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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 2002, when a defective reproductive phenotype was observed in SIRT1^{-/-} mice. Although studies on Sirtuins in reproductive biology have been increasing in the last few years, a recent comprehensive update on this issue is still lacking.

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 nal 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 and 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 homeostasis, 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 homeostasis in somatic cells and organs.

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 1).

Fertility is very sensitive to redox perturbations related to aging and metabolic dysfunctions, which is the link between energy metabolism and reproduction (Seli et al, 2014). Reproductive cells and organs are constantly challenged by stressors and require adaptive responses for their survival. In addition to redox perturbations related to aging or diseases, reproductive cells have to face stress conditions when 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 responses (Tatoneet et al, 2015; Ratoet 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, Kaerberlein and co-workers first identified the evolutionarily conserved gene silent information regulator 2 (Sir2), whose overexpression increased lifespan by 30% in yeast *Saccharomyces cerevisiae* (Kaerberlein et al, 1999). Since then, mammalian 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 (Vtroba 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 (Tatoneet et al, 2016). An increasing number of studies have determined that ability of Sirtuin to influence metabolism and longevity lies 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 ancient 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 | |
|---|---|-----------|---|---|--|--|--|
| I29/Sv | <i>SIRT1</i> 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 <i>et al.</i> , (2003) |
| | | Male | No | Small organs; presence of all stages of spermatogenesis but abnormal spermatids; 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 <i>et al.</i> (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 <i>et al.</i> (2009) |
| I29Svj/C57B6 | <i>SIRT1</i> knockout | Female | Low | Impeded mammary ductal morphogenesis | SIRT1 is part of IGF signalling | Reduced levels of IGF | Li <i>et al.</i> (2007) |
| | | Male | Low | Unknown | Unknown | Unknown | Li <i>et al.</i> (2007) |
| C57BL/6 | <i>SIRT1</i> 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 <i>et al.</i> (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 <i>et al.</i> (2008) |
| I29Svj and I29/CD1 | <i>SIRT1</i> H355Y (point mutation at catalytic domain) | Female | Yes | Unknown | Unknown | This phenotype is less debilitating than | Seifert <i>et al.</i> (2012) |
| | | Male | No | The presence of all stages of spermatogenesis; reduced number of mature sperm that failed to acquire motility | Unknown | <i>SIRT1</i> ^{-/-} supporting the hypothesis that SIRT1 has functions not dependent on its deacetylase activity | |
| <i>SIRT1</i> flox/flox; FVB/N-Tg(Stra8-cre)IReb (Stra8-cre) | Male germ line <i>SIRT1</i> 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 <i>et al.</i> (2014) |
| <i>SIRT1</i> flox/flox; Tnap-Cre | Male germ line <i>SIRT1</i> knockout | Male | Very low | Round head sperm and abnormal acrosome biogenesis | SIRT1 deacetylates LC3 which is essential for autophagosome and ATG7, which promotes acrosome biogenesis | Acrosome biogenesis | Liu <i>et al.</i> (2017) |

Continued

Table 1 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 | Reproductive organs not analysed; delayed sexual maturity | Unknown | Unknown | Bordone et al. (2007) |
| SirBACO | SIRT1 knockin | Male and female | No anatomical or reproductive defects | Unknown | Unknown | Banks et al. (2008) |
| C57BL6 | SIRT3 knockout | Male and female | Reproductive organs not analysed; no evident phenotypic abnormalities | Unknown | Unknown | Kawamura et al. (2010) |
| 129/Sv | SIRT4 knockout | Male and female | Reproductive organs not analysed; no evident phenotypic abnormalities | Unknown | Unknown | Haigs 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 homeostasis, 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).

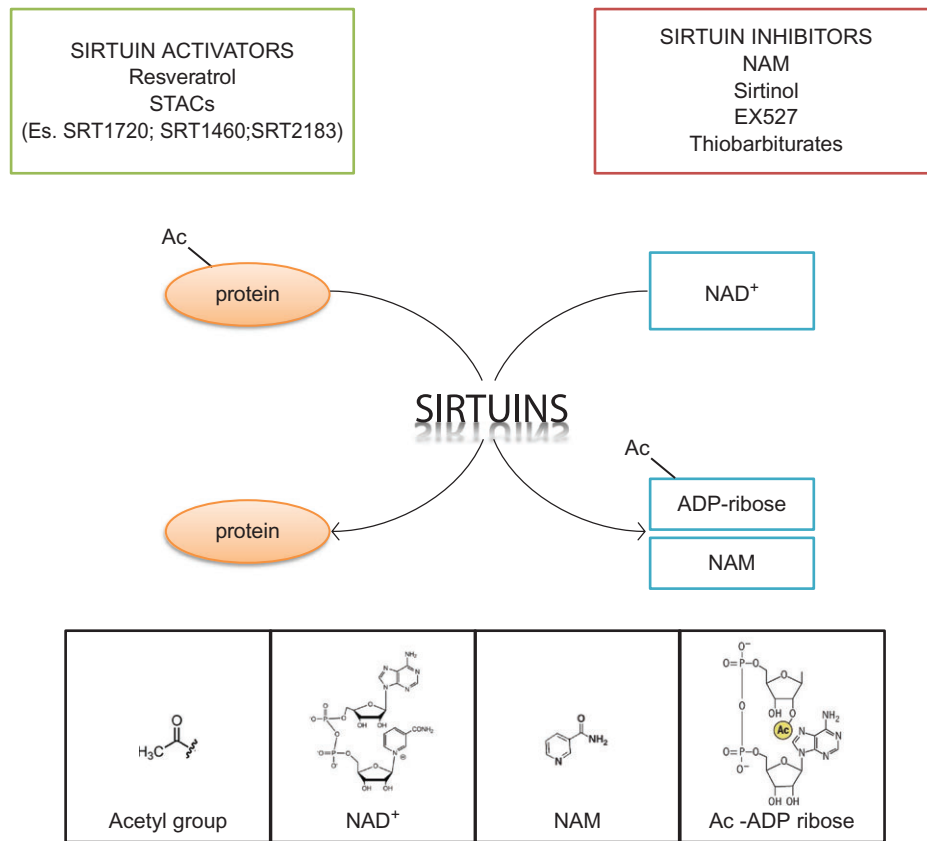


Figure 1 The deacetylase reaction catalyzed by Sirtuins. Sirtuins reverse acetyl modifications of lysine residue in a reaction that consumes NAD⁺ (nicotinamide adenine dinucleotide) releasing NAM (nicotinamide), Acetyl-ADP (adenosine diphosphate) ribose and the deacetylated protein. Sirtuins are activated 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 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 coactivator 1 alpha (PGC1 α), 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 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 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 lipamidase function, through which SIRT4 is thought to regulate the activity of

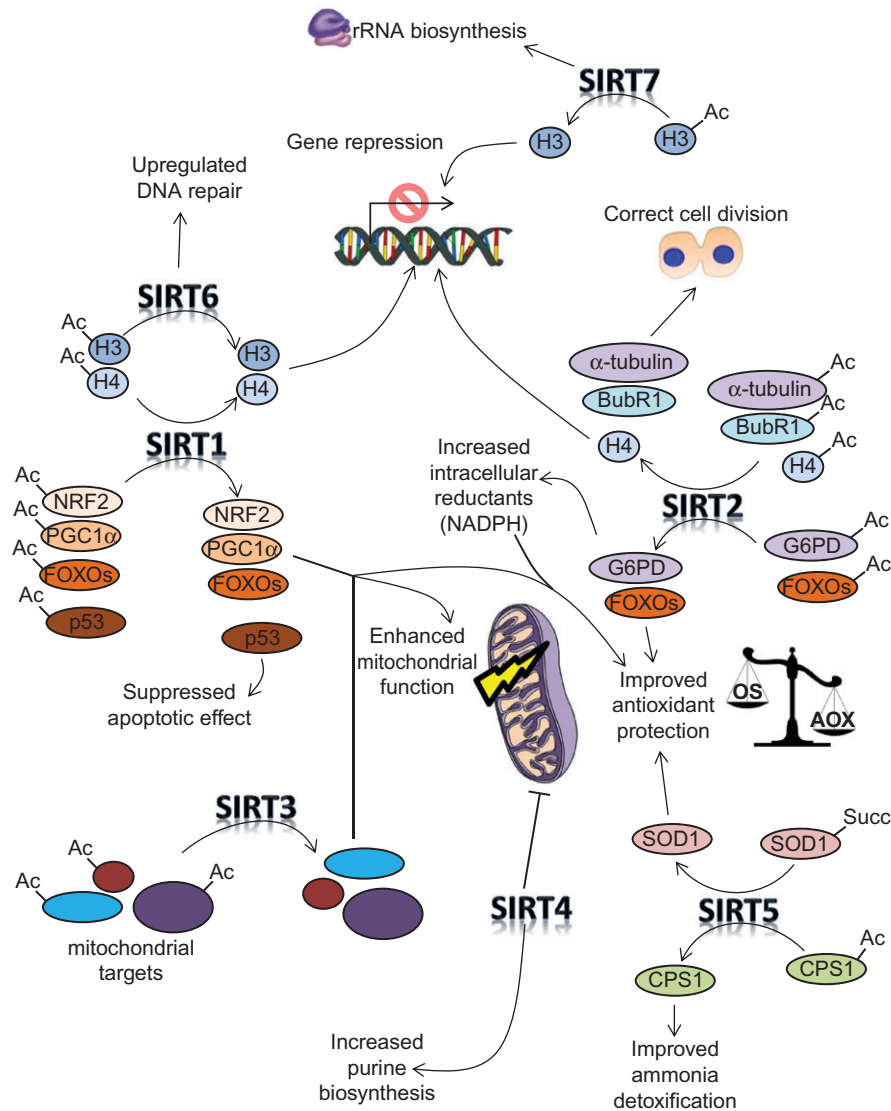


Figure 2 Main cellular functions regulated by mammalian Sirtuins, and major targets of Sirtuin-dependent enzymatic activities. Ac, acetyl group; AOX, antioxidants; Succ, succinyl group; BubR1, budding uninhibited by benzimidazole-related 1; CPS1, carbamoyl phosphate synthetase 1; FOXOs, forkhead box transcription factors; G6PD, glucose-6-phosphate dehydrogenase; H3, histone H3; H4, histone H4; NRF2, nuclear factor erythroid 2-related factor 2; OS, oxidative stress; PGC1 α , 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 carbamoylphosphate 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 homeostasis, by

deacetylating distinct lysine residues on GABP β 1 (GA binding protein β 1), 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 (Contrepolis *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 than 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 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 I (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 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 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 deacetylates 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- κ B, 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 μ M 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 anti-proliferative 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 I (CPSI) 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 spermatogenic 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 spermatogenic 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 PGC1 α in triggering antioxidant defence systems. In particular, when deacetylated and activated by SIRT1, PGC1 α 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/PGC1 α /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 spermatogenic 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 germ 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), pre-clinical 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- κ B 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 | <i>Rat</i> | 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) | |
| | | | <i>Mouse</i> | 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) | |
| | | | <i>Porcine</i> | Follicle atresia | Unknown | Zhao et al. (2014) | |
| | | Granulosa cells | <i>Human</i> | Proliferation; activation of steroidogenesis; response to insulin sensitizers | Visfatin | Reverchon et al. (2013, 2016) | |
| | | | <i>Porcine</i> | Proliferation; secretory activity | p53; NF- κ B; MAPK; ERK1-2 | Pavlová et al. (2013), Sirotkin et al. (2014) | |
| | | | <i>Rat</i> | Mediation of FSH action; activation of steroidogenesis | StAR | Morita et al. (2012) | |
| | | | <i>Bovine</i> | Steroidogenesis | StAR, IGF1R | Reverchon et al. (2016) | |
| | | KGN | | Cell homeostasis; response to metformin; activation of steroidogenesis; proliferation; response to insulin sensitizers | FOXO2; visfatin | Benayoun et al. (2011), Cantó et al. (2009), Caton et al. (2010) and Reverchon et al. (2013, 2016) | |
| | | COV434 | | Cell homeostasis; stress response | FOXO2; HuR | Benayoun et al. (2011) and Di Emidio et al. (2017) | |
| | | Oocyte | <i>Human</i> | Unknown | Unknown | Zhao et al. (2016) | |
| | | | <i>Mouse</i> | 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) | |
| | | | | <i>Porcine</i> | Maturation | Unknown | Li et al. (2016) |
| | | | | <i>Bovine</i> | Chromatin configuration; oocyte maturation | Unknown | Wang et al. (2014a), Labrecque et al. (2015) |
| | | | | <i>Equine</i> | Oocyte maturation | Unknown | Franciosi et al. (2017) |
| | | Embryo | | <i>Porcine</i> | Embryo development; regulation of apoptosis | Unknown | Kwak et al. (2012), Kim et al. (2017) |
| | <i>Bovine</i> | | Embryo development | Unknown | Khan et al. (2017) | | |
| Endometrium | <i>Human</i> | | Embryo implantation; carcinogenesis | E-cadherin | Shirane et al. (2012) and Bartosch et al. (2016) | | |
| SIRT2 | Cytoplasmic nuclear | Oocyte | <i>Human</i> | Unknown | Unknown | Zhao et al. (2016) | |
| | | | <i>Mouse</i> | 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 Endometrium | <i>Porcine</i> | Embryo development; regulation of apoptosis; autophagy | Unknown | Kwak et al. (2012) and Kim et al. (2017) | |
| | <i>Human</i> | Unknown | Unknown | Bartosch et al. (2016) | | | |
| SIRT3 | Mitochondrial | Ovary | <i>Mouse</i> | Folliculogenesis; ovarian aging; stress response | Unknown | Zhang et al. (2016a) and Di Emidio et al. (2017) | |
| | | | <i>Human</i> | Follicle metabolism; aging process; folliculogenesis; luteinisation; progesterone secretion; oxidative stress response | GDH; SOD1; CAT; 17 β HSD1; StAR; P450arom | Pacella-Ince et al. (2014a) and Fu et al. (2014) | |
| | | | COV434 | Folliculogenesis; luteinisation; progesterone secretion | SOD1; CAT; 17 β HSD1; StAR; P450arom | Fu et al. (2014) | |
| | | Cumulus cells | <i>Human</i> | Follicle metabolism; aging process | GDH | Pacella-Ince et al. (2014a) | |
| | | | <i>Human</i> | Oxidative stress response; mitochondrial biogenesis | PGC1- α | Zhao et al. (2016) | |
| | | | <i>Mouse</i> | 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 |
|---------|----------------------------|-------------|----------------|---|----------------------------|---|
| SIRT4 | Mitochondrial | Embryos | <i>Human</i> | Oxidative stress response; mitochondrial biogenesis | Unknown | Zhao et al. (2016) |
| | | | <i>Mouse</i> | Embryo development; oxidative stress response; maintenance of mitochondrial functionality; mitochondrial biogenesis | p53 | Kawamura et al. (2010) and Zhao et al. (2016) |
| | | Endometrium | <i>Porcine</i> | Embryo development; regulation of apoptosis; marker of embryo potential | Unknown | Kwak et al. (2012) and Kim et al. (2017) |
| | | | <i>Human</i> | Unknown | Unknown | Bartosch et al. (2016) |
| SIRT5 | Mitochondrial | Oocyte | <i>Human</i> | Unknown | Unknown | Zhao et al. (2016) |
| | | | <i>Mouse</i> | Unknown | Unknown | Kawamura et al. (2010) |
| | | Endometrium | <i>Human</i> | Unknown | Unknown | Bartosch et al. (2016) |
| SIRT6 | Nuclear | Ovary | <i>Human</i> | Follicle metabolism; aging process | CPS1 | Pacella-Ince et al. (2014b) |
| | | | <i>Human</i> | Unknown | Unknown | Bartosch et al. (2016) |
| | | Oocyte | <i>Rat</i> | Folliculogenesis | mTOR; FOXO3a; NRF-1; SIRT6 | Luo et al. (2012) , Zhang et al. (2013) and Wang et al. (2014b) |
| | | | <i>Mouse</i> | Folliculogenesis; ovarian aging | Unknown | Zhang et al. (2016a) |
| SIRT7 | Nuclear | Oocyte | <i>Human</i> | Unknown | Unknown | Zhao et al. (2016) |
| | | | <i>Mouse</i> | Unknown | Unknown | Kawamura et al. (2010) |
| | | | <i>Human</i> | Carcinogenesis | Unknown | Bartosch et al. (2016) |
| | | Endometrium | <i>Mouse</i> | Follicle development | Unknown | Kawamura et al. (2010) and Wang et al. (2014b) |
| | | | <i>Bovine</i> | Chromatin configuration | Unknown | Labrecque et al. (2015) |

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) Rato et al. (2014) and Rato et al. (2016) |
| | | | | Antioxidant protection | PGC-1 α and SIRT3 | |
| | | Chromatin condensation | BRDT-H4 binding | Bell et al. (2014) | | |
| | | Sumoylation | SUMO1 and SUMO2 | Coussens et al. (2008) | | |
| SIRT3 | Mitochondria | Sertoli cells? | Rat | Acrosome biogenesis | Map1lc3a | Liu et al. (2017) Cakir et al. (2009) and Di Sante et al. (2015) |
| | | | | GnRH neuronal migration | Cortactin | |
| SIRT6 | Nucleus and acrosome | Spermatids | Mouse | Glycolysis control | HIF-1 | Rato et al. (2014) and Rato et al. (2016) |
| | | | | Regulation of OXPHOS and antioxidant protection | ETC complexes | |
| | | | | 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-1 α , peroxisome proliferator-activated receptor γ coactivator 1 α .

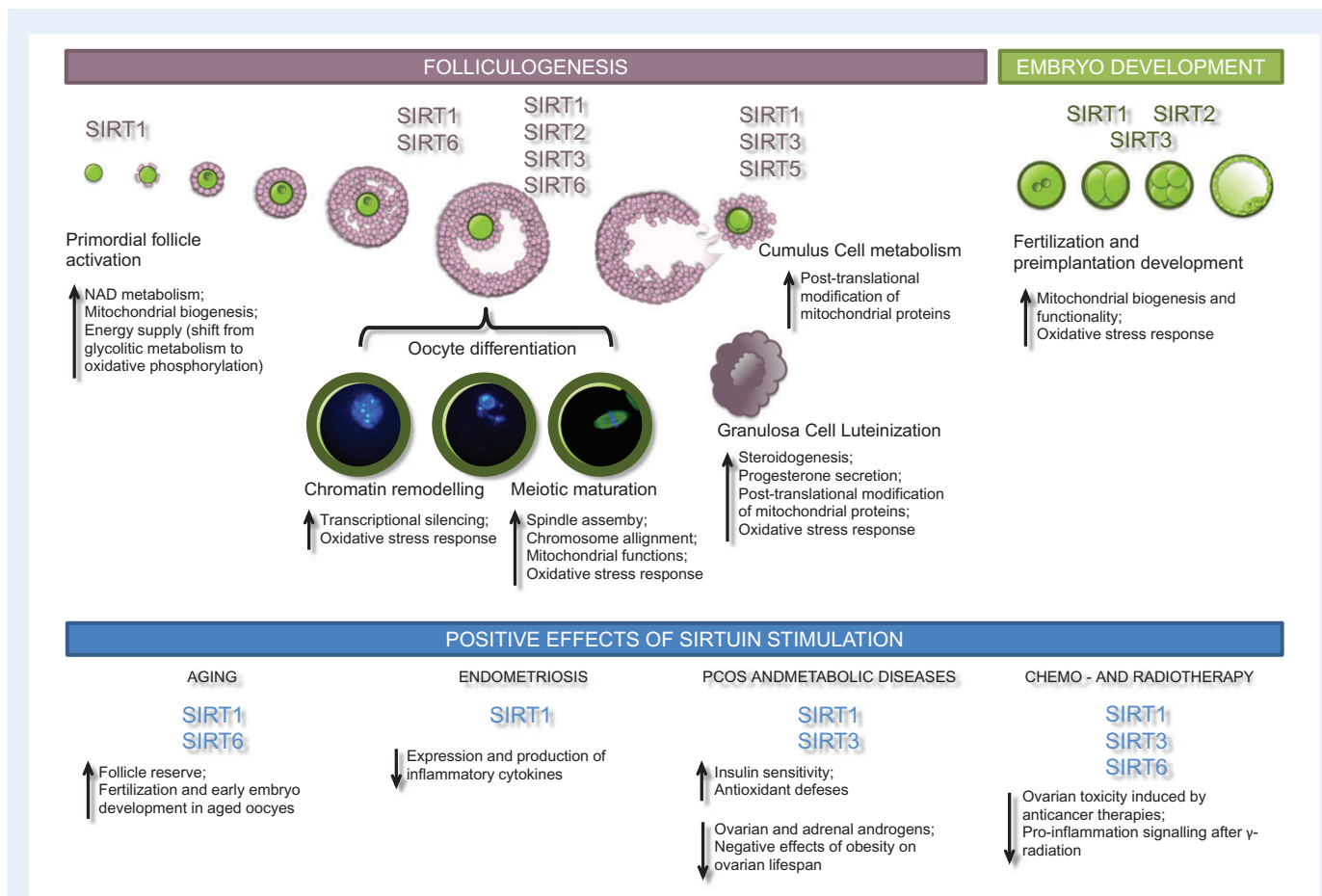


Figure 3 Main Sirtuin functions during different stages of folliculogenesis and embryo development and positive effects of Sirtuin stimulation in aging, endometriosis, PCOS and metabolic diseases, and chemo- and radiotherapy.

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 γ radiations pose high risk of premature ovarian failure (POF) in humans and in animal models (Rones 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 PGC1 α 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 ASI01 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 SIRT1720. 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 homeostasis, 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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References

- Adhikari D, Liu K. Molecular mechanisms underlying the activation of mammalian primordial follicles. *Endocr Rev* 2009;**30**:438–464.
- Adhikari D, Risal S, Liu K, Shen Y. Pharmacological inhibition of mTORC1 prevents over-activation of the primordial follicle pool in response to elevated PI3K signalling. *PLoS One* 2013;**8**:e53810.

- Agarwal A, Majzoub A. Role of antioxidants in assisted reproductive techniques. *World J Mens Health* 2017;**35**:77–93.
- Aitken RJ, Harkiss D, Buckingham DW. Analysis of lipid peroxidation mechanisms in human spermatozoa. *Mol Reprod Dev* 1993;**35**:302–315.
- Allemand I, Anglo A, Jeantet AY, Cerutti I, May E. Testicular wild-type p53 expression in transgenic mice induces spermiogenesis alterations ranging from differentiation defects to apoptosis. *Oncogene* 1999;**18**:6521–6530.
- Andersson RM, Shanmuganayagam D, Weindruch R. Caloric restriction and aging: studies in mice and monkeys. *Toxicol Pathol* 2009;**37**:47–51.
- Andreou A, Tavernarakis N. SUMOylation and cell signalling. *Biotechnol J* 2009;**4**:1740–1752.
- Balen AH, Morley LC, Misso M, Franks S, Legro RS, Wijayarathne CN, Stener-Victorin E, Fauser BC, Norman RJ, Teede H. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. *Hum Reprod Update* 2016;**22**:687–708.
- Banaszewska B, Wrotyńska-Barczyńska J, Spaczynski RZ, Pawelczyk L, Duleba AJ. Effects of resveratrol on polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 2016;**101**:4322–4328.
- Banks AS, Kon N, Knight C, Matsumoto M, Gutiérrez-Juárez R, Rossetti L, Gu W, Accili D. SirT1 gain of function increases energy efficiency and prevents diabetes in mice. *Cell Metab* 2008;**8**:333–341.
- Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011;**21**:381–395.
- Barber MF, Michishita-Kioi E, Xi Y, Tasselli L, Kioi M, Moqtaderi Z, Tennen RI, Paredes S, Young NL, Chen K et al. SIRT7 links H3K18 deacetylation to maintenance of oncogenic transformation. *Nature* 2012;**487**:114–118.
- Barbonetti A, Cinque B, Vassallo MR, Mineo S, Francavilla S, Cifone MG, Francavilla F. Effect of vaginal probiotic lactobacilli on in vitro-induced sperm lipid peroxidation and its impact on sperm motility and viability. *Fertil Steril* 2011;**95**:2485–2488.
- Bartosch C, Monteiro-Reis S, Almeida-Rios D, Vieira R, Castro A, Moutinho M, Rodrigues M, Graça I, Lopes JM, Jerónimo C. Assessing sirtuin expression in endometrial carcinoma and non-neoplastic endometrium. *Oncotarget* 2016;**7**:1144–1154.
- Bell EL, Nagamori I, Williams EO, Del Rosario AM, Bryson BD, Watson N, White FM, Sassone-Corsi P, Guarente L. SirT1 is required in the male germ cell for differentiation and fecundity in mice. *Development* 2014;**141**:3495–3504.
- Benayoun BA, Batista F, Auer J, Dipietromaria A, L'Hôte D, De Baere E, Veitia RA. Positive and negative feedback regulates the transcription factor FOXL2 in response to cell stress: evidence for a regulatory imbalance induced by disease-causing mutations. *Hum Mol Genet* 2009;**18**:632–644.
- Benayoun BA, Georges AB, L'Hôte D, Andersson N, Dipietromaria A, Todeschini AL, Caburet S, Bazin C, Anttonen M, Veitia RA. Transcription factor FOXL2 protects granulosa cells from stress and delays cell cycle: role of its regulation by the SIRT1 deacetylase. *Hum Mol Genet* 2011;**20**:1673–1686.
- Beumer TL, Roepers-Gajadien HL, Gademan IS, van Buul PP, Gil-Gomez G, Rutgers DH, de Rooij DG. The role of the tumor suppressor p53 in spermatogenesis. *Cell Death Differ* 1998;**5**:669–677.
- Boily G, Seifert EL, Bevilacqua L, He XH, Sabourin G, Estey C, Moffat C, Crawford S, Saliba S, Jardine K et al. SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One* 2008;**3**:e1759.
- Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J et al. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* 2007;**6**:759–767.
- Bouliou-Baly C, Hamraoui L, Guibert J, Beaujean N, Szollosi MS, Debey P. Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. *Biol Reprod* 1999;**60**:580–587.
- Bouras T, Fu M, Sauve AA, Wang F, Quong AA, Perkins ND, Hay RT, Gu W, Pestell RG. SIRT1 deacetylation and repression of p300 involves lysine residues 1020/1024 within the cell cycle regulatory domain 1. *J Biol Chem* 2005;**280**:10264–10276.
- Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. *Trends Endocrinol Metab* 2004;**15**:345–350.
- Brown PV, Hwang K, Schlegel PN, Morris PL. Small ubiquitin-related modifier (SUMO)-1, SUMO-2/3 and SUMOylation are involved with centromeric heterochromatin of chromosomes 9 and 1 and proteins of the synaptonemal complex during meiosis in men. *Hum Reprod* 2008;**23**:2850–2857.
- Buckler H. The menopause transition: endocrine changes and clinical symptoms. *J Br Menopause Soc* 2005;**11**:61–65.
- Byles V, Zhu L, Lovaas JD, Chmielewski LK, Wang J, Faller DV, Dai Y. SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. *Oncogene* 2012;**31**:4619–4629.
- Cakir I, Perello M, Lansari O, Messier NJ, Vaslet CA, Nilini EA. Hypothalamic Sirt1 regulates food intake in a rodent model system. *PLoS One* 2009;**4**:e8322.
- Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, Elliott PJ, Puigserver P, Auwerx J. AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity. *Nature* 2009;**458**:1056–1060.
- Caton PW, Nayuni NK, Kieswich J, Khan NQ, Yaqoob MM, Corder R. Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *J Endocrinol* 2010;**205**:97–106.
- Chang HC, Guarente L. SIRT1 and other sirtuins in metabolism. *Trends Endocrinol Metab* 2014;**25**:138–145.
- Chen J, Melton C, Suh N, Oh JS, Horner K, Xie F, Sette C, Belloch R, Conti M. Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (Dazl) at the oocyte-to-zygote transition. *Genes Dev* 2011;**25**:755–766.
- Chen Z, Kang X, Wang L, Dong H, Wang C, Xiong Z, Zhao W, Jia C, Lin J, Zhang W et al. Rictor/mTORC2 pathway in oocytes regulates folliculogenesis, and its inactivation causes premature ovarian failure. *J Biol Chem* 2015;**290**:6387–6396.
- Choy JS, Acuna R, Au WC, Basrai MA. A role for histone H4K16 hypoacetylation in *Saccharomyces cerevisiae* kinetochore function. *Genetics* 2011;**189**:11–21.
- Cinco R, Digman MA, Gratton E, Luderer U. Spatial characterization of bioenergetics and metabolism of primordial to preovulatory follicles in whole ex vivo murine ovary. *Biol Reprod* 2016;**95**:129.
- Clayton AL, Hazzalin CA, Mahadevan LC. Enhanced histone acetylation and transcription: a dynamic perspective. *Mol Cell* 2006;**23**:289–296.
- Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* 2004;**305**:390–392.
- Contrepois K, Thuret JY, Courbeyrette R, Fenaille F, Mann C. Deacetylation of H4-K16Ac and heterochromatin assembly in senescence. *Epigenetics Chromatin* 2012;**5**:15.
- Coussens M, Maresh JG, Yanagimachi R, Maeda G, Allsopp R. Sirt1 deficiency attenuates spermatogenesis and germ cell function. *PLoS One* 2008;**3**:e1571.
- De La Fuente R. Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. *Dev Biol* 2006;**292**:1–12.
- de Mingo Á, de Gregorio E, Moles A, Tarrats N, Tutusaus A, Colell A, Fernandez-Checa JC, Morales A, Marí M. Cysteine cathepsins control hepatic NF- κ B-dependent inflammation via sirtuin-1 regulation. *Cell Death Dis* 2016;**7**:e2464.
- de Oliveira RM, Sarkander J, Kazantsev AG, Outeiro TF. SIRT2 as a therapeutic target for age-related disorders. *Front Pharmacol* 2012;**3**:82.
- Di Emidio G, Falone S, Vitti M, D'Alessandro AM, Vento M, Di Pietro C, Amicarelli F, Tatone C. SIRT1 signalling protects mouse oocytes against oxidative stress and is deregulated during aging. *Hum Reprod* 2014;**29**:2006–2017.
- Di Emidio G, Rossi G, Bonomo I, Alonso GL, Vetuschci A, Artini PG, Provenzani A, Falone S, Carta G, D'Alessandro AM et al. The natural carotenoid crocetin and the synthetic tellurium compound AS101 protect the ovary against cyclophosphamide by modulating SIRT1 and mitochondrial markers. *Oxid Med Cell Longev* 2017;**2017**:8928604.
- Di Sante G, Wang L, Wang C, Jiao X, Casimiro MC, Chen K, Pestell TG, Yaman I, Di Rocco A, Sun X et al. Sirt1-deficient mice have hypogonadotropic hypogonadism due to defective GnRH neuronal migration. *Mol Endocrinol* 2015;**29**:200–212.
- Ding YW, Zhao GJ, Li XL, Hong GL, Li MF, Qiu QM, Wu B, Lu ZQ. SIRT1 exerts protective effects against paraquat-induced injury in mouse type II alveolar epithelial cells by deacetylating NRF2 in vitro. *Int J Mol Med* 2016;**37**:1049–1058.
- Dou X, Sun Y, Li J, Zhang J, Hao D, Liu W, Wu R, Kong F, Peng X, Li J. Short-term rapamycin treatment increases ovarian lifespan in young and middle-aged female mice. *Aging Cell* 2017;**16**:825–836.
- Dryden SC, Nahhas FA, Nowak JE, Goustin AS, Tainsky MA. Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* 2003;**23**:3173–3185.
- Du J, Zhou Y, Su X, Yu JJ, Khan S, Jiang H, Kim J, Woo J, Kim JH, Choi BH et al. Sirt5 is a NAD-dependent protein lysine demalonylase and desuccinylase. *Science* 2011;**334**:806–809.

- Eichenlaub-Ritter U. Oocyte ageing and its cellular basis. *Int J Dev Biol* 2012;**56**: 841–852.
- Ergenoglu M, Yildirim N, Yildirim AG, Yeniel O, Erbas O, Yavasoglu A, Taskiran D, Karadadas N. Effects of resveratrol on ovarian morphology, plasma anti-mullerian hormone, IGF-1 levels, and oxidative stress parameters in a rat model of polycystic ovary syndrome. *Reprod Sci* 2015;**22**:942–947.
- Finley LW, Haigis MC. Metabolic regulation by SIRT3: implications for tumorigenesis. *Trends Mol Med* 2012;**18**:516–523.
- Ford E, Voit R, Liszt G, Magin C, Grummt I, Guarente L. Mammalian Sir2 homolog SIRT7 is an activator of RNA polymerase I transcription. *Genes Dev* 2006;**20**: 1075–1080.
- Franciosi F, Lodde V, Goudet G, Duchamp G, Deleuze S, Douet C, Tessaro I, Luciano AM. Changes in histone H4 acetylation during in vivo versus in vitro maturation of equine oocytes. *Mol Hum Reprod* 2012;**18**:243–252.
- Franciosi F, Goudet G, Tessaro I, Papillier P, Dalbies-Tran R, Reigner F, Deleuze S, Douet C, Miclea I, Lodde V et al. In vitro maturation affects chromosome segregation, spindle morphology and acetylation of lysine 16 on histone H4 in horse oocytes. *Reprod Fertil Dev* 2017;**29**:721–730.
- Fu H, Wada-Hiraike O, Hirano M, Kawamura Y, Sakurabashi A, Shirane A, Morita Y, Isono W, Oishi H, Koga K et al. SIRT3 positively regulates the expression of folliculogenesis- and luteinization-related genes and progesterone secretion by manipulating oxidative stress in human luteinized granulosa cells. *Endocrinology* 2014;**155**:3079–3087.
- Garcia-Cao M, O'Sullivan R, Peters AH, Jenuwein T, Blasco MA. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. *Nat Genet* 2004;**36**:94–99.
- Goldman KN, Chenette D, Arju R, Duncan FE, Keefe DL, Grifo JA, Schneider RJ. mTORC1/2 inhibition preserves ovarian function and fertility during genotoxic chemotherapy. *Proc Natl Acad Sci USA* 2017;**114**:3186–3191.
- Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. *Biogerontology* 2017;**18**:447–476.
- Grob A, Roussel P, Wright JE, McStay B, Hernandez-Verdun D, Sirri V. Involvement of SIRT7 in resumption of rDNA transcription at the exit from mitosis. *J Cell Sci* 2009;**122**:489–498.
- Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem* 2001;**276**:38837–38843.
- Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G et al. SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 2006;**126**:941–954.
- Han L, Ge J, Zhang L, Ma R, Hou X, Li B, Moley K, Wang Q. Sirt6 depletion causes spindle defects and chromosome misalignment during meiosis of mouse oocyte. *Sci Rep* 2015;**5**:15366.
- Hansen LW, Khader A, Yang WL, Prince JM, Nicastro JM, Coppa GF, Wang P. Sirtuin I activator SRT1720 protects against organ injury induced by intestinal ischemia-reperfusion. *Shock* 2016;**45**:359–366.
- Hasegawa K, Wakino S, Yoshioka K, Tatematsu S, Hara Y, Minakuchi H, Washida N, Tokuyama H, Hayashi K, Itoh H. Sirt1 protects against oxidative stress-induced renal tubular cell apoptosis by the bidirectional regulation of catalase expression. *Biochem Biophys Res Commun* 2008;**372**:51–56.
- Hirschey MD, Zhao Y. Metabolic Regulation by Lysine Malonylation, Succinylation, and Glutarylation. *Mol Cell Proteomics* 2015;**14**:2308–23015.
- Hori YS, Kuno A, Hosoda R, Horio Y. Regulation of FOXOs and p53 by SIRT1 modulators under oxidative stress. *PLoS One* 2013;**8**:e73875.
- Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. *Nat Rev Mol Cell Biol* 2012;**13**:225–238.
- Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL et al. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003;**425**:191–196.
- Hui Y, Lu M, Han Y, Zhou H, Liu W, Li L, Jin R. Resveratrol improves mitochondrial function in the remnant kidney from 5/6 nephrectomized rats. *Acta Histochem* 2017;**119**:392–399.
- Ichikawa T, Hayashi R, Suzuki K, Imanishi S, Kambara K, Okazawa S, Inomata M, Yamada T, Yamazaki Y, Koshimizu Y et al. Sirtuin I activator SRT1720 suppresses inflammation in an ovalbumin-induced mouse model of asthma. *Respirology* 2013;**18**:332–339.
- Itami N, Shirasuna K, Kuwayama T, Iwata H. Resveratrol improves the quality of pig oocytes derived from early antral follicles through sirtuin I activation. *Theriogenology* 2015;**83**:1360–1367.
- Iwahara T, Bonasio R, Narendra V, Reinberg D. SIRT3 functions in the nucleus in the control of stress-related gene expression. *Mol Cell Biol* 2012;**32**:5022–5034.
- Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;**293**:1074–1080.
- Jeong SM, Xiao C, Finley LW, Lahusen T, Souza AL, Pierce K, Li YH, Wang X, Laurent G, German NJ et al. SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. *Cancer Cell* 2013;**23**:450–463.
- Jia Y, Zheng Z, Wang Y, Zhou Q, Cai W, Jia W, Yang L, Dong M, Zhu X, Su L et al. SIRT1 is a regulator in high glucose-induced inflammatory response in RAW264.7 cells. *PLoS One* 2015;**10**:e0120849.
- Jiang W, Wang S, Xiao M, Lin Y, Zhou L, Lei Q, Xiong Y, Guan KL, Zhao S. Acetylation regulates gluconeogenesis by promoting PEPCK1 degradation via recruiting the UBR5 ubiquitin ligase. *Mol Cell* 2011;**43**:33–44.
- Jin YH, Kim YJ, Kim DW, Baek KH, Kang BY, Yeo CY, Lee KY. Sirt2 interacts with I4-3-3 beta/gamma and down-regulates the activity of p53. *Biochem Biophys Res Commun* 2008;**368**:690–695.
- Kaeberlein M, McVey M, Guarente L. The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. *Genes Dev* 1999;**13**:2570–2580.
- Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, Nahum L, Bar-Joseph Z, Cohen HY. The sirtuin SIRT6 regulates lifespan in male mice. *Nature* 2012;**483**:218–221.
- Kawamura Y, Uchijima Y, Horike N, Tonami K, Nishiyama K, Amano T, Asano T, Kurihara Y, Kurihara H. Sirt3 protects in vitro-fertilized mouse preimplantation embryos against oxidative stress-induced p53-mediated developmental arrest. *J Clin Invest* 2010;**120**:2817–2828.
- Khan DR, Fournier E, Dufort I, Richard FJ, Singh J, Sirard MA. Meta-analysis of gene expression profiles in granulosa cells during folliculogenesis. *Reproduction* 2016;**151**:R103–R110.
- Khan I, Kim SW, Lee KL, Song SH, Meslam A, Chowdhury MM, Uddin Z, Park KH, Kong IK. Polydatin improves the developmental competence of bovine embryos in vitro via induction of sirtuin I (Sirt1). *Reprod Fertil Dev* 2017;**29**: 2011–2020.
- Kim HS, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD, van der Meer R, Nguyen P, Savage J, Owens KM et al. SIRT3 is a mitochondrial-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. *Cancer Cell* 2010;**17**:41–52.
- Kim MG, Kim DH, Lee HR, Lee JS, Jin SJ, Lee HT. Sirtuin inhibition leads to autophagy and apoptosis in porcine preimplantation blastocysts. *Biochem Biophys Res Commun* 2017;**488**:603–608.
- Kim W, Kim JE. SIRT7 an emerging sirtuin: deciphering newer roles. *J Physiol Pharmacol* 2013;**64**:531–534.
- Kolthour-Seetharam U, Teerds K, de Rooij DG, Wendling O, McBurney M, Sassone-Corsi P, Davidson I. The histone deacetylase SIRT1 controls male fertility in mice through regulation of hypothalamic-pituitary gonadotropin signaling. *Biol Reprod* 2009;**80**:384–391.
- Kong X, Wang R, Xue Y, Liu X, Zhang H, Chen Y, Fang F, Chang Y. Sirtuin 3, a new target of PGC-1alpha, plays an important role in the suppression of ROS and mitochondrial biogenesis. *PLoS One* 2010;**5**:e11707.
- Konsoula Z, Barile FA. Epigenetic histone acetylation and deacetylation mechanisms in experimental models of neurodegenerative disorders. *J Pharmacol Toxicol Methods* 2012;**66**:215–220.
- Krauss V. Glimpses of evolution: heterochromatic histone H3K9 methyltransferases left its marks behind. *Genetica* 2008;**133**:93–106.
- Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci* 2014;**39**:72–81.
- Kumari S, Chaurasia SN, Nayak MK, Mallick RL, Dash D. Sirtuin inhibition induces apoptosis-like changes in platelets and thrombocytopenia. *J Biol Chem* 2015;**290**: 12290–12299.
- Kupis W, Patyga J, Tomal E, Niewiadomska E. The role of sirtuins in cellular homeostasis. *J Physiol Biochem* 2016;**72**:371–380.
- Kwak SS, Cheong SA, Yoon JD, Jeon Y, Hyun SH. Expression patterns of sirtuin genes in porcine preimplantation embryos and effects of sirtuin inhibitors on in vitro embryonic development after parthenogenetic activation and in vitro fertilization. *Theriogenology* 2012;**78**:1597–15610.

- Labrecque R, Lodde V, Dieci C, Tessaro I, Luciano AM, Sirard MA. Chromatin remodelling and histone mRNA accumulation in bovine germinal vesicle oocytes. *Mol Reprod Dev* 2015;**82**:450–462.
- Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell* 2006;**127**:1109–11022.
- Lain S, Hollick JJ, Campbell J, Staples OD, Higgins M, Aoubala M, McCarthy A, Appleyard V, Murray KE, Baker L et al. Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. *Cancer Cell* 2008;**13**:454–463.
- Law IK, Liu L, Xu A, Lam KS, Vanhoutte PM, Che CM, Leung PT, Wang Y. Identification and characterization of proteins interacting with SIRT1 and SIRT3: implications in the anti-aging and metabolic effects of sirtuins. *Proteomics* 2009;**9**:2444–24456.
- Lee J, Hong SW, Chae SW, Kim DH, Choi JH, Bae JC, Park SE, Rhee EJ, Park CY, Oh KW et al. Exendin-4 improves steatohepatitis by increasing Sirt1 expression in high-fat diet-induced obese C57BL/6j mice. *PLoS One* 2012;**7**:e31394.
- Lei ZM, Mishra S, Zou W, Xu B, Foltz M, Li X, Rao CV. Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. *Mol Endocrinol* 2001;**15**:184–200.
- Li H, Rajendran GK, Liu N, Ware C, Rubin BP, Gu Y. Sirt1 modulates the estrogen-insulin-like growth factor-1 signaling for postnatal development of mammary gland in mice. *Breast Cancer Res* 2007;**9**:R1.
- Li L, Fu YC, Xu JJ, Lin XH, Chen XC, Zhang XM, Luo LL. Caloric restriction promotes the reserve of follicle pool in adult female rats by inhibiting the activation of mammalian target of rapamycin signaling. *Reprod Sci* 2015;**22**:60–67.
- Li X. SIRT1 and energy metabolism. *Acta Biochim Biophys Sin (Shanghai)* 2013;**45**:51–60.
- Li Y, Dai D, Lu Q, Fei M, Li M, Wu X. Sirt2 suppresses glioma cell growth through targeting NF- κ B-miR-21 axis. *Biochem Biophys Res Commun* 2013;**441**:661–667.
- Li Y, Wang J, Zhang Z, Yi J, He C, Wang F, Tian X, Yang M, Song Y, He P et al. Resveratrol compares with melatonin in improving in vitro porcine oocyte maturation under heat stress. *J Anim Sci Biotechnol* 2016;**7**:33.
- Lin ZF, Xu HB, Wang JY, Lin Q, Ruan Z, Liu FB, Jin W, Huang HH, Chen X. SIRT5 desuccinylates and activates SOD1 to eliminate ROS. *Biochem Biophys Res Commun* 2013;**441**:191–195.
- Liu C, Song Z, Wang L, Yu H, Liu W, Shang Y, Xu Z, Zhao H, Gao F, Wen J et al. Sirt1 regulates acrosome biogenesis by modulating autophagic flux during spermiogenesis in mice. *Development* 2017a;**144**:441–451.
- Liu M, Yin Y, Ye X, Zeng M, Zhao Q, Keefe DL, Liu L. Resveratrol protects against age-associated infertility in mice. *Hum Reprod* 2013;**28**:707–717.
- Liu WJ, Zhang XM, Wang N, Zhou XL, Fu YC, Luo LL. Calorie restriction inhibits ovarian follicle development and follicle loss through activating SIRT1 signaling in mice. *Eur J Med Res* 2015;**20**:22.
- Liu X, Zhang L, Wang P, Li X, Qiu D, Li L, Zhang J, Hou X, Han L, Ge J et al. Sirt3-dependent deacetylation of SOD2 plays a protective role against oxidative stress in oocytes from diabetic mice. *Cell Cycle* 2017b;**16**:1302–1308.
- Liu Z, Gan L, Liu G, Chen Y, Wu T, Feng F, Sun C. Sirt1 decreased adipose inflammation by interacting with Akt2 and inhibiting mTOR/S6K1 pathway in mice. *J Lipid Res* 2016;**57**:1373–1381.
- Lodde V, Modina S, Galbusera C, Franciosi F, Luciano AM. Large-scale chromatin remodeling in germinal vesicle bovine oocytes: interplay with gap junction functionality and developmental competence. *Mol Reprod Dev* 2007;**74**:740–749.
- Lodde V, Modina S, Maddox-Hyttel P, Franciosi F, Lauria A, Luciano AM. Oocyte morphology and transcriptional silencing in relation to chromatin remodeling during the final phases of bovine oocyte growth. *Mol Reprod Dev* 2008;**75**:915–924.
- Lodde V, Luciano AM, Franciosi F, Labrecque R, Sirard MA. Chromatin remodeling enzymes mRNA and histone mRNA accumulation in oocytes. *Results Probl Cell Differ* 2017;**63**:223–255.
- Lombard DB, Zwaans BM. SIRT3: as simple as it seems? *Gerontology* 2014;**60**:56–64.
- Lombardi PM, Cole KE, Dowling DP, Christianson DW. Structure, mechanism, and inhibition of histone deacetylases and related metalloenzymes. *Curr Opin Struct Biol* 2011;**21**:735–743.
- Lord T, Aitken RJ. Oxidative stress and ageing of the post-ovulatory oocyte. *Reproduction* 2013;**146**:R217–R227.
- Luciano AM, Franciosi F, Modina SC, Lodde V. Gap junction-mediated communications regulate chromatin remodeling during bovine oocyte growth and differentiation through cAMP-dependent mechanism(s). *Biol Reprod* 2011;**85**:1252–1259.
- Luciano AM, Lodde V. Changes of large-scale chromatin configuration during mammalian oocyte differentiation. In: Coticchio G, Albertini DF, De Santis L (eds). *Oogenesis*. London: Springer, 2013;93–108.
- Luo LL, Chen XC, Fu YC, Xu JJ, Li L, Lin XH, Xiang YF, Zhang XM. The effects of caloric restriction and a high-fat diet on ovarian lifespan and the expression of SIRT1 and SIRT6 proteins in rats. *Aging Clin Exp Res* 2012;**24**:125–133.
- Ma P, Schultz RM. Histone deacetylase 1 (HDAC1) regulates histone acetylation, development, and gene expression in preimplantation mouse embryos. *Dev Biol* 2008;**319**:110–120.
- Ma P, Schultz RM. Histone deacetylase 2 (HDAC2) regulates chromosome segregation and kinetochore function via H4K16 deacetylation during oocyte maturation in mouse. *PLoS Genet* 2013;**9**:e1003377.
- Ma R, Zhang Y, Zhang L, Han J, Rui R. Sirt1 protects pig oocyte against in vitro aging. *Anim Sci J* 2015;**86**:826–832.
- Ma X, Dong Y, Matzuk MM, Kumar TR. Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. *Proc Natl Acad Sci USA* 2004;**101**:17294–17299.
- Manosalva I, González A. Aging changes the chromatin configuration and histone methylation of mouse oocytes at germinal vesicle stage. *Theriogenology* 2010;**74**:1539–15347.
- Mao Z, Hine C, Tian X, Van Meter M, Au M, Vaidya A, Seluanov A, Gorbunova V. SIRT6 promotes DNA repair under stress by activating PARP1. *Science* 2011;**332**:1443–1446.
- Marmorstein R, Zhou MM. Writers and readers of histone acetylation: structure, mechanism, and inhibition. *Cold Spring Harb Perspect Biol* 2014;**6**:a018762.
- Martinez-Redondo P, Vaquero A. The diversity of histone versus nonhistone sirtuin substrates. *Genes Cancer* 2013;**4**:148–163.
- Mathias RA, Greco TM, Oberstein A, Budayeva HG, Chakrabarti R, Rowland EA, Kang Y, Shenk T, Cristea IM. Sirtuin 4 is a lipamidase regulating pyruvate dehydrogenase complex activity. *Cell* 2014;**159**:1615–1625.
- McBurney MW, Yang X, Jardine K, Hixon M, Boekelheide K, Webb JR, Lansdorp PM, Lemieux M. The mammalian SIRT2 α protein has a role in embryogenesis and gametogenesis. *Mol Cell Biol* 2003;**23**:38–54.
- Metzler-Guillemain C, Depetris D, Luciani JJ, Mignon-Ravix C, Mitchell MJ, Mattei MG. In human pachytene spermatocytes, SUMO protein is restricted to the constitutive heterochromatin. *Chromosome Res* 2008;**16**:761–782.
- Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J* 2007;**404**:1–13.
- Michishita E, Park JY, Burneski JM, Barrett JC, Horikawa I. Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol Biol Cell* 2005;**16**:4623–4635.
- Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, Cheung P, Kusumoto R, Kawahara TL, Barrett JC et al. SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* 2008;**452**:492–496.
- Mitchell SJ, Martin-Montalvo A, Mercken EM, Palacios HH, Ward TM, Abulwerdi G, Minor RK, Vlasuk GP, Ellis JL, Sinclair DA et al. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Rep* 2014;**6**:836–843.
- Morita Y, Wada-Hiraike O, Yano T, Shirane A, Hirano M, Hiraike H, Koyama S, Oishi H, Yoshino O, Miyamoto Y et al. Resveratrol promotes expression of SIRT1 and StAR in rat ovarian granulosa cells: an implicative role of SIRT1 in the ovary. *Reprod Biol Endocrinol* 2012;**10**:14.
- Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 2006;**124**:315–329.
- Mulligan P, Yang F, Di Stefano L, Ji JY, Ouyang J, Nishikawa JL, Toiber D, Kulkarni M, Wang Q, Najafi-Shoushtari SH et al. A SIRT1-LSD1 corepressor complex regulates Notch target gene expression and development. *Mol Cell* 2011;**42**:689–699.
- Mvunta DH, Miyamoto T, Asaka R, Yamada Y, Ando H, Higuchi S, Ida K, Kashima H, Shiozawa T. Overexpression of SIRT1 is associated with poor outcomes in patients with ovarian carcinoma. *Appl Immunohistochem Mol Morphol* 2016;**25**:415–421.
- Nakamura Y, Ogura M, Ogura K, Tanaka D, Inagaki N. SIRT5 deacetylates and activates urate oxidase in liver mitochondria of mice. *FEBS Lett* 2012;**586**:4076–4081.

- Nakane K, Fujita Y, Terazawa R, Atsumi Y, Kato T, Nozawa Y, Deguchi T, Ito M. Inhibition of cortactin and SIRT1 expression attenuates migration and invasion of prostate cancer DU145 cells. *Int J Urol* 2012;**19**:71–79.
- Napper AD, Hixon J, McDonagh T, Keavey K, Pons JF, Barker J, Yau WT, Amouzegh P, Flegg A, Hamelin E *et al.* Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1. *J Med Chem* 2005;**48**:8045–8054.
- Nayagam VM, Wang X, Tan YC, Poulsen A, Goh KC, Ng T, Wang H, Song HY, Ni B, Entzeroth M *et al.* SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. *J Biomol Screen* 2006;**11**:959–967.
- North BJ, Rosenberg MA, Jeganathan KB, Hafner AV, Michan S, Dai J, Baker DJ, Cen Y, Wu LE, Sauve AA *et al.* SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. *EMBO J* 2014;**33**:1438–1453.
- Oliveira PF, Martins AD, Moreira AC, Cheng CY, Alves MG. The Warburg effect revisited—lesson from the Sertoli cell. *Med Res Rev* 2015;**35**:126–151.
- Ortega I, Duleba AJ. Ovarian actions of resveratrol. *Ann NY Acad Sci* 2015;**1348**:86–96.
- Osborne B, Bentley NL, Montgomery MK, Turner N. The role of mitochondrial sirtuins in health and disease. *Free Radic Biol Med* 2016;**100**:164–174.
- Pacella-Ince L, Zander-Fox DL, Lan M. Mitochondrial SIRT3 and its target glutamate dehydrogenase are altered in follicular cells of women with reduced ovarian reserve or advanced maternal age. *Hum Reprod* 2014a;**29**:1490–1499.
- Pacella-Ince L, Zander-Fox DL, Lane M. Mitochondrial SIRT5 is present in follicular cells and is altered by reduced ovarian reserve and advanced maternal age. *Reprod Fertil Dev* 2014b;**26**:1072–1083.
- Palmer NO, Fullston T, Mitchell M, Satchell BP, Lane M. SIRT6 in mouse spermatogenesis is modulated by diet-induced obesity. *Reprod Fertil Dev* 2011;**23**:929–939.
- Pardo PS, Mohamed JS, Lopez MA, Boriek AM. Induction of Sirt1 by mechanical stretch of skeletal muscle through the early response factor EGR1 triggers an antioxidative response. *J Biol Chem* 2011;**286**:2559–2566.
- Parihar P, Solanki I, Mansuri ML, Parihar MS. Mitochondrial sirtuins: emerging roles in metabolic regulations, energy homeostasis and diseases. *Exp Gerontol* 2015;**61**:130–141.
- Pavlová S, Klucská K, Vašíček D, Ryban L, Harrath AH, Alwasel SH, Sirotkin AV. The involvement of SIRT1 and transcription factor NF- κ B (p50/p65) in regulation of porcine ovarian cell function. *Anim Reprod Sci* 2013;**140**:180–188.
- Qiu D, Hou X, Han L, Li X, Ge J, Wang Q. Sirt2-BubR1 acetylation pathway mediates the effects of advanced maternal age on oocyte quality. *Aging Cell* 2017. doi:10.1111/acel.12698.
- Ramaswamy S, Weinbauer GF. Endocrine control of spermatogenesis: role of FSH and LH/testosterone. *Spermatogenesis* 2014;**4**:e996025.
- Rato L, Alves MG, Dias TR, Lopes G, Cavaco JE, Socorro S, Oliveira PF. High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. *Andrology* 2013;**1**:495–504.
- Rato L, Duarte AI, Tomás GD, Santos MS, Moreira PI, Socorro S, Cavaco JE, Alves MG, Oliveira PF. Pre-diabetes alters testicular PGC1- α /SIRT3 axis modulating mitochondrial bioenergetics and oxidative stress. *Biochim Biophys Acta* 2014;**183**:335–344.
- Rato L, Alves MG, Silva BM, Sousa M, Oliveira PF. Sirtuins: novel players in male reproductive health. *Curr Med Chem* 2016;**23**:1084–1099.
- Rauh D, Fischer F, Gertz M, Lakshminarasimhan M, Bergbrede T, Aladini F, Kambach C, Becker CF, Zerweck J, Schutkowski M *et al.* An acetylome peptide microarray reveals specificities and deacetylation substrates for all human sirtuin isoforms. *Nat Commun* 2013;**4**:2327.
- Reverchon M, Cornuau M, Cloix L, Ramé C, Guerif F, Royère D, Dupont J. Visfatin is expressed in human granulosa cells: regulation by metformin through AMPK/SIRT1 pathways and its role in steroidogenesis. *Mol Hum Reprod* 2013;**19**:313–326.
- Reverchon M, Rame C, Bunel A, Chen W, Froment P, Dupont J. VISFATIN (NAMPT) improves in vitro IGF1-induced steroidogenesis and IGF1 receptor signaling through SIRT1 in bovine granulosa cells. *Biol Reprod* 2016;**94**:54.
- Revollo JR, Li X. The ways and means that fine tune Sirt1 activity. *Trends Biochem Sci* 2013;**38**:160–167.
- Riepsamen A, Wu L, Lau L, Listijono D, Ledger W, Sinclair D, Homer H. Nicotinamide impairs entry into and exit from meiosis I in mouse oocytes. *PLoS One* 2015;**10**:e0126194.
- Rogers RS, Inselman A, Handel MA, Matunis MJ. SUMO modified proteins localize to the XY body of pachytene spermatocytes. *Chromosoma* 2004;**113**:233–243.
- Roichman A, Kanfi Y, Glazz R, Naiman S, Amit U, Landa N, Tinman S, Stein I, Pikarsky E, Leor J *et al.* SIRT6 overexpression improves various aspects of mouse healthspan. *J Gerontol A Biol Sci Med Sci* 2017;**72**:603–615.
- Roness H, Kashi O, Meirrow D. Prevention of chemotherapy-induced ovarian damage. *Fertil Steril* 2016;**105**:20–29.
- Rothgiesser KM, Erenner S, Waibel S, Lüscher B, Hottiger MO. SIRT2 regulates NF- κ B dependent gene expression through deacetylation of p65 Lys310. *J Cell Sci* 2010;**123**:4251–4258.
- Rowlands BD, Lau CL, Ryall JG, Thomas DS, Klugmann M, Beart PM, Rae CD. Silent information regulator 1 modulator resveratrol increases brain lactate production and inhibits mitochondrial metabolism, whereas SIRT1720 increases oxidative metabolism. *J Neurosci Res* 2015;**93**:1147–1156.
- Ryu D, Jo YS, Lo Sasso G, Stein S, Zhang H, Perino A, Lee JU, Zeviani M, Romand R, Hottiger MO *et al.* A SIRT7-dependent acetylation switch of GABP β 1 controls mitochondrial function. *Cell Metab* 2014;**20**:856–869.
- Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. *Nat Med* 2005;**11**:1306–1313.
- Sack MN, Finkel T. Mitochondrial metabolism, sirtuins, and aging. *Cold Spring Harb Perspect Biol* 2012;**4**:a013102.
- Said RS, El-Demerdash E, Nada AS, Kamal MM. Resveratrol inhibits inflammatory signaling implicated in ionizing radiation-induced premature ovarian failure through antagonistic crosstalk between silencing information regulator 1 (SIRT1) and poly (ADP-ribose) polymerase 1 (PARP-1). *Biochem Pharmacol* 2016;**103**:140–150.
- Santos L, Escande C, Denicola A. Potential modulation of sirtuins by oxidative stress. *Oxid Med Cell Longev* 2016;**2016**:9831825.
- Sato D, Itami N, Tasaki H, Takeo S, Kuwayama T, Iwata H. Relationship between mitochondrial DNA copy number and SIRT1 expression in porcine oocytes. *PLoS One* 2014;**9**:e94488.
- Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harb Perspect Biol* 2014;**6**:a018713.
- Scher MB, Vaquero A, Reinberg D. Sirt3 is a nuclear NAD⁺-dependent histone deacetylase that translocates to the mitochondria upon cellular stress. *Genes Dev* 2007;**21**:920–928.
- Schiedel M, Robaa D, Rumpf T, Sippl W, Jung M. The current state of NAD(+)—dependent histone deacetylases (Sirtuins) as novel therapeutic targets. *Med Res Rev* 2017;**38**:147–200.
- Schlicker C, Gertz M, Papatheodorou P, Kachholz B, Becker CF, Steegborn C. Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5. *J Mol Biol* 2008;**382**:790–801.
- Sebastián C, Satterstrom FK, Haigis MC, Mostoslavsky R. From sirtuin biology to human diseases: an update. *J Biol Chem* 2012;**287**:42444–42452.
- Seifert EL, Caron AZ, Morin K, Coulombe J, He XH, Jardine K, Dewar-Darch D, Boekelheide K, Harper ME, McBurney MW. Sirt1 catalytic activity is required for male fertility and metabolic homeostasis in mice. *FASEB J* 2012;**26**:555–566.
- Selesniemi K, Lee HJ, Muhlhauser A, Tilly JL. Prevention of maternal aging-associated oocyte aneuploidy and meiotic spindle defects in mice by dietary and genetic strategies. *Proc Natl Acad Sci USA* 2011;**108**:12319–12324.
- Seli E, Babayev E, Collins SC, Nemeth G, Horvath TL. Minireview: metabolism of female reproduction: regulatory mechanisms and clinical implications. *Mol Endocrinol* 2014;**28**:790–804.
- Shimazu T, Hirschev MD, Hua L, Dittenhafer-Reed KE, Schwer B, Lombard DB, Li Y, Bunkenborg J, Alt FW, Denu JM *et al.* SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production. *Cell Metab* 2010;**12**:654–661.
- Shirane A, Wada-Hiraike O, Tanikawa M, Seiki T, Hiraike H, Miyamoto Y, Sone K, Hirano M, Oishi H, Oda K *et al.* Regulation of SIRT1 determines initial step of endometrial receptivity by controlling E-cadherin expression. *Biochem Biophys Res Commun* 2012;**424**:604–610.
- Sirotkin AV, Dekanová P, Harrath AH, Alwasel SH, Vašíček D. Interrelationships between sirtuin 1 and transcription factors p53 and NF- κ B (p50/p65) in the control of ovarian cell apoptosis and proliferation. *Cell Tissue Res* 2014;**358**:627–632.
- Stankovic-Valentin N, Deltour S, Seeler J, Pinte S, Vergoten G, Guérardel C, Dejean A, Leprince D. An acetylation/deacetylation-SUMOylation switch through a phylogenetically conserved psiKXEP motif in the tumor suppressor HIC1 regulates transcriptional repression activity. *Mol Cell Biol* 2007;**27**:2661–2675.

- Strum JC, Johnson JH, Ward J, Xie H, Feild J, Hester A, Alford A, Waters KM. MicroRNA 132 regulates nutritional stress-induced chemokine production through repression of SirT1. *Mol Endocrinol* 2009;**23**:1876–1884.
- Suchankova G, Nelson LE, Gerhart-Hines Z, Kelly M, Gauthier MS, Saha AK, Ido Y, Puigserver P, Ruderman NB. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. *Biochem Biophys Res Commun* 2009;**378**:836–841.
- Sundaresan NR, Samant SA, Pillai VB, Rajamohan SB, Gupta MP. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. *Mol Cell Biol* 2008;**28**:6384–6401.
- Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A, Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J Clin Invest* 2009;**119**:2758–2771.
- Sundaresan NR, Bindu S, Pillai VB, Samant S, Pan Y, Huang JY, Gupta M, Nagalingam RS, Wolfgeher D, Verdin E et al. SIRT3 blocks aging-associated tissue fibrosis in mice by deacetylating and activating glycogen synthase kinase 3 β . *Mol Cell Biol* 2015;**36**:678–692.
- Taguchi A, Wada-Hiraike O, Kawana K, Koga K, Yamashita A, Shirane A, Urata Y, Kozuma S, Osuga Y, Fujii T. Resveratrol suppresses inflammatory responses in endometrial stromal cells derived from endometriosis: a possible role of the sirtuin 1 pathway. *J Obstet Gynaecol Res* 2014;**40**:770–778.
- Takeo S, Kawahara-Miki R, Goto H, Cao F, Kimura K, Monji Y, Kuwayama T, Iwata H. Age-associated changes in gene expression and developmental competence of bovine oocytes, and a possible countermeasure against age-associated events. *Mol Reprod Dev* 2013;**80**:508–521.
- Takeo S, Sato D, Kimura K, Monji Y, Kuwayama T, Kawahara-Miki R, Iwata H. Resveratrol improves the mitochondrial function and fertilization outcome of bovine oocytes. *J Reprod Dev* 2014;**60**:92–99.
- Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, Kim HS, Flynn CR, Hill S, Hayes McDonald W et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol Cell* 2010;**40**:893–904.
- Tao X, Zhang X, Ge SQ, Zhang EH, Zhang B. Expression of SIRT1 in the ovaries of rats with polycystic ovary syndrome before and after therapeutic intervention with exenatide. *Int J Clin Exp Pathol* 2015;**8**:8276–8283.
- Tatone C. Oocyte senescence: a firm link to age-related female subfertility. *Gynecol Endocrinol* 2008;**24**:59–63.
- Tatone C, Carbone MC, Gallo R, Delle Monache S, Di Cola M, Alesse E, Amicarelli F. Age-associated changes in mouse oocytes during postovulatory in vitro culture: possible role for meiotic kinases and survival factor BCL2. *Biol Reprod* 2006;**74**:395–402.
- Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, Artini PG, Piomboni P, Focarelli R. Cellular and molecular aspects of ovarian follicle ageing. *Hum Reprod Update* 2008;**14**:131–142.
- Tatone C, Di Emidio G, Vitti M, Di Carlo M, Santini S Jr, D'Alessandro AM, Falone S, Amicarelli F. Sirtuin functions in female fertility: possible role in oxidative stress and aging. *Oxid Med Cell Longev* 2015;**2015**:659687.
- Toth KF, Knoch TA, Wachsmuth M, Frank-Stohr M, Stohr M, Bacher CP, Muller G, Rippe K. Trichostatin A-induced histone acetylation causes decondensation of interphase chromatin. *J Cell Sci* 2004;**117**:4277–4287.
- Tremellen K. Oxidative stress and male infertility—a clinical perspective. *Hum Reprod Update* 2008;**14**:243–258.
- Tsai FC, Gardner DK. Nicotinamide, a component of complex culture media, inhibits mouse embryo development in vitro and reduces subsequent developmental potential after transfer. *Fertil Steril* 1994;**61**:376–382.
- Tsai YC, Greco TM, Cristea IM. Sirtuin 7 plays a role in ribosome biogenesis and protein synthesis. *Mol Cell Proteomics* 2014;**13**:73–83.
- Tseng AH, Shieh SS, Wang DL. SIRT3 deacetylates FOXO3 to protect mitochondria against oxidative damage. *Free Radic Biol Med* 2013;**63**:222–234.
- Turner BM. Nucleosome signalling: an evolving concept. *Biochim Biophys Acta* 2014;**1839**:623–626.
- Vachharajani VT, Liu T, Wang X, Hoth JJ, Yoza BK, McCall CE. Sirtuins link inflammation and metabolism. *J Immunol Res* 2016;**2016**:8167273.
- Valente S, Mellini P, Spallotta F, Carafa V, Nebbioso A, Polletta L, Carnevale I, Saladini S, Trisciuglio D, Gabellini C et al. 1,4-Dihydropyridines active on the SIRT1/AMPK pathway ameliorate skin repair and mitochondrial function and exhibit inhibition of proliferation in cancer cells. *J Med Chem* 2016;**59**:1471–1491.
- Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D. Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* 2004;**16**:93–105.
- Vaquero A, Scher M, Erdjument-Bromage H, Tempst P, Serrano L, Reinberg D. SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. *Nature* 2007;**450**:440–444.
- Vigodner M, Ishikawa T, Schlegel PN, Morris PL. SUMO-1, human male germ cell development, and the androgen receptor in the testis of men with normal and abnormal spermatogenesis. *Am J Physiol Endocrinol Metab* 2006;**290**:E1022–E1033.
- Vigodner M. Sumoylation precedes accumulation of phosphorylated H2AX on sex chromosomes during their meiotic inactivation. *Chromosome Res* 2009;**17**:37–45.
- Vigodner M, Morris PL. Testicular expression of small ubiquitin-related modifier-1 (SUMO-1) supports multiple roles in spermatogenesis: silencing of sex chromosomes in spermatocytes, spermatid microtubule nucleation, and nuclear reshaping. *Dev Biol* 2005;**282**:480–492.
- Wagner BA, Buettner GR, Burns CP. Free radical mediated lipid peroxidation in cells: oxidizability a function of cell lipid bis-allylic hydrogen content. *Biochemistry* 1994;**33**:4449–4453.
- Walker WH. Testosterone signaling and the regulation of spermatogenesis. *Spermatogenesis* 2011;**1**:116–120.
- Wang F, Nguyen M, Qin FX, Tong Q. SIRT2 deacetylates FOXO3a in response to oxidative stress and caloric restriction. *Aging Cell* 2007;**6**:505–514.
- Wang F, Tian X, Zhang L, He C, Ji P, Li Y, Tan D, Liu G. Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after in vitro fertilization. *Fertil Steril* 2014a;**101**:577–586.
- Wang H, Jo YJ, Oh JS, Kim NH. Quercetin delays postovulatory aging of mouse oocytes by regulating SIRT expression and MPF activity. *Oncotarget* 2017;**8**:38631–38641.
- Wang N, Luo LL, Xu JJ, Xu MY, Zhang XM, Zhou XL, Liu WJ, Fu YC. Obesity accelerates ovarian follicle development and follicle loss in rats. *Metabolism* 2014b;**63**:94–103.
- Wang Y. Molecular links between caloric restriction and Sir2/SIRT1 activation. *Diabetes Metab J* 2014;**38**:321–329.
- Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 2004;**430**:686–689.
- Wu CC, Bratton SB. Regulation of the intrinsic apoptosis pathway by reactive oxygen species. *Antioxid Redox Signal* 2013;**19**:546–558.
- Xiang Y, Xu J, Li L, Lin X, Chen X, Zhang X, Fu Y, Luo L. Calorie restriction increases primordial follicle reserve in mature female chemotherapy-treated rats. *Gene* 2012;**493**:77–82.
- Wątroba M, Szukiewicz D. The role of sirtuins in aging and age-related diseases. *Adv Med Sci* 2016;**61**:52–62.
- Yamakuchi M. MicroRNA regulation of SIRT1. *Front Physiol* 2012;**3**:68.
- Yamazaki Y, Usui I, Kanatani Y, Matsuya Y, Tsuneyama K, Fujisaka S, Bukhari A, Suzuki H, Senda S, Imanishi S et al. Treatment with SIRT1 activator, ameliorates fatty liver with reduced expression of lipogenic enzymes in MSG mice. *Am J Physiol Endocrinol Metab* 2009;**297**:E1179–E1186.
- Ye X, Li M, Hou T, Gao T, Zhu W, Yang Y. Sirtuins in glucose and lipid metabolism. *Oncotarget* 2017;**8**:1845–1859.
- Yin Y, Stahl BC, DeWolf WC, Morgentaler A. p53-mediated germ cell quality control in spermatogenesis. *Dev Biol* 1998;**204**:165–171.
- Yoo JY, Kim TH, Fazleabas AT, Palomino WA, Ahn SH, Tayade C, Schammel DP, Young SL, Jeong JW, Lessey BA. KRAS activation and over-expression of SIRT1/BCL6 contributes to the pathogenesis of endometriosis and progesterone resistance. *Sci Rep* 2017;**7**:6765.
- Yoshizawa T, Karim MF, Sato Y, Senokuchi T, Miyata K, Fukuda T, Go C, Tasaki M, Uchimura K, Kadomatsu T et al. SIRT7 controls hepatic lipid metabolism by regulating the ubiquitin-proteasome pathway. *Cell Metab* 2014;**19**:712–721.
- Yu J, Sadhukhan S, Noriega LG, Moullan N, He B, Weiss RS, Lin H, Schoonjans K, Auwerx J. Metabolic characterization of a Sirt5 deficient mouse model. *Sci Rep* 2013;**3**:2806.
- Zentner GE, Henikoff S. Regulation of nucleosome dynamics by histone modifications. *Nat Struct Mol Biol* 2013;**20**:259–266.

- Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Mol Endocrinol* 2001;**15**:172–183.
- Zhang FP, Pakarainen T, Zhu F, Poutanen M, Huhtaniemi I. Molecular characterization of postnatal development of testicular steroidogenesis in luteinizing hormone receptor knockout mice. *Endocrinology* 2004;**145**:1453–1463.
- Zhang XM, Li L, Xu JJ, Wang N, Liu WJ, Lin XH, Fu YC, Luo LL. Rapamycin preserves the follicle pool reserve and prolongs the ovarian lifespan of female rats via modulating mTOR activation and sirtuin expression. *Gene* 2013;**523**:82–87.
- Zhang H, Liu K. Cellular and molecular regulation of the activation of mammalian primordial follicles: somatic cells initiate follicle activation in adulthood. *Hum Reprod Update* 2015;**21**:779–786.
- Zhang J, Fang L, Lu Z, Xiong J, Wu M, Shi L, Luo A, Wang S. Are sirtuins markers of ovarian aging? *Gene* 2016a;**575**:680–686.
- Zhang L, Hou X, Ma R, Moley K, Schedl T, Wang Q. Sirt2 functions in spindle organization and chromosome alignment in mouse oocyte meiosis. *FASEB J* 2014;**28**:1435–1445.
- Zhang L, Han L, Ma R, Hou X, Yu Y, Sun S, Xu Y, Schedl T, Moley KH, Wang Q. Sirt3 prevents maternal obesity-associated oxidative stress and meiotic defects in mouse oocytes. *Cell Cycle* 2015a;**14**:2959–2968.
- Zhang L, Ma R, Hu J, Ding X, Xu Y. Sirtuin inhibition adversely affects porcine oocyte meiosis. *PLoS One* 2015b;**10**:e0132941.
- Zhang T, Zhou Y, Li L, Wang HH, Ma XS, Qian WP, Shen W, Schatten H, Sun QY. SIRT1, 2, 3 protect mouse oocytes from postovulatory aging. *Aging (Albany NY)* 2016b;**8**:685–696.
- Zhang Y, Zhang M, Dong H, Yong S, Li X, Olashaw N, Kruk PA, Cheng JQ, Bai W, Chen J *et al*. Deacetylation of cortactin by SIRT1 promotes cell migration. *Oncogene* 2009;**28**:445–460.
- Zhao F, Zhao W, Ren S, Fu Y, Fang X, Wang X, Li B. Roles of SIRT1 in granulosa cell apoptosis during the process of follicular atresia in porcine ovary. *Anim Reprod Sci* 2014;**151**:34–41.
- Zhao HC, Ding T, Ren Y, Li TJ, Li R, Fan Y, Yan J, Zhao Y, Li M, Yu Y *et al*. Role of Sirt3 in mitochondrial biogenesis and developmental competence of human in vitro matured oocytes. *Hum Reprod* 2016;**31**:607–622.
- Zhou XL, Xu JJ, Ni YH, Chen XC, Zhang HX, Zhang XM, Liu WJ, Luo LL, Fu YC. SIRT1 activator (SRT1720) improves the follicle reserve and prolongs the ovarian lifespan of diet-induced obesity in female mice via activating SIRT1 and suppressing mTOR signaling. *J Ovarian Res* 2014;**7**:97.
- Zini A, Libman J. Sperm DNA damage: clinical significance in the era of assisted reproduction. *CMAJ* 2006;**175**:495–500.