Adiponectin and resistin: potential metabolic signals affecting hypothalamo-pituitary gonadal axis in females and males of different species

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Abstract

Adipokines, including adiponectin and resistin, are cytokines produced mainly by the adipose tissue. They play a significant role in metabolic functions that regulate the insulin sensitivity and inflammation. Alterations in adiponectin and resistin plasma levels, or their expression in metabolic and gonadal tissues, are observed in some metabolic pathologies, such as obesity. Several studies have shown that these two hormones and the receptors for adiponectin, AdipoR1 and AdipoR2 are present in various reproductive tissues in both sexes of different species. Thus, these adipokines could be metabolic signals that partially explain infertility related to obesity, such as polycystic ovary syndrome (PCOS). Species and gender differences in plasma levels, tissue or cell distribution and hormonal regulation have been reported for resistin and adiponectin. Furthermore, until now, it has been unclear whether adiponectin and resistin act directly or indirectly on the hypothalamo–pituitary–gonadal axis. The objective of this review was to summarise the latest findings and particularly the species and gender differences of adiponectin and resistin on female and male reproduction known to date, based on the hypothalamo–pituitary–gonadal axis.

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Introduction

Many authors have observed relationships between the energy metabolism and fertility or infertility in various species, including sheep, cattle, pigs, rodents and primates. For example, in cattle selected for high milk production, high negative energy balance in the post-partum period is associated with reduced fertility (Wathes *et al.* 2007). In sheep, it is well known that an increase in availability of energy substrates is associated with an increase in prolificacy (Teleni *et al.* 1989). In pigs, it is also known that a negative energy balance and a decrease in body fat results in a reduction in litter size and viability of piglets (Quesnel *et al.* 2007). Clinical studies have also established some links between insulin resistance and polycystic ovarian syndrome (PCOS) (Gambineri *et al.* 2002). This syndrome is often associated with obesity, metabolic disorders and an imbalance of reproductive hormones in women (Dunaif & Thomas 2001). White adipose tissue (WAT) is one of the main tissues involved in the regulation of energy balance. For a long time, this organ was considered as a simple tissue for the storage of triglycerides. However, it is now well established that white adipose tissue synthesises and secretes numerous cytokines, termed as adipokines that participate in several physiological

and pathological processes, such as food intake and metabolic control, diabetes, atherosclerosis, immunity and also reproductive functions (Reverchon *et al.* 2014). In the present review, we describe and analyse the role of two of these adipokines, adiponectin and resistin, in the female and male hypothalamo–pituitary–gonadal axis.

Structure, expression and role in metabolic functions of adiponectin and resistin

Adiponectin, also known as Acrp30, is mainly secreted by mature adipocytes. It is the most abundant adipokine in the plasma (approximately 1–50 µg/mL) in various species including humans, rats, birds (chicken and turkey), pigs and dairy cows (Chabrolle *et al.* 2007, Hendricks *et al.* 2009, Nishizawa *et al.* 2012, Maleszka *et al.* 2014a, Diot *et al.* 2015, De Koster *et al.* 2016). The plasma adiponectin concentrations are inversely correlated with the adipose tissue reservoir (Kadowaki & Yamauchi 2005, Yamauchi *et al.* 2014). In addition, levels of adiponectin in the plasma were significantly lower in males than those in females in humans and rodents (Nishizawa *et al.* 2002). One explanation is that sexual hormones such oestradiol (E2) and testosterone (T)

could regulate the plasma adiponectin concentration (Nishizawa *et al.* 2002). The adiponectin gene encodes a protein (full-length adiponectin; full AdipoQ) composed of four domains: an N-terminal signal peptide, a variable region, a collagenous domain and a globular domain at the C-terminal end (Fig. 1A). Mammalian adiponectin genes that contain three exons and two introns are highly conserved between species (Hu *et al.* 1996). For example, the homology between pig and mouse, rat and dog was 83, 82 and 90% respectively (Wang *et al.* 2004). The shorter globular adiponectin (g AdipoQ) possesses potent biological activities that have similar properties to full AdipoQ. The circulating adiponectin is found in trimer, hexamer and high-molecular-weight (HMW) forms, the latter is considered the metabolically bioactive form. Two distinct main receptors (AdipoRs) have been described in the literature, namely AdipoR1 (almost ubiquitously expressed, and abundantly so in skeletal muscles; binds the globular form) and AdipoR2 (predominantly expressed in the liver and WAT; binds the full-length protein) (Kadowaki & Yamauchi 2005, Yamauchi *et al.* 2014). Unlike G-coupled protein receptors, AdipoRs are seven-transmembrane domain receptors with an extracellular carboxyl terminus and an intracellular amino terminus. The homology between AdipoR1 and AdipoR2 is 67% amino acid identity. Furthermore, they are structurally conserved from yeast to humans (Yamauchi *et al.* 2014). AdipoR signalling can be modulated by an interaction with two adaptor proteins named adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) and adaptor

protein, phosphotyrosine interacting with PH domain and leucine zipper 2 (APPL2) (Yamauchi *et al.* 2014). Once adiponectin binds to AdipoR1, APPL1 activates various downstream signalling events associated with the adiponectin function. When AdipoR1 is inactive, APPL2 binds and inhibits the APPL1 function. However, APPL2 binding is displaced on AdipoR1 activation. It is well known that adiponectin activates different main signalling pathways in various tissues: AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK): p38, extracellular signal-regulated kinases 1/2 (ERK1/2), serine/threonine protein kinase (Akt) and peroxisome proliferator-activated receptor alpha (PPAR α) (Kadowaki & Yamauchi 2005, Yamauchi *et al.* 2014) (Fig. 1B). Many functions have been described for adiponectin: this hormone can control energy homeostasis and insulin sensitivity, and it affects the lipid metabolism, vasodilatation, atherogenic activity and reproductive functions (Kadowaki & Yamauchi 2005, Brochu-Gaudreau *et al.* 2010, Yamauchi *et al.* 2014).

Resistin is a cysteine-rich, secretory protein, which is also known as Found in Inflammatory Zones (FIZZ) or Adipocyte Secretory Factor (ADSF) (Steppan *et al.* 2001) (Fig. 1A). It is produced by white and brown adipose tissues, but has also been identified in several other peripheral tissues. In adipose tissues, the production of resistin is dependent on the species. Indeed, resistin is produced by the adipocytes in mice, whereas it is predominantly expressed in macrophages in humans (Steppan *et al.* 2001). Human resistin is a 12.5 kDa

Figure 1 (A) Structure of adiponectin and resistin. (B) Signalling pathways of adiponectin receptors (AdipoR1 and AdipoR2) and resistin. AdipoQ, adiponectin; AdipoR1 and AdipoR2, adiponectin receptors; APPL1 and APPL2, adaptor proteins; PPAR α , peroxisome proliferator-activated receptor alpha; PPAR γ , peroxisome proliferator-activated receptor gamma; AMPK, AMP-activated protein kinase; MAPK, mitogen-activated protein kinase p38; ERK1/2, extracellular signal-regulated kinases 1/2; Akt, phosphatidylinositol 3' kinase/Akt; Stat3, signal transducer and activator of transcription 3; TLR4, toll-like receptor; ROR1, tyrosine kinase-like orphan receptor; CAP1, cyclase-associated protein 1; DCN, decorin.

cysteine-rich peptide with a mature sequence consisting of 108 amino acids, whereas rat and mouse resistin has 114 amino acids. There is 55% amino acid identity within the mature segment of human and mouse resistin. The resistin gene is located on chromosome 19 in human, whereas it is located on chromosome 8 and 2 in mouse and pig respectively (Yang *et al.* 2003, Adeghate 2004). Furthermore, the human and mouse resistin genes have markedly divergent promoter regions, suggesting different mechanisms of regulation, tissue distribution and functions (Steppan *et al.* 2001). The comparison of amino acid sequence of bovine resistin with that of human, pig, rat and mouse showed 73, 80, 58 and 57% identity respectively (Kang *et al.* 2006). The plasma and follicular fluid resistin concentrations in humans is from 5 to 50 ng/mL (Munir *et al.* 2005). In pigs, the resistin level in follicular fluid is approximately 0.32 ng/ μ g protein, dependent on the stage of the oestrous cycle (Rak-Mardyla *et al.* 2013). Resistin plasma levels are significantly higher in females when compared with males (Lee *et al.* 2003). The receptor of resistin remains unknown and the molecular mechanism of resistin action is unclear. However, recent reports have suggested potential receptors for resistin such as an isoform of decorin (DCN) (Daquinag *et al.* 2011), mouse receptor tyrosine kinase-like orphan receptor 1 (ROR1) (Sanchez-Solana *et al.* 2012), toll-like receptor 4 (TLR4) (Benomar *et al.* 2013) or adenylyl cyclase-associated protein 1 (CAP1) (Lee *et al.* 2014). Furthermore, it is well known that resistin activates signalling pathways in different tissues (Fig. 1B) such as Akt, MAPK (ERK1/2 and p38), Stat-3 (signal transducer and activator of transcription 3) and PPAR type gamma (PPAR γ). Several studies have identified positive correlations between resistin levels and the pathogenesis of obesity, adipogenesis and insulin resistance (Steppan *et al.* 2001).

Adiponectin and resistin expression and action at hypothalamus–pituitary levels

Despite the conflicting evidence as to whether adiponectin can cross the blood–brain barrier, some studies have reported adiponectin and AdipoR expression in the brain and pituitary of various species, including humans, rats, pigs, rodents and chickens (Rodriguez-Pacheco *et al.* 2007, Wilkinson *et al.* 2007), suggesting that adiponectin may be a factor modulating the reproductive functions. AdipoRs have been identified in the hypothalamic GnRH neuron cells (GT1–7) and in human and rodent hypothalami, including in the paraventricular nucleus and in the periventricular areas. The adiponectin inhibits kisspeptin (KISS-1) gene transcription (Wen *et al.* 2012) and gonadotropin-releasing hormone (GnRH) secretion (Wen *et al.* 2008) (Fig. 2) in GT1–7 cells.

In pigs, pituitary adiponectin levels depend on the phase of the oestrous cycle, and *in vitro* experiments in primary pituitary cells showed that treatment with adiponectin increases follicle-stimulating hormone (FSH) release (Kiezun *et al.* 2014). Conversely, the exposure of rodent pituitary cell cultures to adiponectin resulted in a reduction in luteinising hormone (LH) secretion and GnRH-induced LH release (Rodriguez-Pacheco *et al.* 2007, Lu *et al.* 2008). Moreover, in primary rat pituitary cells, GnRH treatment suppressed pituitary adiponectin expression (Kim *et al.* 2013). AdipoRs have been identified in gonadotropin-producing cells in the pars distalis but not in the pars tuberalis in the human pituitary (Wilkinson *et al.* 2007). To our knowledge, a sexual dimorphism has been described in the levels of circulating plasma adiponectin, with males having lower adiponectin levels than females (Arita *et al.* 1999). However, adiponectin levels in human cerebrospinal fluid (CSF) showed no gender difference (Kos *et al.* 2007).

Figure 2 Effects of adiponectin and resistin on GnRH and LH/FSH expression and/or secretion. GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMPK, AMP-activated protein kinase; KISS-1, kisspeptin; GT1–7, hypothalamic GnRH neuron cells; POMC, pro-opiomelanocortin; NPY, neuropeptide Y; AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; AdipoR1/R2, adiponectin receptors; GH, growth hormone. →, activation; — inhibition. (a) Wen *et al.* 2008, (b) Vazquez *et al.* 2008, (c) Rodriguez-Pacheco *et al.* 2007, Lu *et al.* 2008, (d) Kiezun *et al.* 2014, (e) Nogueiras *et al.* 2004, (f) Rodriguez-Pacheco *et al.* 2007.

Expression of resistin has been reported in the hypothalamus of rodents (Morash *et al.* 2002). Resistin was identified in the human CSF with levels 100-fold lower than that in the serum (Kos *et al.* 2007). However, its role in GnRH neurons remains to be determined. In the pituitary, gender differences of resistin are evident (male > female at postnatal days 28 and 42), and this was not modified by neonatal treatment of female pups with T (Morash *et al.* 2004). Moreover, expression of resistin mRNA in the pituitary is significantly higher in prepubertal mice (Morash *et al.* 2002, Morash *et al.* 2004), whereas it increases until the age of 28 days in rats, suggesting that pituitary resistin expression is age dependent. Moreover, corticosteroids significantly increased pituitary mRNA resistin levels. It was demonstrated that the AdipoRs expression are decreased, whereas the GH secretion is increased after the resistin treatment in *in vitro* rat pituitary cells in culture (Nogueiras *et al.* 2004), but there is a lack of data describing the effect of resistin on gonadotropin secretion. However, Singh and coworkers have shown that serum resistin levels correlated negatively with changes in serum LH level in bats (Singh *et al.* 2014). In addition to the effects of adiponectin and resistin on GnRH neurons in the hypothalamus and/or on the gonadotrophs of the anterior pituitary (Fig. 2), they also affect, in a direct manner, both female and male gonads (Figs 3 and 4).

Adiponectin, AdipoRs and resistin expression in the ovary

As described in Table 1, adiponectin is not only present in the follicular fluid but also in ovarian cells of various species. In granulosa cells, adiponectin

expression is low and almost undetectable in humans, rodents and chickens, suggesting species-specific differences in ovarian expression of the adiponectin gene (Chabrolle *et al.* 2007, 2009, Richards *et al.* 2012). Adiponectin and its receptors are present in the corpus luteum (CL) of mammalian species (rat, cow and sows), including women. In bovine species, the physiological status of the ovary influences the expression pattern of adiponectin and its receptors in follicular and luteal cells (Tabandeh *et al.* 2010). In these species, a positive correlation is also observed between the adiponectin transcript in the ovarian cells of the dominant follicle and follicular fluid oestradiol (E2) levels, indicating an association between adiponectin and follicular dominance and oocyte competence (Tabandeh *et al.* 2012). In humans, ovarian adiponectin and AdipoRs expression are hormonally controlled *in vivo*, as suggested by an increase in adiponectin concentrations in the ovarian follicular fluid of women in response to LH treatment in the *in vitro* fertilisation procedure (Gutman *et al.* 2009). Gonadotrophins modify the expression levels of AdipoR2, but not AdipoR1 and eventually contribute to the enhanced 3 β -hydroxysteroid dehydrogenase (3 β HSD) activity and increased progesterone (P4) secretion in human granulosa cells (Wickham *et al.* 2013). Furthermore, after pregnant mare serum gonadotropin (PMSG) pre-treatment, an injection of human chorionic gonadotropin (hCG) increases the expression of adiponectin and AdipoR1 (but not AdipoR2) genes in rat ovaries (Chabrolle *et al.* 2007). Also, in bovine theca interna cells, LH increases the concentrations of AdipoR2 mRNAs, whereas insulin-like growth factor type 1 (IGF1) suppresses the expression of AdipoR2 (Lagaly *et al.* 2008). Steroids could also affect adiponectin expression. Indeed, in swine, adiponectin

Figure 3 Effects of adiponectin and resistin on granulosa, theca and oocyte function (steroidogenesis, proliferation and apoptosis, oocyte maturation) in different species. P4, progesterone; T, testosterone; E2, estradiol; SF, small follicles; LF, large follicles. ↑, increase, ↓ decrease. (a) Richards *et al.* 2012, (b) Maillard *et al.* 2010, (c) Chabrolle *et al.* 2007, 2009, (d) Maleszka *et al.* 2014, (e) Anuradha *et al.* 2014, (f) Lagaly *et al.* 2008, (g) Spicer *et al.* 2011, (h) Reverchon *et al.* 2013, (i) Rak *et al.* 2015a, (j) Maillard *et al.* 2011, (k) Munir *et al.* 2005, (l) Rak-Mardyła *et al.* 2013, 2014, (m) Singh *et al.* 2015.

Figure 4 Effects of adiponectin and resistin on testicular cell functions (steroidogenesis, proliferation, apoptosis, sperm motility) in different species. P4, progesterone; T, testosterone; AMH, anti-Müllerian hormone; SCF, stem cell factor. (a) Caminos *et al.* 2008, (b) Landry *et al.* 2015, (c) Nogueiras *et al.* 2004, (d) Kadivar *et al.* 2016, (e) Thomas *et al.* 2013.

serum concentrations are higher during the luteal phase than the follicular phase, which suggests that ovarian steroids influence plasma adiponectin levels (Maleszka *et al.* 2014b). However, it remains to be demonstrated whether steroids can locally affect ovarian adiponectin production. Adiponectin can also influence the expression of its receptors differently according to the ovarian cell type. For example, AdipoRs expressions are increased in the cumulus-oocyte complex, but not in granulosa cells (Richards *et al.* 2012).

Similar to adiponectin, resistin is expressed in the ovarian cells of various species (Table 1). Niles and coworkers have demonstrated the resistin expression in human granulosa cells derived from the preovulatory follicles of females undergoing oocyte retrieval during *in vitro* fertilisation, suggesting its role in the follicular development (Niles *et al.* 2012). Resistin is also present in human granulosa cells, cumulus cells and human ovarian granulosa tumour-derived cell line (KGN), as well as in theca cells in large follicles and oocytes in the primary follicles (Reverchon *et al.* 2013). In bovine species, resistin is widely expressed in different-sized follicles (small <6mm and large >6mm) where it is localised in oocytes, cumulus, theca and granulosa cells. In addition, it is present in the CL (Maillard *et al.* 2011). Interestingly, resistin mRNA was undetectable in rat granulosa cell cultures (Maillard *et al.* 2011). 'Species-specific' ovarian resistin expression could contribute to different effects on ovarian follicle functions, such as steroidogenesis and proliferation, as described for adiponectin. In pig ovaries, resistin levels and expression

depend on the stage of the animal reproductive status; differences were observed in the expression and concentration of resistin in follicular fluid collected from small follicles (SFs), medium follicles (MFs) and large follicles (LFs) (Rak-Mardyla *et al.* 2013). Interestingly, in contrast to prepubertal animals, resistin expression and concentration in adult oestrous cycling pigs was independent of follicular size and/or development (Rak-Mardyla *et al.* 2014). Moreover, several hormones can influence ovarian resistin expression. For example, Rak and coworkers reported that gonadotropin and steroid hormone increased, but IGF1 dose-dependent significantly decreased the ovarian resistin mRNA and protein expression in pigs (Rak *et al.* 2015a). In addition, the resistin ovarian expression was decreased by rosiglitazone—a PPAR γ agonist (Rak-Mardyla & Drwal 2016).

Adiponectin and resistin *in vitro* effects on ovarian steroidogenesis and cell survival

The ovarian follicles synthesise steroid hormones that control and maintain female sexual development, behaviour and pregnancy, as well as having important local effects within the ovary. The role of adiponectin has been studied *in vitro* in the steroidogenesis of granulosa and theca cells in several species (Fig. 3). In rats and in women, recombinant adiponectin at physiological doses (5 or 10 μ g/mL) increases the secretion of steroids in IGF1-stimulated cells (Chabrolle *et al.* 2007, 2009). In rats, this increase is due to increased signalling of the

Table 1 Expression of resistin, adiponectin, AdipoR1 and AdipoR2 in ovarian and testicular cells in different species (+: presence; ns: no study).

Adipokine or receptor	Species	Ovarian cells			Testicular cells			References
		Granulosa	Theca	Oocyte	Sertoli	Leydig	Germinal	
Resistin	Human	+	+	+	ns	ns	ns	Nogueiras <i>et al.</i> (2004), Maillard <i>et al.</i> (2011), Niles <i>et al.</i> (2012), Reverchon <i>et al.</i> (2013), Rak-Mardyla <i>et al.</i> (2014), Singh <i>et al.</i> (2015)
	Cattle	+	+	+	ns	ns	ns	
	Rodents	+	+	+	+	+	ns	
	Pig	+	+	ns	ns	ns	ns	
	Birds	ns	ns	ns	ns	ns	ns	
	Fish	ns	ns	ns	ns	ns	ns	
Adiponectin	Bat	+	+	+	ns	ns	ns	Caminos <i>et al.</i> 2008, Chabrolle <i>et al.</i> (2007, 2009), Nishio <i>et al.</i> (2008), Ocón-Grove <i>et al.</i> (2008), Maillard <i>et al.</i> (2010), Maleszka <i>et al.</i> (2014), Gregoraszczuk <i>et al.</i> (2016)
	Human	+	ns	ns	ns	ns	ns	
	Cattle	+	+	+	ns	ns	ns	
	Rodents	+	+	+	+	+	+	
	Pig	+	+	ns	ns	ns	ns	
	Birds	+	+	ns	+	+	+	
AdipoR1	Fish	Whole ovary: +			ns	ns	ns	Chabrolle <i>et al.</i> (2007, 2009), Nishio <i>et al.</i> (2008), Ocón-Grove <i>et al.</i> (2008), Maillard <i>et al.</i> (2010), Comim <i>et al.</i> (2013), Maleszka <i>et al.</i> (2014), Gregoraszczuk <i>et al.</i> (2016)
	Bat	ns	ns	ns	ns	ns	ns	
	Human	+	+	ns	ns	ns	ns	
	Cattle	+	+	+	ns	ns	ns	
	Rodents	+	+	+	+	+	+	
	Pig	+	+	ns	ns	ns	ns	
AdipoR2	Birds	+	+	ns	+	+	+	Chabrolle <i>et al.</i> (2007, 2009), Nishio <i>et al.</i> (2008), Ocón-Grove <i>et al.</i> (2008), Maillard <i>et al.</i> (2010), Comim <i>et al.</i> (2013), Maleszka <i>et al.</i> (2014), Gregoraszczuk <i>et al.</i> (2016)
	Fish	Whole ovary: +			ns	ns	ns	
	Bat	ns	ns	ns	ns	ns	ns	
	Human	+	+	ns	ns	ns	ns	
	Cattle	+	+	+	ns	ns	ns	
	Rodents	+	+	+	+	+	+	
	Pig	+	+	ns	ns	ns	ns	
	Birds	+	+	ns	+	+	+	
	Fish	Whole ovary: +			ns	ns	ns	
	Bat	ns	ns	ns	ns	ns	ns	
	Human	+	+	ns	ns	ns	ns	
	Cattle	+	+	+	ns	ns	ns	

IGF1 receptor and, in women, it is due to an increase in the expression of the CYP19A1 (cytochrome P450 aromatase) enzyme responsible for the oestrogen biosynthesis. In bats, adiponectin at physiological doses (5 and 10 µg/mL) *in vivo* during the period of delayed development causes a significant increase in circulating P4 and E2 levels, together with an increased expression of AdipoR1 in the ovary. In this species, the effects of adiponectin on ovarian steroidogenesis are mediated through increased expression of LH receptor, steroidogenic acute regulatory protein and 3β-HSD (Anuradha & Krishna 2014). In KGN cell lines, specific inactivation of AdipoRs shows that AdipoR1 regulated cell survival, whereas AdipoR2 is preferentially involved in steroidogenesis (Pierre *et al.* 2009). In cows, adiponectin at dose 3 µg/mL *in vitro* decreases the production of androstenedione (A4) by theca cells by reducing the expression of LH receptors and CYP11A1 (cytochrome P450, family 11, subfamily a,

polypeptide 1) and CYP17α1 (cytochrome P450, family 17, subfamily a, polypeptide 1) enzymes mediated by both AdipoR1 and AdipoR2 (Lagaly *et al.* 2008). These data were confirmed by Comim and coworkers showing that knockdown of AdipoRs or the downstream effector protein APPL1 leads to an increase in the secretion of androstenedione (Comim *et al.* 2013). Thus, adiponectin differentially regulates the expression of steroidogenic enzymes in ovarian cells in various species.

The first study showed that treatment with 10 ng/mL of resistin increased the androgen production by stimulation of CYP17 mRNA expression in human theca cells *in vitro* (Munir *et al.* 2005) (Fig. 3). Spicer and coworkers demonstrated that 30 ng/mL of resistin weakly stimulated FSH plus IGF1-induced E2 production, but had no effect on IGF1- or insulin-induced P4 and A4 production by theca cells or P4 production by granulosa cells of LFs (Spicer *et al.* 2011). However, in granulosa cells from SFs, resistin attenuated the stimulatory effect

of IGF1 on P4 and E2 secretion. *In vitro* treatment with resistin on bovine granulosa cells showed a decreased basal, but not IGF1-induced P4 and E2 production. Although in cultured rat granulosa cells, basal and IGF1-induced P4 secretion increased after the treatment with a physiological dose (10 ng/mL) of resistin, with no effects on E2 release (Maillard *et al.* 2011). Similarly, studies on human granulosa cells treated with resistin have also shown reduced steroid hormone secretion in response to IGF1 (Reverchon *et al.* 2013). However, in human theca cells in normal cycling premenopausal women (Munir *et al.* 2005), pig ovary (Rak-Mardyla *et al.* 2013, 2014) and bats (Singh *et al.* 2015), a stimulatory effect of resistin at physiological doses on androgen production was observed. Conversely, in porcine ovarian follicles, resistin decreased gonadotropin- and IGF1-induced steroid hormone secretion by inhibiting the protein expressions of 3 β HSD, 17 β HSD and CYP19A1 (Rak *et al.* 2015a). These contradictory findings may be explained by the presence of various isoforms of resistin, which may explain the functional diversity of resistin in different species. Thus, the resistin receptor remains unknown, and resistin action in ovarian steroidogenesis mechanism remains unclear. However, Rak-Mardyla and Drwal demonstrated that in cultured porcine ovarian follicles, PPAR γ is the key regulator of resistin expression and steroidogenic function (Rak-Mardyla & Drwal 2016). Maillard and coworkers clearly documented that in cultured bovine and rat granulosa cells 10 ng/mL of resistin stimulated the phosphorylation of different kinases such as Akt, MAPKs, P38 and ERK1/2. Furthermore, Reverchon and coworkers demonstrated that resistin decreased the IGF1-induced tyrosine phosphorylation of the IGF1R β subunit and phosphorylation of MAPK ERK1/2 and suggested that the MAPK ERK1/2 signalling pathway regulated *in vitro* steroidogenesis in primary human granulosa cells (Reverchon *et al.* 2013). Recently, Singh and coworkers showed that resistin injection in bats (6.5 μ g/100 g body weight/day for 12 days) increased Stat-3 phosphorylation in the ovaries. Similarly, in cultured porcine ovarian cells, resistin (10 ng/mL) activated not only the phosphorylation of Stat-3 but also MAPK ERK1/2 and Akt (Rak *et al.* 2015b).

Apoptosis is critically important for the survival of multicellular organisms by getting rid of damaged or infected cells that may interfere with normal ovarian function such as oogenesis, folliculogenesis, oocyte loss/selection and atresia. Several pro-survival and pro-apoptotic molecules are involved in ovarian apoptosis with the delicate balance between them being the determinant for the final destiny of the follicular cells. In bovine species, adiponectin at a concentration of 10 μ g/mL increases *in vitro* IGF1-induced granulosa cell proliferation but not basal or insulin-induced proliferation (Maillard *et al.* 2010). In the basal state, similar effects of adiponectin have been described on the proliferation

of LF theca cells. In primary human granulosa cells, treatment with adiponectin at a concentration of 5 μ g/mL does not affect the IGF1-induced cell proliferation (Chabrolle *et al.* 2009). The published *in vitro* data concerning ovarian cell proliferation showed some effects dependent on the resistin doses; no effect at the lower, physiological doses (1 and 10 ng/mL) in pigs (Rak *et al.* 2015b) and rats (Maillard *et al.* 2011), whereas stimulated effects were observed at higher doses (100; 333 and 667 ng/mL). Similarly, *in vivo*, resistin induced a stimulatory effect on the expression of proliferating cell nuclear antigen (PCNA) in the ovaries of seasonally monoestrous bats (Singh *et al.* 2014). However, *in vitro*, resistin reduced [3 H] thymidine incorporation in response to IGF-I in rat granulosa cells (Maillard *et al.* 2011). The previously mentioned observations indicated that resistin, depending on doses and animal models, has different actions on ovarian cell proliferation, which is an important process in the ovarian function.

In the *in vivo* ovaries of bats, resistin decreased protein levels but stimulated caspase-3 activity. Similarly, in pig ovary, resistin decreased pro-apoptotic genes expression, caspase activity and DNA fragmentation (Rak *et al.* 2015b). As a molecular mechanism of resistin action on cell survival, authors have proposed the activation of several signal transduction pathways such as MAPK/ERK1/2, JAK/STAT and PI3K (Rak *et al.* 2015b). These results show that resistin is involved in ovarian apoptosis regulation and could regulate follicular development or atresia.

Adiponectin and resistin effects on oocytes

As described previously, adiponectin and AdipoRs are present in the oocytes of different species (Table 1). In *in vitro* fertilisation protocols in women, mice and pigs, but not in cows, adiponectin at physiological doses improved oocyte maturation and early embryo development (Chappaz *et al.* 2008, Maillard *et al.* 2010, Richards *et al.* 2012) (Fig. 3). In adiponectin-deficient mice, the number of ovulated oocytes drastically decreased as compared with controls with similar body weight (Table 2). However, no experiments to date have specifically inhibited adiponectin and AdipoRs expression in different ovarian cells to determine the involvement of systemic and ovarian adiponectin. In addition, there are no data about the role of resistin in oocyte maturation. These should form the basis of future studies.

Adiponectin, AdipoRs and resistin expressions in testes

In humans, adiponectin concentration in seminal plasma is approximately 66-fold lower than that in serum, and a positive correlation with sperm concentration,

Table 2 Consequences of targeted or non-targeted disruption or overexpression of resistin, adiponectin, AdipoR1 and AdipoR2 on the fertility and reproductive axis in mice.

Components	Tissue/cell type	Effect on fertility	Reproductive axis consequences	References
Resistin	All	Fertile		Pravenec <i>et al.</i> (2003), Banerjee <i>et al.</i> (2004)
Adiponectin	All	Female subfertility	Reduction in retrieval of oocytes, disruption of the estrous cycle, elevated number of atretic follicles, reduction in progesterone, oestradiol and FSH plasma levels, in concentration of LH surge, and in GnRH immunoreactive neurons	Cheng <i>et al.</i> (2016)
Adiponectin	Overexpression dominant negative lacking collagen domain using promoter ap2 (adipose tissue)	Infertile	One of the line displayed 3-fold increased serum adiponectin levels, and was unfertile	Combs <i>et al.</i> (2004)
Adiponectin	All	Fertile		Ma <i>et al.</i> (2002), Nawrocki <i>et al.</i> (2006)
AdipoR1	All	Fertile (male and female)		Yamauchi <i>et al.</i> (2007)
AdipoR1 and AdipoR2	All	Fertile (male and female)		Yamauchi <i>et al.</i> (2007)
AdipoR2	All	Male subfertility	An atrophy of the seminiferous tubules and aspermia associated with reduced testes weight	Bjursell <i>et al.</i> (2007), Lindgren <i>et al.</i> (2013)
AdipoR2	All	Fertile (male and female)		Yamauchi <i>et al.</i> (2007)

sperm count and total normomorphous spermatozoa has been reported (Thomas *et al.* 2013). Heinz and coworkers (Heinz *et al.* 2015) have concluded that in cattle, adiponectin concentration in seminal plasma is likely blood borne and originates from adipose tissues. Therefore, the potential contribution of local secretion from the testes, if any, is only marginal. In rats, adiponectin is mainly present in the Leydig interstitial cells, whereas AdipoR1 is expressed in the seminiferous tubules (Camino *et al.* 2008).

Nogueiras and coworkers (Nogueiras *et al.* 2004) showed that mRNA expression of resistin in rat testes was higher in the Leydig interstitial cells than that in the Sertoli cells within seminiferous tubules, and this expression was regulated by gonadotropins, leptins and nutritional status. Resistin is also expressed in the mouse Leydig cell lines (MA-10 and TM3) and exposure to 8-Br-cAMP (8-Bromoadenosine 3',5'-cyclic monophosphate) increased its mRNA expression in MA-10 Leydig cells (Jean *et al.* 2012). Moretti and coworkers (Moretti *et al.* 2014) showed that in humans, the resistin level was higher in semen than that in serum, and that semen resistin correlated with the sperm quality.

Adiponectin and resistin *in vitro* effects on testes function

Adiponectin treatment decreases the production of T in the presence or absence of hCG in the rat testicular tissue, whereas it has no effect on the expression of the genes encoding anti-Müllerian hormone (AMH) and stem cell factor (SCF) that are specific to the Sertoli cells (Camino *et al.* 2008) (Fig. 4). However, in MA-10 mouse Leydig cells, adiponectin treatment improves

P4 production through an increase in the cholesterol carrier StAR and the CYP11A1 steroidogenesis enzyme, suggesting that high doses of adiponectin (50, 500, or 5000 ng/mL) could promote T production from the Leydig cells (Landry *et al.* 2015). In mice, AdipoR2 deficiency results in atrophy of seminiferous tubules and aspermia without a change in T concentration (Bjursell *et al.* 2007) (Table 2). In chickens, studies show an increase in the testicular adiponectin receptors during sexual maturation and suggest a role for adiponectin in steroidogenesis, spermatogenesis, Sertoli cell function and sperm motility (Ocon-Grove *et al.* 2008). In rams, adiponectin and AdipoR1 mRNA expressions are positively correlated with sperm motility (Kadivar *et al.* 2016). In bulls, adiponectin and its receptors also play vital roles in the structural and functional sperm traits by regulating sperm capacitation (Kasimanickam *et al.* 2013).

To our knowledge, there are limited data on resistin action on testicular cells function. Resistin significantly increased basal and hCG-stimulated T secretion in rat incubated testicular tissues (Nogueiras *et al.* 2004). Exposure to low concentrations of resistin (10 ng/mL), corresponding to a normal physiological condition, contributes to an increased proliferation of MA-10 Leydig cells (Jean *et al.* 2012). In addition, the Sertoli cells may also contribute to the Leydig cells proliferation by secreting resistin (Nogueiras *et al.* 2004). Thus, adiponectin and resistin signalling appears to be present in male gonadal tissues, but the extent to which these hormones contribute to normal human testicular function and fertility potential remains to be determined. In addition to their role in steroidogenesis, adiponectin and resistin could be

involved in sperm capacitation, sperm–egg fusion and fertilisation.

Role of adiponectin and resistin *in vivo* in mice fertility

Although many studies have reported *in vitro* effects of adiponectin and resistin on ovarian and testicular cell functions, the involvement of these two adipokines *in vivo* to control the fertility remains unclear. Several studies have shown that adiponectin-null mice are viable and, in studies where fertility outcomes are mentioned, appear to exhibit normal fertility (Ma *et al.* 2002, Nawrocki *et al.* 2006) (Table 2). However, overexpression of adiponectin leads to increased insulin sensitivity and infertility or subfertility (Combs *et al.* 2004), and a recent study demonstrated that disruption of adiponectin can cause subfertility in female mice (Cheng *et al.* 2016). Indeed, female adiponectin-null mice displayed reduced retrieval of oocytes, a disrupted oestrous cycle, an elevated number of atretic follicles and impaired late folliculogenesis (Table 2). Also, their serum has lower levels of P4 at dioestrus that can be explained by a lower expression of CYP11A1 and a significant reduction in E2 and FSH at pro-oestrus. Adiponectin deficiency also altered the hypothalamo–pituitary axis, as the plasma peak concentrations of LH surged and the number of GnRH immunoreactive neurons was significantly reduced. Concerning AdipoRs, their genetic deletion was not associated with subfertility in one study (Yamauchi *et al.* 2007), but a loss of AdipoR2 was associated with male subfertility in other studies (Bjursell *et al.* 2007, Lindgren *et al.* 2013). Disruption of AdipoR2 in males leads to seminiferous tubules atrophy and aspermia associated with reduced testes weight. Various studies where resistin protein levels were increased in different peripheral tissues (such as adipose tissue or liver) did not report any effect on fertility (Pravenec *et al.* 2003, Banerjee *et al.* 2004).

Thus, these data indicate that adiponectin signalling is important to normal mouse female and male reproduction. However, the role of this adipokine specifically in each gonadal cell remains to be determined. Concerning resistin, however, much less is known about its involvement in *in vivo* reproductive function and, therefore, this remains to be investigated.

Potential involvement of adiponectin and resistin in some pathologies associated with gonadal dysfunctions such as PCOS

PCOS is a metabolic disorder in humans that is linked to insulin resistance and obesity. It is characterised by anovulation, hyperandrogenism and hyperinsulinemia (Dunaif & Thomas 2001). Excessive production of insulin in women in PCOS, with its subsequent induction of

theca cell steroidogenesis, is thought to be the primary cause of hyperandrogenism.

Adipokines such as adiponectin and resistin could act as a link between obesity and PCOS (Spritzer *et al.* 2015). Many studies have investigated the concentration of adiponectin and resistin in both plasma and follicular fluid in patients with PCOS and control groups. However, data are still controversial (Spritzer *et al.* 2015). Several studies have documented a lack of any difference in resistin concentration in serum or follicular fluid of patients with PCOS when compared to the control groups, even though serum adiponectin was significantly lower in obese than that in normal-weight women. However, Munir and coworkers have suggested that resistin is involved in PCOS because the concentration of resistin was significantly increased in patients with PCOS and was positively correlated with body mass index and T levels. Concerning adiponectin, some authors have also analysed the different forms of adiponectin in patients with PCOS and they observed that there were low levels of HMW adiponectin in both serum and follicular fluid of PCOS patients with controlled ovarian hyperstimulation compared to controls (Artimani *et al.* 2016). Furthermore, the expression pattern of the adiponectin system (adiponectin, AdipoRs and APPL1) has been studied in patients with PCOS, and a reduction of APPL1 and the adiponectin system was observed in human granulosa cells (Dehghan *et al.* 2016). Furthermore, recently Yuan and coworkers showed that brown adipose tissue (BAT) transplantation activated endogenous BAT and increased the circulating level of adiponectin in a dehydroepiandrosterone (DHEA)-induced PCOS rat (Yuan *et al.* 2016). Comim and coworkers also showed that a lower proportion of theca cells expressed AdipoRs in polycystic ovaries than that in normal ovaries (Comim *et al.* 2013). Many studies have also described the association of PCOS with polymorphisms of the adiponectin or adiponectin receptor genes. The resistin gene polymorphism is associated with body mass index in women with PCOS, suggesting that resistin might be related to adiposity in PCOS.

Conclusions

In conclusion, resistin, adiponectin and AdipoRs are present and active in the hypothalamo–pituitary–gonadal axis. Their expressions can be regulated by various factors (such as gender, age, nutritional and hormonal status). Many *in vitro* studies have shown that these two adipokines can regulate gonadal steroidogenesis and gametogenesis. Adiponectin can also exert effects on GnRH synthesis and the pituitary secretory functions that could then indirectly affect gonadal functions. The *in vitro* effects of resistin on GnRH and gonadotropin secretion are still unknown. In mice, adiponectin deficiency leads to female subfertility associated

with ovarian and hypothalamo–pituitary dysfunction and AdipoR2 deficiency leads to male subfertility with aspermia and atrophy of tubules, suggesting an important role of the adiponectin system in the normal reproduction. If these data are confirmed in human and other species, adiponectin or its analogues (recombinant adiponectin, adiponectin receptor agonist) could be used in the treatment of certain infertilities, similar to how they are used in the metabolic syndrome. Furthermore, whether these *in vivo* adipokine effects act directly on the hypothalamo–pituitary–gonadal axis needs to be studied. For example, mice with targeted disruption of the adiponectin system or resistin in ovarian and testicular cells could be developed to analyse the metabolic and reproductive phenotypes. Finally, the role of other adipokines (visfatin, chemerin, omentin and so forth) in reproductive diseases related to insulin resistance and obesity should be investigated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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