et al.*, 2014). Moreover, SIRT3 has been revealed to be an important sensor of metabolic state in human GCs and CCs by targeting mitochondrial enzymes, such as glutamate dehydrogenase (GDH). A decrease in SIRT3 activity along with an increase of the inactive acetylated GDH form characterizes the process of GC and CC aging, whereas SIRT5 expression and activity decreased with aging along with alterations of activity of the SIRT5 target carbamoyl phosphate synthase 1 (CPS1) in GCs and CCs. Therefore, alterations to mitochondrial Sirtuins may affect post-translational modifications of mitochondrial proteins thus producing metabolic alterations in the aged follicle (Pacella-Ince *et al.*, 2014a,b).

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 most *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. (McBurney *et al.*, 2003; Coussens *et al.*, 2008). Mutant mice displayed infertility with decreased testes size, but the rare spermatozoa recovered from their cauda epididymis were immotile, exhibited abnormal morphology and were not as efficient in IVF experiments as wild-type sperm (McBurney *et al.*, 2003; Coussens *et al.*, 2008). 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. Most of the data about the possible role of Sirtuins in male fertility have been produced in the knockout mouse with a whole body of defective SIRT1 activity (Table I). Nevertheless these models suffer from a major limitation: they do not discriminate whether the spermatogenesis damage results from the loss of spermatogenetic cell-specific SIRT1 activities or the lack of an extrinsic positive control exerted by SIRT1 on spermatogenesis. In this regard, studies based on germ cells male germ line *SIRT1* knockout have been carried out along with investigation of the role of SIRT1 in the hypothalamic-pituitary-gonadotropin (HPG) axis signalling.

The spermatogenetic process

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

Histological analyses of testes from $SIRT1^{-/-}$ mice revealed dramatically reduced numbers, or even 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 with severe morphological abnormalities, apoptotic features and increased DNA damage within the seminiferous epithelium (McBurney *et al.*, 2003; Coussens *et al.*, 2008; Kolthur-Seetharam *et al.*, 2009; Bell *et al.*, 2014).

Searching for molecular mechanisms by which *SIRT1* deficiency leads to spermatogenesis derangement, many explanatory attempts

have been made. As testicular apoptosis is dependent on the activity of p53 (Beumer et al., 1998; Yin et al., 1998; Allemand et al., 1999), which is triggered by acetylation, male germ cell death in *SIRT1* knockout mice could reflect an up-regulation of p53 activity (McBurney et al., 2003). Sperm defects observed in *SIRT1*$^{-/-}$ mice could also result from oxidative stress. Indeed SIRT1 co-operates with SIRT3 and PGC1α in triggering antioxidant defence systems. In particular, when deacetylated and activated by SIRT1, PGC1α induces the transcription of gene encoding SIRT3 (Kong et al., 2010) which is also expressed in mammalian testicular tissue (Michishita et al., 2005). The relationship between derangement of the SIRT1/PGC1α/SIRT3 and imbalance of ROS and antioxidant defences in testes has been demonstrated by Rato et al. (2014) in a high-energy-diet induced pre-diabetic rat model. Consistent with the association between decreased expression of SIRT1 and SIRT3 and higher glycolytic activity in many tissues (Ye et al., 2017), decreased SIRT3 levels were found to promote glycolysis in rat testis (Rato et al., 2013, 2014), revealing the role of Sirtuins as key sensors of testis metabolism. Although glucose metabolism and resultant lactate production are crucial for normal spermatogenetic process (Boussouar and Benahmed, 2004; Oliveira et al., 2015), an increased glycolytic activity may promote mitochondrial overproduction of ROS (Rato et al., 2013). This would be further favoured by the fact that the loss of *SIRT1* and *SIRT3* leads to a dysfunctional electron transport chain, while lowering antioxidant defences (Rato et al., 2016). Mammalian spermatozoa, indeed, are uniquely sensitive to oxidative stress, mainly due to the high polyunsaturated fatty acid (PUFA) content of their membranes (Tremellen, 2008). PUFAs play a major role in maintaining membrane fluidity and fusogenicity, which are required for sperm acrosomal exocytosis and sperm-oolemma interactions. Unfortunately, they are also particularly vulnerable to lipid peroxidation (Aitken et al., 1993; Wagner et al., 1994; Barbonetti et al., 2011).

Oxidative stress could also mediate the association between *SIRT1* silencing and gem cell apoptosis. ROS, indeed, can potentially impact the mitochondrial pathway of apoptosis in many ways (Wu and Bratton, 2013). Both oxidative stress and apoptosis could be responsible for the DNA damage, which has been revealed in germ cells from *SIRT1*$^{-/-}$ mice by TUNEL (Kolthur-Seetharam et al., 2009; Bell et al., 2014) and comet assays (Coussens et al., 2008; Bell et al., 2014). Notably, germ cell-specific *SIRT1* knockout mice exhibit defects in histone to protamine transition and altered chromatin condensation (Bell et al., 2014), which increases the susceptibility of sperm DNA to apoptotic/oxidative damage (reviewed in Zini and Libman, 2006). SIRT1 could cooperate with SIRT6 in driving chromatin condensation, as a poor sperm protamination has been also reported in high-fat diet-fed obese mice, which exhibited a significant decrease in SIRT6 expression in the nucleus of spermatids (Palmer et al., 2011). Very recently, a role of SIRT1 in spermiogenesis has been confirmed in a germ cell-specific *SIRT1* knockout mouse model, where Liu and collaborators (2017a) demonstrated an accumulation of acetylated LC3 in the spermatid nucleus, which affected the recruitment of several acrosome biogenesis-related proteins to the acrosomal vesicles: these findings pointed to a novel function for SIRT1 during acrosome biogenesis.

To better clarify the role of SIRT1 in the spermatogenesis, Coussens and collaborators (2008) carried out a microarray analysis of global gene expression in the testis from *SIRT1* deficient mice, revealing aberrant expression of several genes involved in spermatogenesis and in sumoylation of proteins. Sumoylation, indeed, can be modulated by the SIRT1 deacetylase activity (Bouras et al., 2005; Stankovic-Valentin et al., 2007) and this represents a post-translational protein modification which, in somatic cells, has been implicated in a number of processes, such as transcription, nuclear transport, DNA repair, mitochondrial activity, plasma membrane ion transport, cell cycle and chromatin structure (reviewed in Andreou and Tavernarakis, 2009). Intriguingly, it has been reported that sumoylation also play multiple roles in testicular function and spermatogenesis, such as spermatogonial proliferation, meiotic sex chromosome inactivation, centromeric heterochromatin organization and reshaping of the spermatid nucleus (Rogers et al., 2004; Vigodner and Morris, 2005; Vigodner et al., 2006; Brown et al., 2008; Metzler-Guillemain et al., 2008; Vigodner, 2009).

HPG axis

The biological plausibility of an involvement of Sirtuins in the HPG axis function arises from the evidence that SIRT1 is highly expressed in the hypothalamus (Cakir et al., 2009) and in particular in gonadotropin-releasing hormone (GnRH) neurons (Di Sante et al., 2015). Kolthur-Seetharam and collaborators (2009) observed first that in testes from *SIRT1* knockout mouse, spermatogenesis arrest and apoptosis of germ cells were associated with abnormal Leydig and Sertoli cell maturation and strongly reduced intratesticular testosterone levels. They demonstrated that this phenotype is the consequence of reduced hypothalamic GnRH secretion, resulting in a significant reduction of FSH and LH levels (Kolthur-Seetharam et al., 2009). A closer analysis showed that several Leydig cell-expressed genes involved in steroidogenesis, such as *StAR*, were also down-regulated (Kolthur-Seetharam et al., 2009). Under physiological conditions, gonadotropins cooperate to drive normal spermatogenesis; FSH binds to its receptor (FSHR) expressed by Sertoli cells and regulates their development and function, while LH binding to its cognate receptor (LHR) on Leydig cells promotes testosterone biosynthesis (Ramaswamy and Weinbauer, 2014). In particular, intratesticular testosterone has a pivotal role in initiation and maintenance of spermatogenesis by acting on androgen receptors expressed in Sertoli cells (Walker, 2011). Interestingly, the blockage of testosterone biosynthesis by inactivating the genes encoding LH or LHR, leads to a phenotype similar to that of *SIRT*$^{-/-}$ mice (Lei et al., 2001; Zhang et al., 2001, 2004; Ma et al., 2004). Recently, a hypogonadotropic hypogonadism has been confirmed in *SIRT1* knockout mice and attributed to a failure in the GnRH neuronal migration from the vomeronasal organ towards the hypothalamus (Di Sante et al., 2015). The authors demonstrated that in GnRH neuronal cell lines, SIRT1 binds and deacetylates cortactin (Di Sante et al., 2015). As previously demonstrated in different cell lines, cortactin, upon deacetylation by SIRT1, promotes cell migration by modulating F-actin polymerization (Zhang et al., 2009; Byles et al., 2012; Nakane et al., 2012). Interestingly, in the study by Di Sante and collaborators (2015), *SIRT1*$^{-/-}$ mice were also affected by anosmia, thus exhibiting a phenotype overlapping the Kallmann's Syndrome, the congenital hypogonadotropic hypogonadism resulting from failed GnRH neuronal migration in humans.

Sirtuins in post-fertilization events

The role of Sirtuins in post-fertilization events has been 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) induce 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 decreases 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 foetal development, increased postnatal mortality rates and smaller size with developmental defects including reduced development of mammary glands (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 maintaining mitochondrial functionality. Fertilization and blastocyst formation rates significantly decreased when *SIRT3* null oocytes are subjected to IVF regardless of the sperm genotype, supporting the conclusion that storage of *SIRT3* during oogenesis is essential for fertilization and preimplantation 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.

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 Sirtuin 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 ~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 exerts 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 in insulin levels (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 GCs, 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 GCs.

The role of Sirtuin 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 gynaecological condition frequently associated with pelvic pain and infertility. Recently, SIRT1 has been suggested as a key driver of the progesterone resistance contributing to pathophysiology and survival of ectopic lesions and proposed as a valuable endometrial marker in women with endometriosis (Yoo *et al.*, 2017). Pro-inflammatory mediators are involved in the progression of endometriosis and therefore inhibiting inflammation is important in controlling of the disease. Experiments carried out in primary endometriotic stromal cells and peritoneal immune cells exposed to resveratrol or sirtinol have revealed an important role of SIRT1 in regulating the expression and production of inflammatory cytokines (Taguchi *et al.*, 2014; Mvunta *et al.*, 2016). Overall, these data represent a strong starting point for further investigations of the role of Sirtuin activators as negative regulators of the inflammatory response in endometriosis, thereby determining their potential as novel agents in endometriosis therapy.

Significant evidence from experiments on high-fat diet induced obese mice and rats supports the potential of SIRT1 as a therapeutic target for preventing the negative effects of obesity on ovarian lifespan. Consistent with reduced levels of ovarian SIRT1 and SIRT6 expression in obese animals, oral administration of resveratrol or SRT1720, a 1000 times more potent SIRT1 activator, preserves follicle reserve and suppress follicle atresia by activating SIRT1, suppressing mTOR signalling, and modulating NF-κB signalling (Luo *et al.*, 2012; Wang *et al.*, 2014b; Zhou *et al.*, 2014; Liu *et al.*, 2015). Therefore, a better understanding of the relationship between SIRT1 and mTOR signalling could promote the development of new pharmacological approach to treat metabolic diseases associated with obesity.

Table III 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), Bartosch *et al.* (2016) and Wang *et al.* (2014b)
			Mouse	Folliculogenesis; ovarian aging; glycolytic/oxidative metabolic shift during primordial to primary oocyte transition; stress response	HuR	Zhang *et al.* (2016), Zhang *et al.* (2016a), Cinco *et al.* (2016) and 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	Pavlová *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 homoeostasis; response to metformin; activation of steroidogenesis; proliferation; response to insulin sensitizers	FOXL2; visfatin	Benayoun *et al.* (2011), Cantó *et al.* (2009), Caton *et al.* (2010) and Reverchon *et al.* (2013, 2016)
		COV434		Cell homoeostasis; stress response	FOXL2; HuR	Benayoun *et al.* (2011) and 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 González (2010), Di Emidio *et al.* (2014) and Zhang *et al.* (2016b)
			Porcine	Maturation	Unknown	Li *et al.* (2016)
			Bovine	Chromatin configuration; oocyte maturation	Unknown	Wang *et al.* (2014a),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) and Bartosch *et al.* (2016)
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) and Zhang *et al.* (2016b)
		Embryo	*Porcine*	Embryo development; regulation of apoptosis; autophagy	Unknown	Kwak *et al.* (2012) and Kim *et al.* (2017)
		Endometrium	*Human*	Unknown	Unknown	Bartosch *et al.* (2016)
SIRT3	Mitochondrial	Ovary	*Mouse*	Folliculogenesis; ovarian aging; stress response	Unknown	Zhang *et al.* (2016a) and Di Emidio *et al.* (2017)
		Granulosa cells	*Human*	Follicle metabolism; aging process; folliculogenesis; luteinisation; progesterone secretion; oxidative stress response	GDH; SOD1; CAT; 17βHSD1; StAR; P450arom	Pacella-Ince *et al.* (2014a) and Fu *et al.* (2014)
		COV434		Folliculogenesis; luteinisation; 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.* (2016b), Zhao *et al.* (2016) and Liu *et al.* (2017)

Continued

Table III *Continued*

Sirtuin	Intracellular localization	Cell type	Species	Proposed function	Proposed mediator	References
		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) and Zhao *et al.* (2016)
			Porcine	Embryo development; regulation of apoptosis; marker of embryo potential	Unknown	Kwak *et al.* (2012) and Kim *et al.* (2017)
		Endometrium	Human	Unknown	Unknown	Bartosch *et al.* (2016)
SIRT4	Mitochondrial	Oocyte	*Human*	Unknown	Unknown	Zhao *et al.* (2016)
			Mouse	Unknown	Unknown	Kawamura *et al.* (2010)
		Endometrium	*Human*	Unknown	Unknown	Bartosch *et al.* (2016)
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.* (2016)
SIRT6	Nuclear	Ovary	*Rat*	Folliculogenesis	mTOR; FOXO3a; NRF-1; SIRT6	Luo *et al.* (2012), Zhang *et al.* (2013) and Wang *et al.* (2014b)
			Mouse	Folliculogenesis; ovarian aging	Unknown	Zhang *et al.* (2016a)
		Oocyte	*Human*	Unknown	Unknown	Zhao *et al.* (2016)
			Mouse	Follicle development	Unknown	Kawamura *et al.* (2010) and Wang *et al.* (2014b)
			Bovine	Chromatin configuration	Unknown	Labrecque *et al.* (2015)
		Endometrium	*Human*	Unknown	Unknown	Bartosch *et al.* (2016)
SIRT7	Nuclear	Oocyte	*Human*	Unknown	Unknown	Zhao *et al.* (2016)
			Mouse	Unknown	Unknown	Kawamura *et al.* (2010)
		Endometrium	*Human*	Carcinogenesis	Unknown	Bartosch *et al.* (2016)

Table IV 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)
				Antioxidant protection	PGC-1α and SIRT3	Rato *et al.* (2014) and 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) and Di Sante *et al.* (2015)
SIRT3	Mitochondria	Sertoli cells?	*Rat*	Glycolysis control	HIF-1	Rato *et al.* (2014) and Rato *et al.* (2016)
				Regulation of OXPHOS and antioxidant protection	ETC complexes	Rato *et al.* (2016)
SIRT6	Nucleus and acrosome	Spermatids	*Mouse*	Chromatin condensation		Palmer *et al.* (2011)

BRDT, testis-specific bromodomain protein; ETC, electron transport chain; GnRH, Gonadotropin-releasing Hormone; H4, histone 4; Hif-1, hypoxia-inducible factor-1; Map1lc3a, microtubule-associated protein light chain (Lc3); Oxphos, oxidative phosphorylation; Pgc-1α, peroxisome proliferator-activated receptor γ coactivator 1 α.

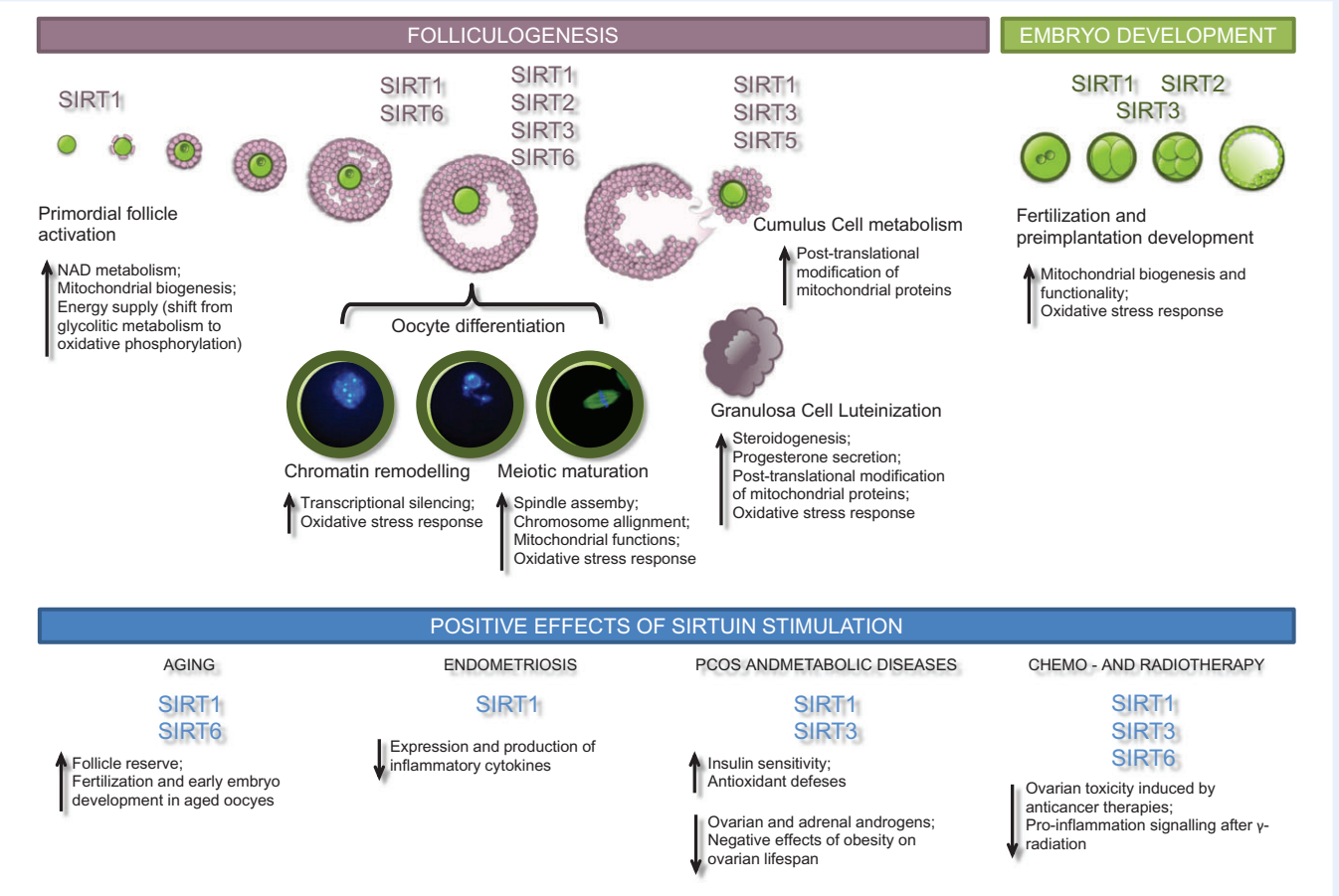

Figure 3 Main Sirtuin functions during different stages of folliculogenesis and embryo development and positive effects of Sirtuin stimulation in aging, endometriosis, PCOS and metabolic diseases, and chemo- and radiotherapy.

Recently, gonadotoxicity exerted by chemotherapy and radiotherapy treatments has been associated with reduced levels of ovarian Sirtuins. Accordingly, administration of Sirtuin stimulators provides protection against ovarian toxicity induced by anticancer therapies in animal models. It is well known that alkylating agents, i.e. CPM, and /or radiations pose high risk of premature ovarian failure (POF) in humans and in animal models (Roness *et al.*, 2016). In rats, the ovarian damage induced by these treatments results in the decline of SIRT1, SIRT3 and SIRT6 expression and can be counteracted by Sirtuin activating strategies, i.e. resveratrol administration or CR (Xiang *et al.*, 2012; Said *et al.*, 2016; Luo *et al.*, 2012). In particular, resveratrol-activated SIRT1 expression was associated with 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 CPM 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 responses to CPM insults, 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, while SOD2 and the mitochondrial biogenesis activator PGC1α decrease, providing the first evidence of CPM induces mitochondrial damage in the ovary. 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 the Sirtuin induction and loss of ovarian follicles (Di Emidio *et al.*, 2017)

As reported above, improved expression of ovarian Sirtuins can be achieved by means of CR, rapamycin, resveratrol and SRT1720. All these strategies have the potential to prolong the reproductive lifespan in aging female mice. Anti-aging dietary strategies associated with increased SIRT1 expression include NAC supplementation for two months. Indeed increased rates of fertilization and early embryo development were observed in association with higher expression levels of *SIRT1* and *SIRT2* and increased telomerase activity lengths (Liu *et al.*, 2013). All these data need to be extended in future studies in order to determine safety and potential side effects with the long term use of these strategies, before being taken into account to delay the occurrence of menopause.

Concluding remarks and future perspectives

In the present study, we have provided an update and overview of Sirtuin function in the reproductive system, revealing the role of these enzymes in the regulation of energy homoeostasis, mitochondrial biogenesis, chromatin remodelling and protection against oxidative stress. We also showed that the evaluation and manipulation of Sirtuin activity may have a great therapeutic potential in the field of reproductive medicine.

As evolutionary conservative NAD^+-dependent HDACs, 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, participation of Sirtuin in the regulation of ovarian reserve, follicle development and luteinisation has clearly emerged (Table III). The role of Sirtuins in oocyte function seems to be 2-fold 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 correct spindle assembly and chromosome alignment, mitochondrial activity and biogenesis, to face the oxidative stress response. Also, oocyte Sirtuins appear to be crucial in preimplantation embryo development. Although deserving more attention and specific investigation, Sirtuins have been found to regulate specific functions of male germ cells, Sertoli cells and Leydig cells (Table IV) and male germ cell *SIRT1* knockout models have clearly demonstrated the essential role of this Sirtuin in spermatogenesis (Table I). Finally, evidence from preclinical models supports the potential of Sirtuin modulators for ameliorating fertility in PCOS, diabetes, endometriosis, xenobiotic stress and aging (Fig. 3).

Nevertheless, validation of hypotheses reported above would require further studies based on a multidisciplinary approach. At present, the lack of sensitive methods for direct quantification of Sirtuin enzymatic activity makes it difficult to investigate Sirtuins in germ cells and embryos. On the other, hand high-throughput screening methods for the study of lysine acetylome, Sirtuin signalling, targets and modulators could be highly promising. Moreover, given the pleiotropic role of Sirtuins, a female germ cell Sirtuin knockout would represent a valuable approach.

Overall, 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.

Authors' roles

C.T. was the main supervisor for this review, providing key research direction, overview and detailed review of the work. G.D., S.F., A.M.L., A.B. and C.T. contributed to the synthesis of the literature and drafting of the manuscript. G.C. contributed to manuscript revision and critical discussion. F.A. was involved in design and review of the complete manuscript. All authors approved the final version of the manuscript.

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