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# Energy sensors and reproductive hypothalamo-pituitary ovarian axis (HPO) in female mammals: Role of mTOR (mammalian target of rapamycin), AMPK (AMP-activated protein kinase) and SIRT1 (Sirtuin 1)

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1           **Energy sensors and reproductive hypothalamo-pituitary ovarian axis (HPO)**  
2           **in female mammals: role of mTOR (mammalian target of rapamycin), AMPK (AMP-**  
3           **activated protein kinase) and SIRT1 (Sirtuin 1)**

4

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21 **Abstract**

22           In female, energy metabolism influences reproductive function by modulating the  
23 Hypothalamic Pituitary Ovarian axis including the hypothalamic GnRH neuronal network, the  
24 pituitary gonadotropin secretion and the ovarian follicle growth and steroidogenesis. Several  
25 hormones and neuropeptides or metabolites are important signals between energy balance and  
26 reproduction. These energy sensors mediate their action on reproductive cells through specific  
27 kinases or signaling pathways. This review focuses on the role of three main enzymes-  
28 specifically, mTOR, AMPK, and SIRT1 at the hypothalamic pituitary and ovarian axis in  
29 normal female fertility and then we discuss their possible involvement in some women  
30 reproductive disorders known to be associated with metabolic complications, such as  
31 polycystic ovary syndrome (PCOS) and premature ovarian failure (POF).

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## 33        **1. Introduction**

34            In female animals, reproductive functions are highly dependent on successful  
35 regulation of energy metabolism. Indeed, the physiological processes including puberty,  
36 pregnancy and lactation have an important energetic costs. They are dependent on the cyclic  
37 production of sex hormones at different levels of the hypothalamus-pituitary-ovarian (HPO).  
38 This production relies on female ability in saving oxidizable fuels. Several examples in  
39 various animal species show this tight interconnection between energy metabolism and  
40 reproduction. Over 2000 years ago, Hippocrates observed that hard-working slim servant  
41 women were more fertile than their overfed and sedentary Roman employers (cited by  
42 (Franks, et al. 1996). More recently, large literature reported relationships between nutritional  
43 status and/or diet and fertility/infertility in agronomic species (sheep, cattle, chicken...),  
44 rodents and human. In females, when this physiological balance between reproduction and  
45 metabolism is disrupted reproductive disorders occur. In cattle selected for high milk  
46 production, negative energy balance in the post-partum period is associated with reduced  
47 fertility (Wathes, et al. 2007). In women, obesity and anorexia, which are an important health  
48 issue nowadays, play a significant role in reproductive disorders (Gambineri, et al. 2019,  
49 Pinheiro, et al. 2007) . They are associated with anovulation, menstrual disorders, infertility,  
50 difficulties in assisted reproduction, and adverse pregnancy outcomes. Moreover, clinical  
51 studies in women demonstrated associations between obesity, insulin resistance and  
52 polycystic ovarian syndrome (PCOS) (Shang, et al. 2020, Zeng, et al. 2020). PCOS represents  
53 the most common hormonal abnormality in reproductive-age women affecting about 10% of  
54 this population (Teede, et al. 2018b). It is a set of reproductive and metabolic symptoms  
55 associated with an imbalance of reproductive hormones. Premature ovarian failure (POF) is  
56 another reproductive disorder that can be associated with metabolic complications (Wang et  
57 al., 2014).

58 Energy metabolism may influence reproductive axis through different signals : hormone  
59 including Insulin-like Growth Factor-1 (IGF-1)/insulin, adipokines (leptin, adiponectin and  
60 new emerging adipokine such as chemerin...), metabolites (glucose, lipid, amino acid) and  
61 neuropeptides. These signals through modulation of various signaling pathways including  
62 mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and Sirtuin  
63 1 (SIRT1) are able to regulate HPO axis (McIlwraith and Belsham 2020). Many studies have  
64 highlighted the importance of mTOR, AMPK and SIRT1 in a wide range of human diseases  
65 like type 2 diabetes, obesity and metabolic syndrome (Boutouja, et al. 2019, Kitada, et al.  
66 2019, Madhavi, et al. 2019). More recently, there are a large number of researches on these  
67 three main signaling pathways related to fertility and reproductive disorders (Correia, et al.  
68 2020, Nguyen 2019, Tatone, et al. 2018). In this review, we will summarize the physiological  
69 regulatory roles of mTOR, AMPK and SIRT 1 in female at hypothalamic, pituitary and  
70 ovarian levels to provide molecular insight of these signaling pathways in female fertility.  
71 Finally, we will discuss their potential role in women reproductive disorders including PCOS  
72 and POF.

## 73 **2. mTOR**

74 mTOR was first discovered as the cellular target of rapamycin (Brown, et al. 1994), a  
75 Streptomyces–derived compound which is currently used clinically as an immunosuppressive  
76 drug. mTOR is modulated by a wide range of nutrients (especially amino acids), growth  
77 factors, hormones and stressors, with an essential role in the control of cell proliferation,  
78 growth and metabolism (Martinez de Morentin, et al. 2014, Xu, et al. 2020) (**Figure 1A**).

### 79 **2.1 Structure of mTOR**

80 The protein mTOR is a serine/threonine kinase highly conserved that belongs to the family of  
81 phosphatidylinositol-3-kinase (PI3K) -related kinase (PIKK). As recently reviewed, it is  
82 composed of several domains (Xu, et al. 2020). Moreover, the TOR system includes two  
83 structurally and functionally distinct multi-molecular complexes: mTOR complex 1  
84 (**mTORC1**) and mTOR complex 2 (**mTORC2**) that are composed by several regulatory  
85 proteins (Figure 1A) (Xu, et al. 2020). MTORC1 is mainly involved in the regulation of  
86 cellular anabolic processes, such as protein synthesis and lipid synthesis, to promote cell  
87 metabolism and cell growth (**Figure 1A**). Activation of this complex can be carried out in  
88 different ways: (1) by signaling pathways involving PI3K / Akt / TSC1-TSC2 (tuberous  
89 sclerosis protein 1 and 2) and Ras / Raf / Extracellular Regulated Kinase (ERK), which are  
90 often associated with tyrosine kinase receptors (e.g. IGF-1, insulin and Epidermal Growth  
91 Factor (EGF) receptors); (2) by the availability of the cell in certain amino acids, such as  
92 leucine or arginine. Conversely, an energy deficit (lack of ATP) activates the AMPK protein  
93 which phosphorylates the mTORC1 complex and inhibits it. Thus, AMPK is also a key  
94 energy sensor as described in the present review (section 3).

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96 The mTORC2 complex (**Figure 1A**) is less well understood than mTORC1, although it is  
97 known to be activated in a PI3K-dependent fashion and it thus is also responsive to growth  
98 factors. Furthermore, mTORC2 regulates cell survival and growth (Laplane and Sabatini  
99 2012) and it has also an indirect role in metabolism, not only in terms of mitochondrial  
100 physiology (Betz, et al. 2013) but also by being able to control glucose metabolism in AKT-  
101 dependent (Masui, et al. 2014) and FOXO-dependent (Masui, et al. 2013) manners. Finally,  
102 mTORC2 is also able to modulate the hexosamine biosynthetic pathway, thus affecting  
103 protein and lipid glycosylation, as well as glutamine metabolism both involved in anabolic  
104 processes (Moloughney, et al. 2016).

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## 2.2 Hypothalamic mTOR and female reproduction

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mTOR is highly expressed in the hypothalamus (Cota, et al. 2006). In the hypothalamic arcuate nucleus (ARC), mTOR plays an important role in sensing nutrient (mainly amino acids) availability and mediating the anorectic effects of leptin (Cota, et al. 2006). At the reproductive level, intracerebroventricular administration of rapamycin in rats inhibits the puberty onset (**Figure 2**). Indeed, in these animals, plasma LH and estradiol levels are decreased, vaginal opening is delayed, and ovaries and uterus are atrophied (Roa, et al. 2009). At the molecular levels, rapamycin suppressed ARC Kiss1 levels (Roa, et al. 2009)(**Figure 2**). The *Kiss1* gene encodes Kisspeptins that are a family of hypothalamic peptides (Ruohonen, et al. 2020). They are neuromodulators that act upstream of GnRH, and they are sensitive to sex steroid feedback and metabolic cues (Ruohonen, et al. 2020). Kisspeptins are now recognized as crucial regulators of the onset of puberty, the regulation of sex hormone-mediated secretion of gonadotrophins, and the control of fertility (Pinilla, et al. 2012). Inactivation of mTOR was also shown to diminish the positive effect of leptin on puberty onset in food-restricted females demonstrating that this mTOR pathway plays a critical role in the neuroendocrine control (Roa, et al. 2009).

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Interestingly, mTOR signaling seems to regulate Kiss1 neurons indirectly, since pS6 (the downstream of mTOR and S6K1) is not expressed in Kiss1 neurons (Quennell, et al. 2011). Although the significant effects of rapamycin on puberty onset were determined in animal experiments, patients clinically treated with rapamycin before menarche were found to have a similar menarche time as patients administered rapamycin post-menarche (Sparagana, et al. 2017). The difference could be explained by the fact that rapamycin administered orally has little effects on mTOR signaling in the brain (Zhang, et al. 2014), although rapamycin is

130 believed to cross the blood–brain barrier (Franz, et al. 2006).The hypothalamus is a region  
131 that integrates different signals that regulate appetite, fertility and thermogenesis. At this  
132 level, the neurons with neuropeptide Y (NPY) which intervene in the regulation of food intake  
133 but also inhibit the activity of neurons with GnRH, are sensitive to the mTOR pathway. In  
134 organotypic cultures of hypothalamic explants, inhibition of the mTOR pathway by  
135 rapamycin leads to an increase in the level of NPY transcripts (Shimizu, et al. 2010). Other  
136 neuropeptides involved in the metabolic control of the HPO including neurokinin B and the  
137 products of POMC neurons (Manfredi-Lozano, et al. 2018) could be *in vivo* regulated by  
138 mTOR signaling. It therefore seems that, in the central structures regulating the onset of  
139 puberty and / or the pulsatility of gonadotropic hormones, inhibition of mTOR leads to  
140 deregulation of the Kiss/GnRH system. In addition, recently PTEN deletion specifically in  
141 kisspeptin-expressing cells resulted in a female-specific hyperactivation of mTOR signaling  
142 in anteroventral periventricular and ARC kisspeptin neurons (Negron, et al. 2020). PI3K-  
143 mTOR hyperactivity was associated with higher hypothalamic kisspeptin protein expression  
144 and higher plasma LH levels in fasted females compared to controls. This deletion of the *Pten*  
145 gene specifically in kisspeptin-expressing cells results in a brain region-specific hypertrophy,  
146 accompanied by decreased fertility in females and reduced gonadotropin responses to  
147 gonadectomy in both sexes. PTEN acts both as a lipid and protein phosphatase and as a  
148 regulator of several signaling cascades, most notably as the direct negative regulator of  
149 phosphatidylinositol 3-kinase (PI3K). PI3K activates downstream molecules like the  
150 serine/threonine-specific protein kinase, Akt, and the mammalian target of rapamycin  
151 (mTOR) in response to insulin or leptin (Tsou and Bence 2012).

152 Altogether, these observations suggest that modulations of mTOR signaling in the brain  
153 are indispensable for proper functioning of the HPO axis and fertility.

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### 2.3 Role of mTOR in pituitary and female reproduction

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The mTOR protein is also expressed and active in normal pituitary cells and pituitary adenomas (Sajjad, et al. 2013), for review (Monsalves, et al. 2014). Several studies have examined the antiproliferative properties of rapamycin treatment in pituitary and pituitary adenomas cells. In normal rat pituitary cells, rapamycin inhibits basal proliferation and insulin-, cAMP-, and estradiol-induced proliferation of cells (Kawashima, et al. 2000) (**Figure 2**). Using human pituitary anterior cells in primary culture it has also demonstrated that rapamycin induces mTOR inhibition in mTOR-active GH- and ACTH cells (Sajjad, et al. 2013). Human PRL gene is expressed in the GH3 cell line and its transcription was shown to be inhibited by rapamycin (Wera, et al. 1995). In an animal model using rats that carry an inactivating germline mutation of the *TSC2* gene that results in pituitary tumor formation, rapamycin induced regression of the pituitary tumors and a concomitant decrease in the levels of phosphorylated-S6 (the target of p70S6K; (Kenerson, et al. 2005).

In vitro experiments on a mouse pituitary cell line have shown that the stimulation of protein synthesis induced by GnRH is inhibited by rapamycin (Sosnowski, et al. 2000) (**Figure 2**). Using the L $\beta$ T2 gonadotrope cell line it has been demonstrated a specific role for mTORC2 in regulating membrane remodeling events (Edwards, et al. 2017). More precisely, pharmacological inhibition of mTORC2-blunted GnRH-mediated actin reorganization and similarly attenuated activation of ERK and LH $\beta$  gene expression (Edwards, et al. 2017) (**Figure 2**). Thus, mTORC2 is an important signaling intermediate in regulating membrane remodeling events and subsequent MAPK activation in gonadotrop cells.

### 2.4 Role of mTOR in ovary

180 The mTOR protein is expressed in most ovarian cells, with higher levels in the surface  
181 epithelium of the ovary, in granulosa cells and in immature oocytes. Granulosa cells are  
182 somatic cells that surround the follicle; they are steroidogenic and allow follicular growth and  
183 maturation. The outermost cells follicle, theca cells, androgens, precursors estradiol  
184 synthesized by granulosa cells. Phosphorylated mTOR (serine 2448) is strongly enriched  
185 within mitotic granulosa cells.

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### 187 ***Proliferation***

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189 In rat, the activated mTOR pathway stimulates proliferation (FSH-induced) granulosa  
190 cells (Kayampilly and Menon 2007, Yu, et al. 2011) and that of theca cells (induced by LH  
191 and insulin) (Palaniappan and Menon 2012) (**Figure 2**). Inhibition of mTORC1 in theca  
192 primary cells induces a decrease in proliferation markers expression, such as PCNA  
193 (proliferating cell nuclear antigen) or cyclin D3, despite the induction of proliferation by hCG  
194 (human chorionic gonadotropin) which mimics the effect of the LH (Palaniappan and Menon  
195 2010). In addition, intraperitoneal injection of rapamycin in mice increases abnormal mitosis  
196 of granulosa cells preantral follicles (Yu, et al. 2011). The abnormal proliferation of these cells  
197 leads to a proportional decrease at the dose used of ovulated follicles. However, these follicles  
198 do not show any functional abnormalities *in vitro* ; oocytes can be fertilized and develop up to  
199 the blastocyst stage (Yu, et al. 2011). Pharmacological study in rats showed that while causing  
200 an accumulation of primordial follicles, long-term rapamycin treatment also resulted in  
201 reduced antral follicles and ovulation rates. In mice, rapamycin-sensitive mTORC1 signaling  
202 is also involved in physiological primordial follicle activation in mouse ovary (Tong, et al.  
203 2013). Short-term rapamycin treatment increases ovarian lifespan in young and middle-aged  
204 female mice (Dou, et al. 2017). Culture of human ovarian cortex is also sensitive to

205 rapamycin (McLaughlin, et al. 2011). Indeed, the addition rapamycin during the six days of  
206 culture inhibits follicle growth and increases the number of small or even oocyte-free follicles  
207 (McLaughlin, et al. 2011). This inhibition is not correlated with an increase apoptosis, but a  
208 decrease in cell proliferation. Thus, mTOR regulates cell growth in ovarian cells.

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### 210 *Steroidogenesis*

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212 The mTOR signaling also participates in the regulation of ovarian steroidogenesis. In  
213 2004, Alam et al. demonstrated that differentiation granulosa cells, induced by FSH involves  
214 the PI3K / mTOR pathway (Alam, et al. 2004). Indeed, the addition rapamycin to primary cell  
215 cultures of granulosa from prepubertal rats inhibits expression of proteins involved in  
216 granulosa cell differentiation: inhibin alpha, VEGF (vascular endothelial growth factor) and  
217 the LH receptor. Subsequently, numerous works completed this study demonstrating the  
218 involvement of the mTOR pathway in steroidogenesis (induced by the combination FSH  
219 +TGFbeta1) of granulosa cells from cultured rats *in vitro*. However, in other study, mTOR is  
220 not involved in the synthesis of estradiol triggered by simple stimulation by FSH. In women  
221 as in rodents, Cure et al. does show no change in synthesis estradiol following treatment with  
222 sirolimus and tacrolimus (Cure, et al. 2004).

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224 The use of transgenic animal models has improved the understanding of the mTOR  
225 role *in vivo* in ovary (**Table 1**). Indeed, specific deletion of Rictor (rapamycin-insensitive  
226 companion of mTOR, a key component of mTORC2) in oocyte of primordial follicles leads to  
227 massive follicular death, excessive loss of functional ovarian follicles and abnormal gonadal  
228 hormone secretion (Chen, et al. 2015). Furthermore, deletion of TSC1 or TSC2 (tumor  
229 suppressor tuberous sclerosis complex 1), negative regulators of mTORC1 activity, promotes

230 the growth of all primordial follicles in neonatal animals, leading to the exhaustion of the  
231 entire follicle pool, followed by a premature ovarian failure phenotype (Adhikari, et al. 2009,  
232 Adhikari, et al. 2010). Specific disruption of TSC1 in granulosa cells of secondary follicles  
233 improves the follicle growth, leading to increased ovulatory capacity and delivery of more  
234 pups followed by a premature ovarian failure phenotype (Huang, et al. 2013). At the  
235 molecular level, a recent paper identified miR-92b-3p as a novel regulator of primordial  
236 follicle assembly by negatively regulating TSC1 in mTOR/Rps6 signaling (Li, et al. 2019).  
237 Also, the caseinolytic peptidase P (Clpp) could be a negative regulator of mTOR signaling in  
238 the ovary since Clpp  $-/-$  mice ovaries show accelerated depletion of follicular reserve,  
239 associated with mTOR pathway activation (Wang, et al. 2018).

### 240 **3. AMPK**

241 As is the case for mTOR, other cellular energy-sensing systems participate in the control  
242 of the female reproduction through the HPO axis. For example, AMPK, a member of the  
243 metabolite-sensing protein kinase family, also contributes to the metabolic gating of fertility  
244 (Bertoldo, et al. 2015a, Tosca, et al. 2008). In addition, it is well known that AMPK is able to  
245 interfere with mTOR activation (Gwinn, et al. 2008, Xu, et al. 2012). Thus, it is somewhat  
246 predictable that the above described phenomena concerning mTOR could involve also  
247 changes in AMPK signaling.

#### 248 **3.1 Structure of AMPK**

249 AMPK is a highly conserved heterotrimeric serine/threonine kinase (Carling, et al. 2012).  
250 (**Figure 1B**). AMPK is regarded as the genuine (and indispensable) cellular energy sensor. In  
251 fact, in contrast to other putative metabolic gauges, AMPK is able to directly sense changes in  
252 availability of the real energy sources of the cells, namely, adenine nucleotides. Any changes

253 that affect intracellular ATP levels, such as glucose deprivation, hypoxia, ischemia, and  
254 oxidative stresses, will activate AMPK. A number of hormones that regulate energy  
255 homeostasis (leptin, ghrelin, adiponectin, and resistin) have also been shown to exhibit tissue-  
256 specific stimulation or inhibition of AMPK kinase (for review (Kahn, et al. 2005)) (**Figure**  
257 **1B**).

### 258 **3.2 Hypothalamic/brain AMPK and female reproduction**

259 Besides this ubiquitous role at the cellular level, and as described earlier for the mTOR  
260 pathway, brain AMPK signaling plays also a relevant role in the central control energy  
261 balance and food intake. As expected for a signaling system that becomes activated in  
262 conditions of energy shortage, activation of AMPK stimulates appetite (Kahn, et al. 2005), as  
263 a mean to increase calorie incorporation. In keeping with such function, leptin suppresses  
264 hypothalamic AMPK activity, while ghrelin stimulates it (Kahn, et al. 2005). In fact, AMPK  
265 and mTOR are mutually regulated; for instance, AMPK can inhibit mTOR signaling in  
266 different cell systems (Inoki, et al. 2003). Hence, these two metabolic cell sensors have been  
267 proposed to act as functional antagonists, so that they reciprocally cooperate in the central  
268 control of energy homeostasis. Considering the reciprocal regulation and function of mTOR  
269 and AMPK in brain centers to regulate metabolic homeostasis, it is tempting to hypothesize  
270 that this interaction may also be relevant for the dynamic modulation of the HPO related to  
271 fertility.

272 Thus, activation of AMPK has been shown to inhibit GnRH secretion in the mouse  
273 hypothalamic GnRH neuronal cell line (GT1-7 cells) *in vitro*, where it may act as transducer  
274 of the effects of globular adiponectin (Coyral-Castel, et al. 2008) (Wen, et al. 2008) (**Figure**  
275 **2**). In addition, adiponectin has been shown to inhibit *Kiss1* gene expression in GT1-7 cells  
276 apparently via activation of AMPK (Wen, et al. 2012). In addition, activation of hypothalamic

277 AMPK *in vivo* after AICAR intracerebro-ventricular injection has been shown to alter estrous  
278 cyclicity in adult female rats, thus resulting in the lowering of the time interval between  
279 consecutive estrous cycles (Coyral-Castel, et al. 2008). More recently, Roa et al., 2018  
280 showed that hypothalamic AMPK signaling plays a key role in the metabolic control of  
281 puberty acting via a repressive modulation of ARC Kiss1 neurons in conditions of negative  
282 energy balance in female rodents (Roa, et al. 2018) (**Table 1**). Other studies show that  
283 malnutrition-induced AMPK activation of ependymocytes of the lower brainstem might be  
284 involved in suppression of GnRH/LH release and then gonadal activities (Minabe, et al.  
285 2015). In addition, a recent study shows that hindbrain AMPK mediates short-term food  
286 deprivation inhibition of the GnRH/LH axis (Shakya, et al. 2018).

287 Thus, these findings are suggestive of a role of brain AMPK in the metabolic control  
288 of reproduction, and are compatible with a predominant inhibitory role of AMPK pathways in  
289 the central regulation of the HPO axis. Such inhibitory action is in agreement with the role of  
290 AMPK as sensor of energy insufficiency and functional antagonist of mTOR.

### 291 **3.3 AMPK and pituitary**

292 In rat, the different subunits of AMPK are expressed in pituitary. In particular, AMPK  
293 alpha 1 is detected mainly in gonadotrophs and thyrotrophs, is less abundant in lactotrophs  
294 and somatotrophs, and is undetectable in corticotrophs (Tosca, et al. 2011). In rat pituitary  
295 cells, metformin-induced AMPK activation decreases gonadotropin secretion and MAPK  
296 ERK1/2 phosphorylation induced by GnRH and FSH release, FSHbeta subunit expression,  
297 and SMAD2 phosphorylation induced by activin (Tosca, et al. 2011) (**Figure 2**). In LbetaT2  
298 gonadotropes, adiponectin-induced AMPK activation also decreases LH secretion (Lu, et al.  
299 2008). In a good agreement with a suppressive effect of AMPK activation in gonadotrop cells,

300 a recent study shows that AMPK activation reduces the transcriptional activity of the murine  
301 luteinizing hormone  $\beta$ -subunit gene (Moriyama, et al. 2020) (**Figure 2**).

### 302 **3.4 AMPK and ovarian functions**

303 AMPK protein exists in different mammalian ovarian cells, including oocytes, granulosa  
304 cells, theca cells, and corpus luteum (Tosca, et al. 2007, Tosca, et al. 2005). Several data show  
305 that AMPK is involved in the regulation of primordial follicle activation (Lu, et al. 2017) and  
306 also in different biological functions of ovarian cells. In situ ovarian intrabursal administration  
307 of AMPK inhibitor Compound C in mice, primordial follicle activation and stimulation of  
308 follicle growth were observed, and thus more healthy pups were delivered (Lu, et al. 2017).  
309 AMPK inhibition by Compound C promotes the growth of cultured ovary in vitro by  
310 activating mTOR and increasing the expression of Ctgf (connective tissue growth factor) in  
311 the YAP (hippo signaling Yes-Associated Protein) signaling pathway (Lu, et al. 2017).

### 312 *Proliferation/apoptosis*

313 AMPK participates in regulating balance between ovarian cells proliferation and  
314 apoptosis (**Figure 2**). As showed metformin inhibited proliferation by AMPK in response to  
315 IGF-1 in bovine granulosa cells (Tosca, et al. 2010). Whereas, in rat granulosa cells FSH  
316 stimulated proliferation by reducing p27 via AMPK inhibition (Kayampilly and Menon  
317 2009). Additionally, authors observed that dihydrotestosterone decreased mitogenesis by  
318 AMPK activation. Furthermore, inhibition of AMPK by compound C reversed the DHT-  
319 mediated reduction in positive cell cycle regulator, cyclin D2, and 5-bromo-2'-deoxyuridine  
320 incorporation (Kayampilly and Menon 2012). On the other hand, Tosca et al. (2005) did not  
321 show essential effect of AMPK on rat granulosa cell proliferation (Tosca, et al. 2005). Some  
322 experiment about AMPK involvement in metformin effect on cell proliferation was done also

323 in rat theca interna cells and showed that metformin inhibited insulin-induced proliferation  
324 and the up-regulation of the cell cycle regulatory proteins, cyclin D3 and CDK4 by activation  
325 AMPK. Effect was reversed with the addition of AMPK inhibitor, compound C (Will, et al.  
326 2012). Thus, most of the studies show that modulation of AMPK activation is involved in the  
327 regulation of granulosa and theca cell proliferation in response to various factors.

### 328 *Steroidogenesis*

329 AMPK action on ovarian steroidogenesis was described by several papers (**Figure 2**).  
330 Literature showed that AMPK inhibits progesterone (P4) production by decreasing 3 $\beta$ -  
331 Hydroxysteroid dehydrogenase (HSD3B) mRNA and protein expression, with no effect on  
332 oestradiol (E2) secretion in rat granulosa cells (Tosca, et al. 2005). Beside metformin-  
333 induced P4 inhibition and decreasing in HSD3B, CYP11A1 (Cytochrome P450 Family 11  
334 Subfamily A Member 1) and STAR (steroidogenic acute regulatory protein) were mediated  
335 by AMPK activation in primary rat granulosa cells (Tosca, et al. 2006). Moreover, P4 and E2  
336 production by bovine granulosa cells was decreased in response to metformin via AMPK  
337 pathway (Tosca, et al. 2007). Additionally, AMPK activation could be involved in the  
338 negative effects of one adipokine called apelin on steroidogenesis in porcine ovarian follicles  
339 (Rak, et al. 2017) and those of vitamin D3 in cultured granulosa cells of  
340 dehydroepiandrosterone-induced PCOS mice (Bakhshalizadeh, et al. 2018). Thus, in most of  
341 species, AMPK activation is associated to a negative effect on the *in vitro* ovarian  
342 steroidogenesis.

### 343 *Oocyte maturation*

344 Some evidence indicated a key role of AMPK action in oocyte maturation (**Figure 2**).  
345 AMPK activation by AICAR induced oocyte maturation in mice (Chen, et al. 2006). In



346 contrast, AMPK activation by metformin or AICAR caused oocyte arrest in germinal vesicle  
347 stage in porcine and bovine cumulus-oocyte complexes (COCs), but only in the presence of  
348 cumulus cells (Mayes, et al. 2007). Cumulus cells are necessary for promoting the inhibitory  
349 effect of AMPK activation on the recovery of swine and bovine oocyte meiosis. At the  
350 opposite, in mice, phospho-AMPK provides a meiotic maturation signal in mouse COCs and  
351 induces oocyte maturation. This discrepancy shows that the role of AMPK in follicular  
352 development is species dependent. Thus, the species difference in AMPK regulation in oocyte  
353 maturation is worth in-depth studies.

#### 354 **4. SIRT1**

355 Several reports have suggested that AMPK interacted with the NAD<sup>+</sup>-dependent  
356 deacetylase, SIRT1, another enzyme crucial in the regulation of glucose metabolism (Canto,  
357 et al. 2009, Silvestre, et al. 2014). AMPK and SIRT1 share many target molecules and  
358 regulate each other, which suggests that both enzymes cooperate in a cycle (Ruderman, et al.  
359 2010). AMPK $\alpha$  elevates the expression of SIRT1 by up-regulating the intracellular levels of  
360 its co-substrate NAD<sup>+</sup> or the activity of nicotinamide (Canto, et al. 2009). Similarly, SIRT1  
361 can activate AMPK via deacetylation of LKB1, which promotes LKB1 translocation from the  
362 nucleus to the cytosol, where it is activated and phosphorylates and activates AMPK  
363 (Rogacka, et al. 2018). Under stress conditions, SIRT1 cooperates with AMPK in order to  
364 restore energy balance or promoting cell death (Fulco and Sartorelli 2008). Concerning the  
365 reproductive process, our laboratory showed that AMPK/SIRT1 signalling pathway was  
366 involved in the regulation of visfatin expression in response to metformin (Reverchon, et al.  
367 2013).

368

#### 369 **4.1 Structure of SIRT1**

370 Sirtuins are part of the family of class 3 histone deacetylases (HDACs), which, unlike  
371 class 1 and 2 HDACs, use the cofactor NAD + (nicotinamide adenine dinucleotide) as  
372 substrate. In mammals, the sirtuins family includes seven isoforms (SIRT1 to 7) (Haigis and  
373 Sinclair 2010). Among the seven sirtuins, SIRT1 is the most studied and well-characterized  
374 sirtuin with respect to its physiological functions. Several studies have suggested that  
375 resveratrol is an agonist of sirtuin type 1 (SIRT1) and exerts a wide variety of beneficial  
376 effects in a SIRT1-dependent manner to prevent the development of many illnesses, through,  
377 e.g., antidiabetic, antioxidant and antiinflammatory approaches (**Figure 1 C**). SIRT1 is  
378 ubiquitously expressed and has many important functions in peripheral metabolic tissues,  
379 including adipose tissue, liver, and muscle, that underlie its role in sensing energy balance  
380 (Boutant and Canto 2016). Indeed, SIRT1 is activated during fasting or caloric restriction to  
381 increase fatty acid oxidation and gluconeogenesis and suppress insulin secretion, insulin  
382 action, and adipogenesis (**Figure 1 C**). Like AMPK and mTOR, SIRT1 functions as a cellular  
383 energy sensor. In addition to its well-characterized effects in peripheral tissues, evidence  
384 suggests that it plays a role in the interactions between metabolism and reproductive  
385 functions.

386

#### 387 **4.2 SIRT1 and hypothalamic function in reproduction**

388 SIRT1 is expressed in different neurons involved in the metabolism regulation in  
389 hypothalamus (Yamamoto and Takahashi 2018). Concerning the reproductive function,  
390 SIRT1 knockout mice exhibit a diminished hypothalamic GnRH expression and in turn  
391 reduced serum LH and FSH levels and spermatogenesis arrest, suggesting an important role  
392 of SIRT1 in the HPG axis (Kolthur-Seetharam, et al. 2009). SIRT1 is expressed in  
393 hypothalamic Kiss1 neurons and suppresses Kiss1 expression (Vazquez, et al. 2018). Thus, in

394 female, it acts as a molecule that restrains female puberty via epigenetic repression of the  
395 puberty-activating gene, *Kiss1* (Vazquez, et al. 2018). Using an inducible, conditional  
396 deletion of the SIRT1 deacetylase domain or conditional overexpression of wild-type SIRT1  
397 in astrocytes, Choi et al recently showed that SIRT1 regulates female reproductive functions  
398 (Choi, et al. 2019). Indeed, astrocyte SIRT1 promotes and lack of SIRT1 impairs estrus  
399 cycles. SIRT1 inactivation reduces ovary size and number of corpora lutea and a greater  
400 proportion of SIRT1 overexpressing mice showed LH surges. Regarding potential central  
401 actions of SIRT1 in the control of the reproductive axis, mice with congenital *Sirt1* deficiency  
402 displayed central hypogonadism due to defective GnRH neuronal migration to the  
403 hypothalamus (Di Sante, et al. 2015). Thus, hypothalamic SIRT1 seems to play an important  
404 role in the regulation of female fertility.

405

### 406 **4.3 SIRT1 and gonadotropin expression and secretion**

407

408 SIRT1 KO mice have smaller pituitary than wild-type mice (Lemieux, et al. 2005). In  
409 normal animals, the distribution of SIRT1 among the hormone-secreting pituitary cells in  
410 mice and humans demonstrates that SIRT1 is abundantly localized in TSH-positive cells but  
411 is barely detectable in the ACTH-, FSH-, LH/GH-, or prolactin-positive cells (Akieda-Asai, et  
412 al. 2010). GnRH treatment decreases SIRT1 level via the miR-132/212 induction in the  
413 pituitary. This results in the downregulation of SIRT1-dependent FOXO1 deacetylation and a  
414 decrease in the FOXO1-mediated inhibition of *Fsh $\beta$*  transcription that ultimately increases the  
415 *Fsh $\beta$*  expression in rat primary pituitary cells and L $\beta$ T2 cell line (Lannes, et al. 2015).

416

### 417 **4.4 SIRT1 and ovarian functions**

418 SIRT1 is involved in control of basic ovarian functions (see (Tatone, et al. 2015) for  
419 review). In ovary, SIRT1 is expressed in stroma, large ovarian follicles, particularly in the  
420 oocyte and granulosa cells (Morita, et al. 2012), and loss of SIRT1 causes small ovaries with  
421 early-stage follicular development but no evidence of ovulation (Tatone, et al. 2015). Indeed,  
422 SIRT 1 overexpression is associated with promotion of mice ovarian folliculogenesis and  
423 fecundity (Long, et al. 2019), while its deletion has an opposite effect (Tatone, et al. 2018). In  
424 human follicle, SIRT1 is detected in follicular fluid, and its concentration is independent of its  
425 serum levels. In addition, ovarian hyperstimulation resulted in significantly higher serum  
426 SIRT1 levels in pregnant women compared with non-pregnant women suggesting a role of  
427 ovarian SIRT1 in the reproductive success (Bodis, et al. 2019).

#### 428 *Proliferation/apoptosis*

429 The amount of SIRT1s in ovarian cells is associated with their state and health. Atresia of  
430 porcine ovarian follicles was associated with a decrease in SIRT1 expression (Zhao, et al.  
431 2014). Zhang et al. reported that the expression levels of SIRT1 were decreased in the ovaries  
432 of aged mice and mice treated with chemotherapy, but increased in calorie-restricted mice  
433 (Zhang, et al. 2016). The transfection-induced overexpression of SIRT1 in cultured porcine  
434 ovarian granulosa cells reduces their proliferation, increases insulin-like growth factor I (IGF-  
435 I) release and modified the response of granulosa cells to exogenous follicle-stimulating  
436 hormone (Pavlova, et al. 2013, Sirotkin, et al. 2014). In human luteinized granulosa cells,  
437 SIRT1 also play an anti-apoptotic role (Han, et al. 2017). Recently, Luo et al showed that  
438 overexpression of miR-23a inhibits the expression of SIRT1 and increases apoptosis in  
439 human granulosa cells (Luo, et al. 2019).

440

#### 441 *Steroidogenesis*

442 SIRT1 plays a role in the activation of steroidogenesis associated with luteinization  
443 and the terminal differentiation of rat granulosa cells (Morita, et al. 2012). Thus, stimulation  
444 of SIRT1 by resveratrol could potentially be beneficial in the treatment of luteal phase  
445 deficiency. Studies in human luteinized granulosa cells and KGN cells showed that SIRT1  
446 signalling is involved in the response of ovarian cells to the insulin sensitizer metformin  
447 (Reverchon, et al. 2013). In cultured bovine granulosa cells, visfatin improves basal and  
448 IGF1-induced steroidogenesis and IGF1 receptor signaling through SIRT1 (Reverchon, et al.  
449 2016).

450

#### 451 ***Oocyte and Oxydative stress***

452 SIRT 1 is expressed in mouse ovulated metaphase II (MII) oocytes, and its expression  
453 gradually decreases upon fertilization until the blastocyst stage suggesting that SIRT1 is  
454 supposed to be relevant to oogenesis rather than fertilization and early embryo development.  
455 SIRT1 mRNA levels are significant decreased in MII oocytes aged *in vivo* or *in vitro*, when  
456 compared to fresh MII oocytes (Ma, et al. 2015). More recently, Ma et al., 2018 showed that a  
457 SIRT1/Nrf2 (Nuclear factor erythroid 2-related factor 2) pathway is involved in oocyte aging  
458 by regulating Cyclin B1 (Ma, et al. 2018). In mouse GV and MII oocytes, SIRT1 expression  
459 has been associated with changes in chromatin configuration (Manosalva and Gonzalez  
460 2010), oxidative stress response, reproductive aging, and postovulatory aging (Di Emidio, et  
461 al. 2014). In mouse oocyte, resveratrol treatment through SIRT1 increases resistance of mouse  
462 oocytes to postovulatory aging *in vivo* (Liang, et al. 2018). Experimental evidence in mouse  
463 oocytes demonstrated the role of redox signalling in the regulation of SIRT1 (Di Emidio, et al.  
464 2014). Indeed in oocytes arrested at GV stage the gene transcript encoding SIRT1 is induced  
465 in response to *in vitro* exposure to H<sub>2</sub>O<sub>2</sub>. By changing its intracellular localization, activating  
466 GV chromatin rearrangement, and modulating antioxidant enzymatic response, SIRT1 has

467 been shown to orchestrate the adaptive response to oxidative stress in mouse oocytes (Di  
468 Emidio, et al. 2014). More recently, it was shown that continuous overexpression of SIRT1 in  
469 oocytes by suppressing mTOR enhances reproductive capacity, preserves ovarian reserve and  
470 extends ovary lifespan in mice (Long, et al. 2019). These results are in good agreement with  
471 data showing that SIRT1 activator (SRT1720) improves the follicle reserve and prolongs the  
472 ovarian lifespan of diet-induced obesity in female mice via activating SIRT1 and suppressing  
473 mTOR signaling (Zhou, et al. 2014). In addition, activation or overexpression of SIRT1 not  
474 only partly prevents the deficient phenotypes of aged oocytes but also ameliorates the meiotic  
475 anomalies and oxidative stress in NMNAT2-depleted oocytes (Wu, et al. 2019). NMNAT2  
476 belongs to the NMNAT family members that can catalyze the synthesis of NAD<sup>+</sup> both in the  
477 de novo pathway and recycling pathway.

478

## 479 **5. Energy sensors involved in ovarian pathological disorders**

480

481 As a consequence of physiological roles of mTOR, AMPK and SIRT1, changes in their  
482 activity have been observed in some reproductive pathologies, such as PCOS (Tatone, et al.  
483 2018) and POF.

### 484 **5.1 PCOS**

485 PCOS is a reproductive disorder affecting about 10% of women of reproductive age  
486 (Azziz, et al. 2016). Its diagnosis requires the presence of at least two of the following  
487 features; menstrual irregularities (oligo-/anovulation), signs of androgen excess and  
488 polycystic ovaries on ultrasound (corresponding to a follicle number per ovary  $\geq 20$  and/or an  
489 ovarian volume  $\geq 10$  mL in either ovary) (Teede, et al. 2018a). PCOS is typically associated  
490 with metabolic features like insulin resistance, abdominal obesity and an increased risk of  
491 developing type 2 diabetes obesity. At the reproductive neuroendocrine level, women with

492 PCOS exhibit reduced sensitivity to the inhibitory effects of sex steroids on gonadotropin  
493 secretion (Christman, et al. 1991, Eagleson, et al. 2000) and increased pituitary  
494 responsiveness to GnRH (Patel, et al. 2004), with both contributing to LH hypersecretion.

495         Some data have suggested that the mTOR signaling system could be an important  
496 pathophysiological basis of PCOS. Indeed, mTOR expression and phosphorylation, Rictor  
497 expression and its downstream effectors are increased in DHEA-treated PCOS mouse model  
498 (Yaba and Demir 2012). Moreover, a recent study showed that mTOR expression and  
499 phosphorylation in granulosa cells were higher in PCOS than non PCOS patients (Kuang, et  
500 al. 2020). Since DHEA increases mTOR expression in proliferative and differentiating cells  
501 (premature luteinization of granulosa cells), the mTOR signal pathway in DHEA metabolism  
502 could be important in the PCOS mouse ovary, possibly impairing selection of the dominant  
503 follicle and leading to abnormal follicular development (Yaba and Demir 2012). However,  
504 another study observed that the mTOR protein amount in luteal granulosa cells was similar in  
505 PCOS and healthy patients but less mTOR protein expression was found in luteal granulosa  
506 cells with PCOS compared to that in healthy patients upon stimulation with insulin (Song, et  
507 al. 2018). Moreover, the level of mTOR mRNA in granulosa cells from PCOS patients are  
508 decreased in response to berberine that is an isoquinoline alkaloid used in Chinese medicine  
509 (Kuang, et al. 2020). In humans, the lipid-lowering and insulin-resistance improving actions  
510 of berberine have clearly been demonstrated in numerous randomized clinical trials  
511 (Imenshahidi and Hosseinzadeh 2019). Thus, mTOR could be linked to PCOS through its  
512 involvement in the regulation of insulin sensitivity.

513         In addition, as described previously, PCOS patients have high levels of LH and  
514 blockade of central mTOR signaling by rapamycin causes decreased LH secretion in rodents  
515 (Roa, et al. 2009). Thus, rapamycin treatment is expected to be used to eliminate metabolic  
516 syndrome with PCOS and reduce LH secretion for preventing or treating anovulatory PCOS

517 despite potential adverse effects on fertility and short-term metabolism. Additional studies are  
518 needed to explore the role of mTOR in the PCOS syndrome.

519         The interest in the involvement of AMPK and SIRT1 in PCOS development and  
520 progression has been increasing (Tatone, et al. 2018). AMPK inhibitor Compound C  
521 significantly reduced the effect of metformin and pioglitazone combination therapy on  
522 restoring ovarian follicular development in PCOS rats (Wu, et al. 2018). In PCOS patients  
523 with hyperinsulinemia, treatment with metformin enhances endometrial AMPK $\alpha$  expression  
524 (Carvajal, et al. 2013). In addition, compared with non-PCOS patients, AMPK $\alpha$   
525 phosphorylation of the endometrium in PCOS patients is reduced (Li, et al. 2015). AMPK  
526 activation is also observed in human endometrial cells exposed to a PCOS environment in  
527 response to myo-inositol that is an insulin-sensitizing compound (Cabrera-Cruz, et al. 2020).  
528 In KGN human granulosa cells, AMPK signaling pathways is activated in response to  
529 berberine that is known to improve insulin sensitivity and ovulation function in PCOS  
530 patients (Li, et al. 2020). Recently, Zhang et al showed that AMPK pathway is involved in the  
531 reduction of the embryo loss rate in response to adiponectin treatment in PCOS mice (Zhang,  
532 et al. 2020). In the well-established DHEA-induced PCOS mouse model, expression of SIRT1  
533 and activation of AMPK are largely enhanced in ovary suggesting that SIRT1 through AMPK  
534 activation could modulate autophagy in PCOS ovaries (Di Emidio, et al. 2020). The  
535 AMPK $\alpha$ -SIRT1 pathway has also been demonstrated to be involved in the beneficial effects  
536 of metformin and exenatide (a GLP-1 receptor agonist) on the reproductive and endocrine  
537 functions of rats with PCOS (Tao, et al. 2019). In rats PCOS, combined treatment with  
538 metformin and resveratrol improves ovarian follicular cell architecture by inducing  
539 antioxidant and antiinflammatory systems via SIRT1 and AMPK activation (Furat Rencber, et  
540 al. 2018). The SIRT1 agonist, resveratrol, can reduce oxidative stress in rats with  
541 hyperandrogen-induced polycystic ovary syndrome (PCOS) through inhibiting p66Shc



542 (Wang, et al. 2020). Taken together, these results emphasized the significance of the  
543 AMPK/SIRT1 pathway in the treatment of PCOS. In addition, in PCOS human granulosa  
544 cells, SIRT1 expression is enhanced to inhibit excessive mitophagy in response to melatonin  
545 treatment suggesting a protective role of SIRT1 (Yi, et al. 2020).

## 546 **5.2 POF**

547 POF, is a challenging reproductive issue causing loss of ovarian function in women  
548 younger than 40 years of age (Torrealday, et al. 2017). POF is characterized by  
549 hypoestrogenism; increased serum gonadotropin levels; and, most important, amenorrhea  
550 (Nelson 2009). Ovaries of patients with POF are characterized by loss of secondary follicles  
551 and arrested folliculogenesis, which leads to a decrease or elimination of estrogen production  
552 and infertility. The roles of mTOR signaling in follicular development have been largely  
553 investigated (cf **Table 1**); abnormalities in this process lead to a series of pathologies such as  
554 POF and infertility (Liu, et al. 2018). Concerning the role of AMPK in POF, oocyte-specific  
555 deletion of LKB1 (an AMPK Kinase) from the primordial follicle stage leads to severe female  
556 fertility defects due to defective follicular development (Jiang, et al. 2016). At the molecular  
557 level, the loss of LKB1 in oocytes leads to enhanced mTORC1-S6K-rpS6 signaling with  
558 reduced AMPK activity (Jiang, et al. 2016). Thus, the AMPK/mTOR signaling may play a  
559 key role in the POF in women. However, it remains to be demonstrated in human since most  
560 of the experiments have been performed in mice. The role of SIRT1 remains to be deepened  
561 in human reproductive HPO axis (Zbroch, et al. 2018). Indeed, a recent study shows that loss  
562 of SIRT1 specifically in mouse oocyte induces premature ageing (Iljas, et al. 2020). In  
563 addition, Ma et al suggested a role of SIRT1 in the protection of premature ovarian  
564 insufficiency in mice in response to melatonin (Ma, et al. 2017).

565

## 566 **6 Conclusions**

567 Mammalian TOR, AMPK and SIRT1 are three energy sensors expressed in the  
568 reproductive cells of the HPO axis. It is now clear that a modulation of their expression by  
569 using conditional mutagenesis in mouse models or a modulation of their activation by using  
570 pharmacological approaches can impact the function of cells from HPO and consequently  
571 affect female fertility. However, if the activation level of mTOR and AMPK is frequently  
572 determined in the studies, those of SIRT1 is rarely evaluated. In addition, the molecular  
573 mechanisms of these energy sensor in the reproductive cells of the HPO axis remain to be *in*  
574 *vivo* investigated. Indeed, most of the reported effects have been obtained *in vitro* by using  
575 sometimes pharmacological or chemicals (rapamycin for mTOR, metformin or AICAR for  
576 AMPK and resveratrol for SIRT1). Indeed, only few specific deletion in mouse of these  
577 energy sensors mainly in oocytes have been performed in reproductive cells. For example no  
578 animals models with specific deletion for AMPK in gonadotrop cells (where AMPK $\alpha$ 1 is  
579 strongly expressed) have been yet reported. Thus, some tools like genome editing could be  
580 used to make targeted genetic modifications of the mTOR, AMPK or SIRT1 in order to  
581 address their role in HPO axis. In rodents, some evidence shows that mTOR, AMPK and  
582 SIRT1 are involved and could be proposed as therapeutic target for reproductive pathologies  
583 such as PCOS (Liu, et al. 2016, Nejabati, et al. 2020, Tao, et al. 2017). We described here the  
584 involvement of mTOR, AMPK and SIRT1 in HPO axis but it is important to precise that  
585 these components also play a crucial role in the fertilization events, embryo development and  
586 uterine implantation processes in normal and pathological conditions (Correia, et al. 2020,  
587 Griffiths, et al. 2020, Martin-Hidalgo, et al. 2018, Ochiai and Kuroda 2020, Tatone, et al.  
588 2018).

589

590 **Figure legends**

591 **Figure 1 : Role of mTOR (A), AMPK (B) and SIRT1 (C) in the cellular homeostasis.**

592 **A. mTOR system includes two structurally and functionally distinct multi-molecular**  
593 **complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)**A. The  
594 mTORC1 complex is composed of mTOR protein and several regulatory proteins: raptor,  
595 mLST8 (mammalian lethal with sec13 protein8), dector (DEP domain-containing mTOR-  
596 inte-racting protein) and PRAS40 (proline-rich Akt substrate of 40-kDa). The mTORC2  
597 complex consists of the proteins mTOR, rictor, Sin1 (stress-activated map kinase-interacting  
598 protein 1), protor (protein observed with rictor) and mLST8. **B. AMPK is composed of one**  
599 **catalytic  $\alpha$ -subunit, and two regulatory subunits,  $\beta$  and  $\gamma$ .** It is activated by  
600 phosphorylation at Thr-172 of the  $\alpha$ -subunit, which is promoted by either increased  
601 AMP/ATP and ADP/ATP ratios or by the serine/threonine kinase 11 (STK11/LKB1),  
602 calcium/calmodulin-dependent protein kinase kinase (CAMKK), and also, the transforming  
603 growth factor- $\beta$ -activated kinase (TAK1). Upon AMPK phosphorylation, ATP-producing  
604 catabolic processes such as fatty acid oxidation, glycolysis, and autophagy by regulating  
605 downstream factors, including mTOR signaling are activated, while ATP-consuming anabolic  
606 phenomena such as protein synthesis, glycogenolysis, and lipogenesis are inhibited, thereby  
607 restoring the AMP/ATP balance. **C. SIRT1 is a NAD<sup>+</sup>-dependent deacetylase.** It is  
608 activated by phosphorylation and sumoylation and inhibited by methylation and nitrosylation.  
609 SIRT1 inhibits apoptosis, oxidant stress, hypoxia, and inflammation whereas it protects  
610 against metabolic stress and improves lifetime.

611

612 **Figure 2 : Role of mTOR, AMPK and SIRT1 activation in the female reproductive**  
613 **functions at the Hypothalamic Pituitary and Ovary axis level.** Involvement of mTOR has  
614 been mainly demonstrated by using the mTOR inhibitor, Rapamycin.

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**Table 1 : The reproductive consequences of targeted disruption or overexpression of mTOR, AMPK and SIRT1 or associated components in mice HPG axis.**

Component modified	Targeted cell	Genetic transformation	Physiological and biochemical consequences	References
Raptor (regulatory-associated protein of mTORC1)	Oocyte (of primordial follicles)	Specific deletion	Follicular development and fertility not affected, loss of mTORC1 signalling in oocytes triggered compensatory activation of the PI3K signalling.	(Gorre, et al. 2014)
Rictor (rapamycin-insensitive companion of mTOR, a key component of mTORC2)	Oocyte (of primordial follicles)	Specific deletion	POF phenotype, massive follicular death, abnormal gonadal hormone secretion	(Chen, et al. 2015)
TSC1 or TSC2 (tumor suppressor tuberous sclerosis complex 1), negative regulators of mTORC1 activity	Oocyte	Specific deletion	Promotes the growth of all primordial follicles in neonatal animals, leading to the exhaustion of the entire follicle pool, followed by a POF phenotype	(Adhikari, et al. 2009, Adhikari, et al. 2010)
double deletion of TSC1 and PTEN	oocyte	Specific deletion	synergistic enhancement of oocyte growth and follicle activation when compared with singly mutated mice indicating that mTORC1 activation and increased AKT signaling synergistically stimulate the growth of primordial follicles	(Adhikari, et al. 2010)
Disruption of TSC1	granulosa cells of secondary follicles	Specific deletion	Enhancement of follicle growth, leading to increased ovulatory capacity and delivery of more pups followed by a POF phenotype	(Huang, et al. 2013)
Conditional knockout of mTOR	primordial or growing oocytes	Specific deletion	MTOR-dependent pathways in primordial or growing oocytes differentially affected downstream processes including follicular development, sex-specific identity of early granulosa cells, maintenance of oocyte genome integrity, oocyte gene expression, meiosis, and preimplantation developmental competence	(Guo, et al. 2018)
Disruption of LKB1	Oocyte from the primordial follicle stage	Specific deletion	Mice were severely subfertile with significantly enlarged ovaries. The entire primordial follicle pool was activated but failed to mature and ovulate, subsequently causing POF	(Jiang, et al. 2016)
Disruption of AMPK ( $\alpha$ 1AMPK)	Oocyte	Specific deletion	a decrease of 27% in litter size was observed. Absence of AMPK, modifies oocyte quality through energy processes and oocyte/somatic cell communication	(Bertoldo, et al. 2015b)
Disruption of AMPK $\alpha$ 2	Kiss1 neurons	Specific deletion	females are fertile and have normal litter size.	(Torsoni, et al. 2016)
Disruption of AMPK $\alpha$ 2	Kiss1 neurons	Specific deletion	conditional ablation of the AMPK $\alpha$ 1 subunit in Kiss1 cells, largely prevented the delay in puberty onset caused by chronic subnutrition	(Roa, et al. 2018)
Disruption of both AMPK $\alpha$ 1 and $\alpha$ 2	Gonadotropes, and peri-ovulatory follicles	Specific deletion	normal estrous cyclicity, normal steroidogenesis during pregnancy, and no ovulatory defect but a delay in the timing of embryo implantation	(Griffiths, et al. 2020, McCallum, et al. 2018)
SIRT1	Whole body	Knockout	Mice are infertile, small ovaries, lack of ovulation, they have decreased levels of the gonadotropic hormones indicating a defect in the hypothalamic-pituitary-gonadal (HPG) axis	(Kolthur-Seetharam, et al. 2009, McBurney, et al. 2003)
SIRT1	Oocytes	Specific deletion	no effect in young females (no impact on ovarian reserve, oocyte chromosome segregation, mitochondrial function, antioxidant defence or litter sizes ) but 50% of females lacking oocyte-Sirt1 become prematurely sterile between 9 and 11 months of age. Thus, loss of Sirt1 induces premature ageing.	(Iljas, et al. 2020)
SIRT1 H355Y	Whole body	Mutation of the catalytic domain	Animals have lower postnatal mortality than Sirt1 $-/-$ animals. Unlike Sirt1 $-/-$ females, homozygosity for the H355Y mutation did not affect female fertility	(Seifert, et al. 2012)
SIRT1	Oocytes	Specific over-expression	Sustains the ovarian follicular pool and/or oocyte quality during ageing. Thus, increasing Sirt1 function appears to combat deleterious effects of reproductive ageing in vivo	(Long, et al. 2019)