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Il sistema adipocitochine/recettori nei tessuti riproduttivi maschili dei mammiferi
Adipocytokines/cognate receptors system in mammalian male reproductive tissues

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SUMMARY

In this Thesis, a review of adiponectin initially carried out, as an important component within adipokines, from its discovery to functionality studies on reproductive function.

Subsequently, the available data on the distribution and function, discovered or proposed, of this bioprotein in the functionality in reproductive tissues of the male, from the hypothalamus and other nervous centers to the gonads and their attached glands, are established.

It taken into consideration that most of the discoveries of adiponectin in reproduction have made on the female and its correlation with different pathologies.

Subsequently, the current information available on this adipokine in livestock animals is referenced, the knowledge available in the ovine species is established, the importance of its reproductive seasonality and the absence of available data on adiponectin, in relation to this hormonal peculiarity.

Finally, the work carried out, sent to a Q1 Journal, "Animals" (IF 2022: 3.231), included in the annexes for consideration by the Editorial at this time.

With the results found in the study, it was possible to verify not only the expression but also the distribution of the AdipoR1 receptor in reproductive tissues in rams outside the reproductive season. In addition to the novelties and lack of evidence in other studies of this species, the correlation between the arrangement of the receptors reported by us in comparison with other studies carried out in other species that also have reproductive cycles, manipulated with photoperiod, is also important.

RIASSUNTO

Nella presente tesi è stata inizialmente effettuata una revisione dell'adiponectina, come componente importante all'interno delle adipocitochine, dalla sua scoperta agli studi di funzionalità nei processi riproduttivi. Successivamente, vengono stabiliti i dati disponibili sulla distribuzione e sulla funzione, scoperta o proposta, di questa bioproteina nella funzionalità nei tessuti riproduttivi del maschio, dall'ipotalamo e altri centri nervosi alle gonadi e alle loro ghiandole annesse. Si è tenuto conto del fatto che la maggior parte delle scoperte sull'adiponectina nella riproduzione sono state fatte sulla donna e sulla sua correlazione con diverse patologie. Successivamente, si fa riferimento alle informazioni attualmente disponibili su questa adipocitochina negli animali da reddito, si stabiliscono le conoscenze disponibili nella specie ovina, l'importanza della sua stagionalità riproduttiva e l'assenza di dati disponibili sull'adiponectina, in relazione a questa peculiarità ormonale.

Infine, il lavoro svolto, per essere pubblicato, è stato inviato ad *Animals* (IF 2022: 3.231; *quartile ranking* Q1). Con i risultati riscontrati nello studio, è stato possibile verificare non solo l'espressione ma anche la distribuzione del recettore ADIPOR1 nei tessuti riproduttivi degli arieti al di fuori della stagione riproduttiva. Oltre alle novità e alla mancanza di evidenze in altri studi su questa specie, è importante anche la correlazione tra la disposizione dei recettori da noi riportata rispetto ad altri studi effettuati in altre specie che hanno anch'esse cicli riproduttivi, manipolati con il fotoperiodo.

GENERAL INTRODUCTION

It is well known that adipose tissue is an endocrine organ. It secretes adipokines, which act at endocrine, paracrine, and autocrine levels (Coelho et al. 2013). These adipokines are not only synthesized and secreted mainly by adipocytes, but also synthesized and secreted by the other cells that make up the adipose tissue, such as macrophages, lymphocytes, and fibroblasts (Antuna-Puente et al. 2008; Thomas et al 2013). Moreover, proinflammatory cytokines are secreted mainly by nonadipose cells in adipose tissue (Antuna-Puente et al. 2008).

The adipose tissue metabolism plays a crucial role in the support of reproduction as well as lactation in farm animals as a result of the close connection between metabolism and reproductive function (Trayhurn et al. 2006; Rosen & Spiegelman 2006).

The study of the adipokines functions in the reproductive tract is required because energy hemostasis is highly important in the reproduction of farm animals under different management techniques. The correlations between adipokines and reproduction have been demonstrated in humans, rodents, and other animals (Tersigni et al. 2011).

The APLN/APJ was found in the HHG axis and it has been widely illustrated that APLN is an advantageous peptide regulating reproduction in females and males (Li et al. 2006). GnRH secretion in different nuclei of the hypothalamus is the start of the endocrine roles of reproduction. A study in rats showed that APLN/APJ genes were expressed in the supraoptic nucleus and the magnocellular and parvocellular parts of the paraventricular nucleus (Reaux et al. 2002) and also in the periventricular, suprachiasmatic, ventromedial, dorsomedial, nucleic, and retrochiasmatic regions. The expression of APLN/APJ genes in the hypothalamus shows their role in the control of releasing hormones (Newson et al. 2009). The mRNA expression of APLN/APJ was also identified in the anterior and posterior, intermediate lobes of the pituitary in rats (O'Carroll et al. 2000). The wide expression of APLN/APJ in the same hypothalamus nucleus group reveals their essential functions in reproduction control (Pope et al. 2012). Intracerebroventricular and intraperitoneal infusion of APLN-13 caused suppression of FSH and LH secretion in frontal hypophysis of rats, but it does not lead to disruption of the GnRH concentration (Taheri et al. 2002; Tekin et al. 2017).

The sheep is a highly important productive species in food production. Several studies of adiponectin in the reproductive tract of the breeding male have been carried out since 2012, but until now the significance of the reproductive seasonality of the species and its relationship with the functionality of adiponectin have not been considered.

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1. DISCOVERY, PROTEIN STRUCTURE, AND RECEPTORS

1.1 History

In 1995, a year after the discovery of leptin, Scherer et al. published the initial report on adiponectin. Utilizing subtractive cDNA screening, mRNAs induced during 3T3-L1 adipocyte differentiation identified. One specific mRNA induced greater than 100-fold during adipocyte differentiation, and its encoded proteinwas discovered to be novel. The protein had sequence and structural homology to complement factor C1q, and therefore adiponectin was originally named adipocyte complement-related protein of 30 kDa, or Acrp30 (Scherer et al. 1995).

In 1996, Hu et al. utilized mRNA differential display to isolate a novel adipose cDNA they named AdipoQ. Specifically expressed in rodent adipose tissue and encoded a 247 amino acid protein with significant homology to complement factor C1q. The encoded protein contained a collagenous structure in its N-terminus as well as a globular domain in its C-terminus. AdipoQ strongly induced during adipocyte differentiation and downregulated in adipose tissue collected from obese mice and humans (Hu et al. 1996).

Also in 1996, Maeda et al. constructed a cDNA library from human adipose tissue and used it to identify adiponectin as the most abundant transcript. They named the transcript adipose most abundant gene transcript 1, or apM1 (Maeda et al. 1996). Further analysis using RNA from numerous different human tissues revealed that apM1 was highly enriched in adipose tissue. During the same year, Nakano et al. utilized gelatin-based affinity chromatography to identify a novel protein from human plasma they named gelatin-binding protein of 28 kDa, or GBP28 (Nakano et al., 1996). In agreement with previous work, Nakano et al. (1996) showed that GBP28 exists as multiple complexes with different molecular weights (Scherer et al., 1995; Nakano et al., 1996). Further analysis revealed that the cDNA clone apM1 encoded GBP28. The amino acid sequence for GBP28 showed 82.7% homology to Acrp30, which is the murine equivalent of GBP28 (Nakano et al., 1996).

1.2 Protein Structure

Adiponectin is a 30-kDa monomeric glycoprotein (Berg et al. 2002). Mouse adiponectin contains 247 amino acids and human adiponectin consists of 244 amino acids, with 83% homology between them (Nakano et al., 1996). Adiponectin is composed of an N-terminal signal sequence, a nonhomologous or hypervariable region, a collagenous domain containing 22 collagen repeats (8 Gly-X-Pro and 14 Gly-X-Y), and a C-terminal C1q-like globular domain (Fig. 1). The N-terminal hypervariable region of adiponectin contains a single cysteine residue (Cys-39 in mice or Cys-36 in humans) followed by a collagenous domain containing several conserved lysine and proline residues (Frizzell et al. 2009). The cysteine residue is critical to the formation of the multimeric species of adiponectin through disulfide bonding of trimers (Frizzell et al. 2009). The globular domain is similar in structure to other proteins, including complement factor C1q, type VIII and X collagens, and TNF-α (Hu et al. 1996, Shapiro & Scherer, 1998). Specifically, studies utilizing the crystal structure of homotrimeric adiponectin demonstrated close homology to TNF-α, including conservation of key residues, identical folding topologies and similar intron positions, and trimer interfaces (Shapiro & Scherer, 1998). (Fig. 1)

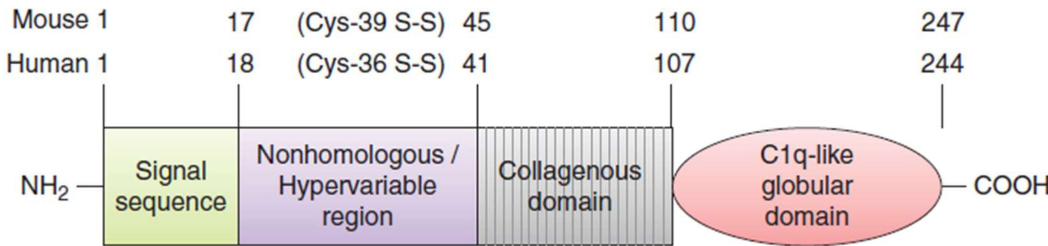

Figure 1 - Adiponectin protein structure. Human adiponectin consists of 244 amino acids and mouse adiponectin consists of 247 amino acids. Adiponectin is composed of an N-terminal signal sequence, a nonhomologous or hypervariable region, a collagenous domain, and a C1q-like globular domain. Cys-39/36 (mouse/human) is required for multimer formation (Fang & Judd, 2018).

Adiponectin primarily produced in adipocytes, where the monomeric protein posttranslationally modified into different multimers (Wang et al. 2002). Recombinant adiponectin produced by Escherichia *coli* consists of only monomeric adiponectin, demonstrating that adiponectin multimer formation

requires posttranslational processing by mammalian adipocytes (Wang et al. 2002).

Mammalian-produced adiponectin can be separated utilizing velocity sediment centrifugation into three complexes or multimers (Waki et al. 2003; Pajvani et al. 2003; Schraw et al. 2008). These multimeric complexes of adiponectin have been identified as low molecular weight form (LMW, trimer, ~90 kDa), middle molecular weight form (MMW, hexamer, ~180 kDa), and high molecular form (HMW, 12-18 monomers, ~360-540 kDa), though the nomenclature can vary among different research groups (Fig. 2).

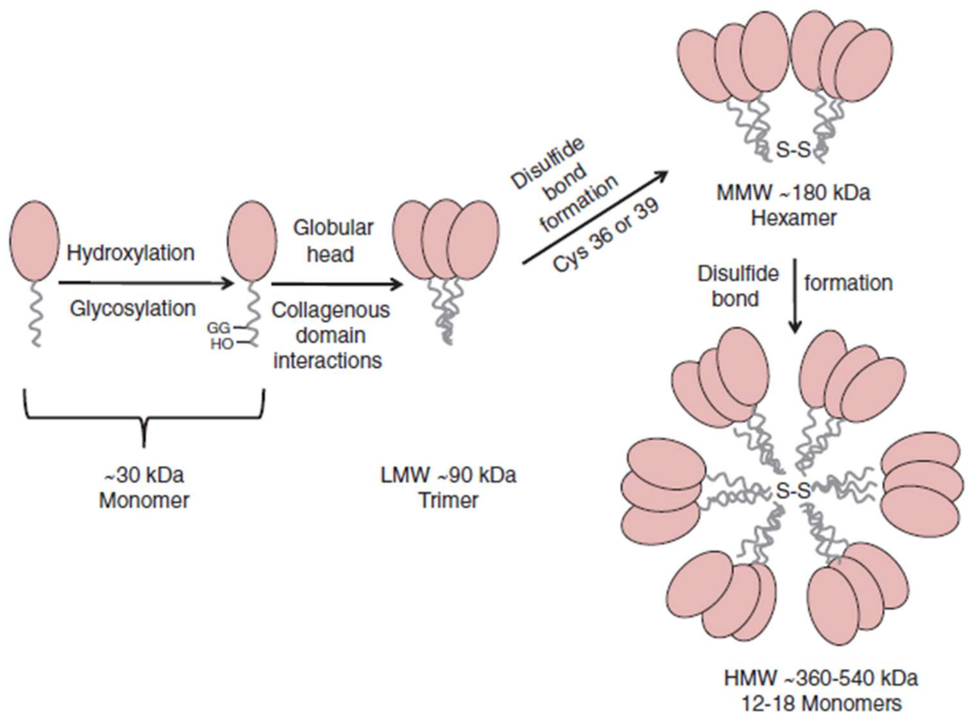

Figure 2 - Adiponectin multimer formation. In the endoplasmic reticulum, monomeric adiponectin is first hydroxylated and glycosylated on conserved lysine residues in the collagenous domain. Monomers form trimers through initial globular head attractions, which stabilized by interactions in the collagenous domain. Trimers (LMW) form hexamers (MMW) through disulfide bond formation between single cysteine residues in the N-terminal hypervariable region. Hexameric adiponectin converted to larger multimers of 12 to 18 monomers by additional disulfide bonding in the same region (Fang & Judd, 2018).

Multimeric complexes contribute significantly to the numerous biological effects of adiponectin, with multimer formation dependent on disulfide bond formation mediated by Cys-39 in the N-terminal hypervariable region (Pajvani et al. 2003; Waki et al. 2003). Hexameric and HMW multimer formation is impaired

when Cys-39 is mutated, including human mutants G84R, G90S, R112C, and I164T which are associated with hypoadiponectinemia and impaired multimer assembly and secretion (Pajvani et al. 2003; Waki et al. 2003). Cys-39 is also a key site of succination of adiponectin (Frizzell et al. 2009).

Succinated adiponectin not incorporated into multimeric forms in the cell or secreted from the cell (Frizzell et al. 2009). Assembly of adiponectin multimers occurs through a complex series of steps in the endoplasmic reticulum (ER) and Golgi apparatus (Xie et al. 2006; Wang et al. 2008). Monomers of adiponectin form trimers through hydrophobic interactions with the globular heads which stabilized by noncovalent interactions within the collagenous domains (Wang et al. 2008; Frizzell et al. 2009).

Trimeric adiponectin retained in the ER by the ER-resident chaperone ERp44. This retention is thiol-mediated and allows higher order complex formation through disulfide bond formation at Cys-39. Such complex formation is stimulated by ER oxidoreductase, Ero1, which displaces adiponectin from ERp44 and triggers Cys-39 disulfide bond formation to a hexamer and then to a HMW complex (Qiang et al. 2007). As such, Ero1 is critical for adiponectin complex formation and secretion. Another ER chaperone with oxidoreductase-like activity, DsbA-L, later discovered to play a significant role in HMW formation, stability, and secretion (Liu et al. 2008, Wang & Scherer 2008). DsbA-L is localized in the ER in adipocytes and plays a critical role in suppressing ER stress and promoting adiponectin multimerization and secretion (Liu et al. 2015). Therefore, ER lumen and associated chaperones provide a critical environment for the folding and assembly of adiponectin.

Physiological and pharmacological factors can have a tremendous impact on the ER environment, including the synthesis of ER chaperones. Obesity, diabetes, and other disease states are associated with ER stress and the unfolded protein response (UPR), designed to remove incorrectly folded proteins which may interfere with normal cell function (Ozcan et al. 2004; Marciniak & Ron, 2006). In 3T3-L1 adipocytes, induction of ER stress promotes autophagy-dependent adiponectin degradation (Zhou & Liu, 2000).

Suppressing ER stress in 3T3-L1 adipocytes increases adiponectin levels and alleviates high fat diet (HFD)-induced adiponectin downregulation in mice (Zhou & Liu, 2000; Zhou et al. 2010). Chemical or HFD-induced ER stress and associated adiponectin downregulation can be reversed by overexpression of DsbA-L (Zhou & Liu, 2000; Zhou et al. 2010; Liu et al. 2016). PPARγ agonists, such as TZDs, increase ER chaperone production and enhance the folding environment for adiponectin (Qiang et al. 2007; Wang et al. 2007; Jin et al. 2015). Specifically increasing DsbA-L in the adipose tissue increases the HMW form of adiponectin (Liu et al. 2012). On the other hand, DsbA-L reduced under obese conditions in both rodents and humans (Liu et al. 2008). TNF-α suppresses the multimerization and secretion of adiponectin by inhibiting the expression of Ero1, ERp44, and DsbA-L (He et al. 2016).

Mitochondrial dysfunction is also associated with ER stress, suggesting it may also play a role in adiponectin multimerization and function (Koh et al. 2007; Zhou et al. 2010). Adiponectin expression and mitochondrial content in adipose tissue reduced in obese *db/db* mice, and these changes reversed by the TZD, rosiglitazone (Koh et al. 2007). Increased mitochondrial biogénesis increases adiponectin synthesis, whereas impairment in mitochondrial function decreases it (Koh et al. 2007). Higher intracelular ROS levels produced by mitochondrial dysfunction results in an impairment in adipocyte function, including the secretion of adiponectin (Wang et al. 2013). Caveolin-1 null mice, which have altered metabolic and mitochondrial function in adipose tissue, display decreased circulating levels of total and high molecular weight adiponectin (Asterholm et al. 2012). Enhanced mitocondrial function, associated with a lower mitochondrial membrane potential and lower ROS levels, promotes adiponectin production and secretion (Kusminski et al. 2012). Therefore, there is a link between mitochondrial function and adiponectin biosynthesis, which may involve ER stress and/or other physiological mechanism links (Koh et al. 2007; Zhou et al. 2010; Liu & Liu 2014).

In addition to ER chaperones and disulfide bonding, the multimerization of adiponectin also regulated by hydroxylation and glycosylation (Richards et al. 2006, Wang et al. 2006; 2008). Hydroxylation and subsequent glycosylation by glucosyl-α (1-2) galactosyl group occurs on four conserved lysine residues (mouse: Lys- Fasshauer et al. 2002; Ferre 2004; Gao et al. 2013; Holland et al.

2007/human: Lys- Fasshauer et al. 2002; Ho et al. 2014; Fang et al. 2015) in the collagenous domain of adiponectin, each having the surround motif of GXKGE (D) (Wang et al. 2002b; Richards et al. 2006; Wang et al. 2008). Replacement of the lysine residues with arginine residues inhibits the formation and secretion of HMW adiponectin (Wang et al. 2002; 2006). Lysyl hydroxylase 3 (LH3), a multifunctional enzyme with lysyl hydroxylase, galactosyltransferase and glucosyltransferase activity and ER localization, is required for lysine hydroxylation and glycosylation (Ruotsalainen et al. 2002; Wang et al. 2002). In LH (luteinizing hormone) mutant mice, in which there is a point mutation in Asp669Ala leading to enzyme inactivation, a significant decrease in the HMW/total adiponectin ratio is observed (Ruotsalainen et al. 2002). Proline hydroxylation may also contribute to the formation of adiponectin multimers, but further supporting evidence remains to be discovered (Richards et al. 2006).

1.3 Adiponectin Mediated Signaling Pathways

Adiponectin was independently identified by four research groups using different methods nearly three decades ago and was thus assigned a series of names, including apM1 (adipose most abundant gene transcript 1), Acrp30 (adipocyte complement-related protein of 30 kDa), AdipoQ, and GBP28 (gelatin-binding protein of 28 kDa; Scherer et al. 1995; Maeda et al., 1996; Nakano et al. 1996). The adiponectin gene consists of three exons and two introns, spanning a 17-kb region, and is located on chromosome 3q27 in a region recently mapped as a susceptibility locus for type II diabetes and adiposity (Scherer et al., 1995; Hu et al. 1996). Human adiponectin contains 244 amino acid residues and consists of a 20-residue signal sequence, an N-terminal region, a collagen-like region, and a C-terminal globular domain. This polypeptide of 30 kDa is assembled into an array of complexes composed of multimers of 30 kDa polypeptide. Adiponectin subunits assemble into trimers, called low molecular weight (LMW) complexes, hexamers or middle molecular weight (MMW), or a more elaborate HMW complex composed of nine hexamers (>300 kDa; Liu & Liu, 2010) (Fig. 2). Full-length adiponectin (fAd) can cleaved by leucocyte elastase and can exist in serum as a globular form, thus creating trimers (gAd; Hada et al.

2007). The formation of HMW adiponectin in cells regulated by posttranslational modifications such as hydroxylation, glycosylation, and disulfide bond formation. Impairments in such modifications lead to a marked reduction in the intracellular levels of adiponectin and its subsequent secretion (Wang et al. 2008). Even if adiponectin protein contains a signal peptide, the molecular mechanisms of adiponectin secretion are not, as of yet, completely understood. The NAD-dependent deacetylase SIRT1 activity seems to be implicated (Qiang et al. 2007).

1.4 Gene Expression and Secretion

Adiponectin circulates in the plasma in remarkably high concentrations (for examples in humans, BMI<25: males 4-26 μg/mL, females 5-37 μg/mL—Mayo Medical Libraries), constituting approximately 0.01% to 0.05% of total serum proteins (Pajvani et al. 2003). This is roughly three orders of magnitude greater than most hormones, including leptin and insulin, in humans (Arita et al. 1999; Lui & Lui 2014). A gender-related difference in circulating adiponectin concentrations been observed, with females having higher total and HMW adiponectin than males in both rodents and humans (Nishizawa et al. 2002; Pajvani et al. 2003; Combs et al. 2003; Peake et al. 2005). The differences are due primarily to the inhibition of HMW adiponectin production by circulating testosterone in males (Nishizawa et al. 2002; Bottner et al. 2004).

Full-length adiponectin circulates in serum as three major forms: trimer, hexamer, and HMW adiponectin (Fruebis et al. 2001; Tsao et al. 2002; Waki et al. 2003; Pajvani et al. 2003; Waki et al. 2005; Qiang et al. 2007). These multimers have distinct biochemical characteristics and do not interconvert once present in the circulation (Schraw et al. 2008). The globular domain of adiponectin (alone, without the collagen stalk) also has biological activity, but is present at much lower levels than the other multimers of adiponectin (Fruebis et al. 2001; Hug & Lodish 2002; Chandran et al. 2003). Globular adiponectin generated locally through the activity of the enzyme leukocyte elastase, which cleaves adiponectin in its collagenous domain, resulting in a globular fragment of 18 to 25 kDa (Waki et al. 2005). Adiponectin has a relatively long half-life in serum (2.5-6

h), and these levels determined in part by ultradian patterns (Hoffstedt et al. 2004; Pajvani et al. 2004).

Adiponectin secreted with diurnal variation, with serum levels higher during the day (Gavrila et al. 2003). Plasma adiponectin concentrations influenced by factors regulate adiponectin gene expression and secretion. The role of insulin in adiponectin synthesis been investigated in detail, yet remains somewhat controversial.

A number of studies have demonstrated that insulin acutely stimulates adiponectin gene expression and secretion from 3T3-L1 adipocytes (Scherer et al. 1995; Pereira & Draznin 2005; Blumer et al. 2008). However, there have been reports insulin downregulating adiponectin gene expression in a dose- and time-dependent manner in a similar adipocyte model system (Fasshauer et al. 2002). Insulin-like growth factor (IGF-1) and growth hormone (GH) positively regulate adiponectin gene expression and secretion in murine and human adipose tissue (Halleux et al. 2001; Xu et al. 2004). Leptin has also been shown to increase adiponectin mRNA and protein in a dose-dependent manner using human white preadipocytes (Singh et al. 2016). In contrast, TNF-α and interleukin-6 (IL-6) inhibit adiponectin gene expression and secretion from 3T3-L1 adipocytes (Fasshauer et al. 2002; Fasshauer et al. 2003).

The regulation adiponectin gene expression is tightly controlled by number transcription factors (Shehzad et al. 2012). These transcription factors have binding sites on both the mouse and human adiponectin promoter, including peroxisome proliferator-activated receptor (PPAR)-response elements, C/EBP sites, sterol regulatory elements (SREs), and E-boxes. PPARγ, which expressed mainly in adipose tissue, the major positive regulator of adiponectin gene expression (Gustafson et al. 2003).

Deletion of PPARγ in adipose tissue results in decreased levels of adiponectin (He et al. 2003). PPARγ and its ligands, the TZDs, increase both adiponectin gene expression and secretion in rodents and humans (Maeda et al. 2001; Yu et al. 2002; Hammarstedt et al. 2005). These effects are PPARγ specific, as point mutations of the PPARγ binding site on the adiponectin

promoter results in reduced TZD-mediated promoter activation (Seo et al. 2004). CCAAT/enhancer-binding protein alpha (C/EBPα) also been identified an important adiponectin transcription factor in various adipocyte cell lines (Park et al. 2004). C/EBPα interacts with a response element in the intronic enhancer of the human adiponectin gene, resulting in full gene activation (Qiao et al. 2005). Forkhead box protein O1 (FOXO1) interacts with C/EBPα to form a transcription complex at the mouse adiponectin promotor that upregulates adiponectin gene transcription (Qiao & Shao, 2006). FOXO1 activation and formation of the FOXO1-C/EBPα complex increased by sirtuin, silent mating type information regulation 2 homolog (SIRT1) (Qiao & Shao, 2006).

Sterol regulatory element binding protein (SREBP)-1c is another activator of adiponectin transcription (Seo et al. 2004). SREBP-1c mediates adiponectin expression in differentiating 3T3-L1 adipocytes and OP9 mouse stromal cells by interacting with the E-protein, E47, on the adiponectin promoter (Doran et al. 2008). cAMP response element binding protein (CREB) plays an important role in the regulation of adiponectin gene expression in response to growth factors, including IGF-1 (Kim et al. 2010). CREB increases the activity of adiponectin promoter, resulting increased levels of adiponectin mRNA and HMW form adiponectin (Qi et al. 2009).

Unlike most other adipokines, adiponectin gene expression and blood concentrations are inversely associated with fat mass and obesity (Swarbrick & Havel 2008; Lee & Shao 2014). Adiponectin mRNA and plasma concentrations decreased in both obese mice and humans compared with normal controls (Hu et al. 1996; Arita et al. 1999). Weight loss (≈10-20%) due to caloric restriction in obese humans dramatically increases adiponectin adipose tissue gene expression and plasma concentrations toward normal lean levels (Hotta et al. 2000; Bruun et al. 2003). Low caloric intake associated with anorexia nervosa is also associated with increases in plasma adiponectin concentrations, suggesting that energy intake plays an important role in the regulation of adiponectin gene expression (Delporte et al. 2003). However, adipose mass itself also plays a role in plasma adiponectin concentrations, with visceral adipose tissue mass negatively associated with plasma adiponectin concentrations (Cnop et al. 2003,

Gavrila et al. 2003). Interestingly, this does not appear to be the case with subcutaneous adipose tissue, which positively correlated with plasma adiponectin concentrations (Addy et al. 2003). In support of this, bariatric surgery-induced weight loss results in a significant increase in the circulating concentration of adiponectin (Yang et al. 2001; Linscheid et al. 2008). Pharmacological treatment of obese mice and insulin-resistant overweight humans with TZDs also increases systemic adiponectin concentrations (Maeda et al. 2001). In all of these cases, increases in adiponectin correlated with improved insulin sensitivity (Yang et al. 2001; Bruun et al. 2003).

1.5 Receptors and Signal Transduction

In 2003, through a sequence of expression cloning experiments, AdipoR1 and AdipoR2 first identified as receptors for adiponectin (Yamauchi et al. 2003) (Fig. 3). AdipoR1 and R2 ubiquitously expressed, but AdipoR1 is more highly expressed in skeletal muscle and AdipoR2 is mostly restricted to the liver (Yamauchi et al. 2003). AdipoR1 is high-affinity receptor for globular adiponectin and a low-affinity receptor for full-length adiponectin, whereas AdipoR2 is an intermediate receptor for both globular and full-length adiponectin (Yamauchi et al. 2003). AdipoR1 and R2 structurally related to one another, with a 67% homology at the protein level. Similar to other G proteincoupled receptors (GPCRs), both AdipoR1 and R2 contain seven transmembrane-spanning domains. However, they are structurally and functionally opposite all known GPCRs, with the N-terminus of the adiponectin receptor oriented intracellularly, the C-terminus extracellularly and no association with G proteins (Yamauchi et al. 2003). The adiponectin receptor belongs to the progesterone and adiponectin Q receptor (PAQR) family (Tang et al. 2005).

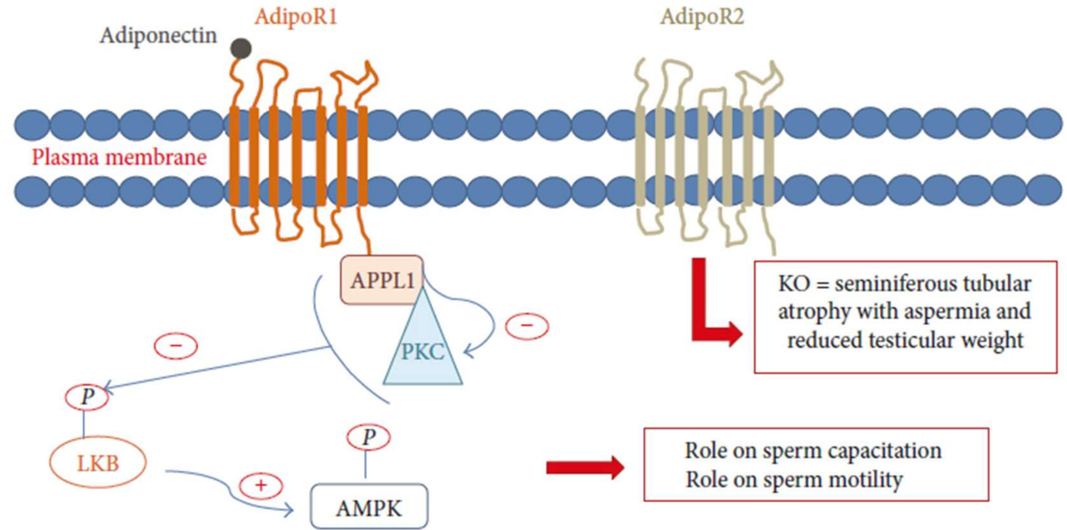

Figure 3. Adiponectin receptors and its possible interactions to fertility. Modified from Elfassy et al. (2018) based on Kasimanickam et al. (2013), Kawwass et al. (2015) and Kadivar et al (2016).

Crystallization of human AdipoR1 and R2 demonstrated that these receptors represent a novel structure class in which there is a large cavity enclosed by the seven-transmembrane helices in both AdipoR1 and R2 (Tanabe et al. 2015). The ligand and receptor interaction takes places between the globular domain of adiponectin and the extracellular surface of the receptors.

AdipoR1 and R2 contain a zinc-binding catalytic site coordinated by three histamine residues and located near the inner surface of the plasma membrane (Tanabe et al. 2015). Adiponectin binding to the zinc-binding motif of AdipoR1 and R2 initiates a series of downstream signaling events in many target tissues, including skeletal muscle, liver, heart, kidney, and pancreas (Holland et al. 2011). Activation of AdipoR1 and R2 by globular and full-length adiponectin increases adenosine monophosphate protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation and increases peroxisome proliferator-activated receptor alpha (PPARα) ligand activity, resulting in increased fatty acid oxidation and glucose utilization (Yamauchi et al. 2003). In genetically obese *ob/ob* mice, adenovirus overexpression of AdipoR1 activates AMPK, leading to decreased gluconeogenic gene expression (Yamauchi et al. 2007). Adenovirus overexpression of AdipoR2 in *ob/ob* mice increases AMPK activation and PPARα activity (Yamauchi et al. 2007).

In 2006, an adaptor protein containing a pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif (APPL1), identified for adiponectin (Mao et al. 2006). APPL1 directly binds to the intracellular regions of both AdipoR1 and R2, through its C-terminal PTB and CC domains (Mao et al. 2006). This engagement of AdipoR1 and R2 by APPL1 mediates the downstream effects of AMPK activation, including increased glucose uptake and lipid oxidation (Zhou et al. 2009; Deepa et al. 2011). APPL1 stimulates adiponectin-mediated AMPK activation by activating protein phosphatase 2A (PP2A) and inactivating protein kinase Cζ (PKCζ), which dephosphorylates liver kinase B1 (LKB1) (Deepa et al. 2011). This allows LKB1 to translocate from the nucleus to the cytosol and phosphorylate AMPK (Zhou et al. 2009; Deepa et al. 2011). Adiponectin also stimulates the interaction between APPL1 and Rab5 (a small GTPase downstream of APPL1) and p38 MAPK, leading to an increase in glucose uptake (Mao et al. 2006; Xin et al. 2011). More recent *in vitro* and *in vivo* mouse studies have demonstrated that APPL1 can promote the interaction of IRS1/2 to the insulin receptor, and that this interaction stimulated by both insulin and adiponectin binding to their respective receptors (Deepa & Dong 2009; Ryu et al. 2014). Wholebody knockout of APPL1 impairs adiponectin signaling and results in insulin resistance in major insulin target tissues (Ryu et al. 2014). Thus, APPL1 is a critical regulator of adiponectin action in whole-body energy homeostasis. It is important to note that an isoform of APPL1, APPL2, negatively modulates adiponectin signaling in murine skeletal muscle cells (Wang et al. 2009). APPL2 directly binds to AdipoR1 and R2 via its BAR domain, thereby competitively preventing the interaction of APPL1 with AdipoRs and blocking adiponectin signaling (Wang et al. 2009; Ruan & Dong, 2016). APPL1 and APPL2 also form heterodimers impair adiponectin action (Wang et al. 2009; Ruan & Dong, 2016). Adiponectin stimulates the dissociation of the APPL1/APPL2 heterodimer, freeing up APPL1 to engage AdipoR1 and R2 (Wang et al. 2009; Ruan & Dong, 2016). These opposing actions of APPL1 and APPL2 considered the “Ying & Yang” modulatory concept of adiponectin signaling (Wang et al. 2009).

Adiponectin can also act independently of APPL1 to activate AMPK. Adiponectin primarily does this by increasing the intracellular concentrations of

calcium, either by inducing release of calcium from intracellular stores or increasing the extracellular flux of calcium into the cell. Adiponectin activation of phospholipase C, which subsequently produces inositol 3-phosphate (IP3), leads to calcium release from the ER (Zhou et al. 2009). Calcium released from the ER stimulates the upstream kinase Ca^{2+}/calmodulin-dependent protein kinase (CaMKK-β), which activates AMPK in an APPL- 1-independent manner (Zhou et al. 2009). Adiponectin can also promote extracellular calcium influx by activating AdipoR1, though the precise mechanism not well understood (Iwabu et al. 2010).

Calcium stimulates peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1-α) through both the calmodulin-dependent protein kinase (CaMK) pathway and via AMPK, which activates SIRT1 (Iwabu et al. 2010). These calciummediated effects on CaMKK-β and AMPK observed primarily in the skeletal muscle, where they result in an overall increase in mitochondrial biogenesis and oxidative capacity (Zhou et al. 2009; Iwabu et al. 2010).

In addition to downstream signaling events, adiponectin activation of AdipoR1 and R2 lowers cellular ceramide, a sphingolipid that has been implicated in insulin resistance, cell death, inflammation, and atherosclerosis (Holland et al. 2008; Chaurasia et al. 2015). Ceramides are increased in diabetes, and compounds which increase adiponectin, including fibroblast growth factor 21 (FGF21) and TZDs, are associated with a decrease in ceramide levels (Holland et al. 2013; Chaurasia et al. 2015; Warshauer et al. 2015). Specifically, adiponectin administration decreases ceramide levels in the liver and other tissues, resulting in increased hepatic insulin sensitivity (Holland et al. 2011). Mechanistically, this is due to the association of ceramidase with the adiponectin receptors independent of AMPK (Holland et al. 2011).

Ceramidase hydrolyses ceramide into sphingosine, which phosphorylated to sphingosine 1-phosphate (S1P). S1P is a beneficial lipid promotes cell survival and proliferation (Holland et al. 2008; Wang & Scherer, 2016). Knockout of both AdipoR1 and R2 in mouse embryonic fibroblasts (MEFs) decreases adiponectin induced ceramidase activity, leading to lower S1P concentrations (Holland et al.

2011). In contrast, overexpression of AdipoR1 and AdipoR2 in the liver is associated with an increase in ceramidase activity, resulting in a lowering of ceramide content and an improvement in insulin sensitivity (Holland et al. 2011). Adiponectin binding to AdipoR1 and R2 can sequester and induce catalytic ceramidase activity and/or the adiponectin receptors themselves can catalyze ceramidase activity (Holland et al. 2011). Structural studies have demonstrated that AdipoR2 has an internal tunnel, which allows ceramide to enter from the intracellular side upon receptor activation (Vasiliauskaite-Brooks et al. 2017). Once ceramide enters the receptor tunnel, it hydrolyzed to sphingosine, which is release into the cell and phosphorylated to S1P (Vasiliauskaite-Brooks et al. 2017). AdipoR1 does not appear to have the same internal tunnel span, but does have adiponectin-dependent inherent ceramidase activity (Vasiliauskaite-Brooks et al. 2017).

AdipoR1 and R2 expression levels altered in different pathophysiological states, including diabetes and obesity. AdipoR1 and R2 expression increased in skeletal muscle of streptozotocin (STZ) diabetic mice, a model of type 1 diabetes (Tsuchida et al. 2004). Insulin treatment reduces elevated expression levels of both adiponectin receptors (Tsuchida et al. 2004). Hepatic expression of AdipoR1 and R2 in STZ diabetic mice is not significantly different from non-STZ mice (Tsuchida et al. 2004). However, insulin decreased hepatic expression of both receptors in STZ diabetic mice (Tsuchida et al. 2004). In insulin-resistant obese *ob/ob* mice (decreased leptin production), the expression of both AdipoR1 and R2 is decreased in muscle and adipose tissue but not in the liver (Tsuchida et al. 2004). Additional studies in *Lepr−/−* mice (mutated leptin receptor, *db/db*), a genetic animal model of obesity and diabetes, demonstrated that both AdipoR1 and R2 expression in the liver is decreased and correlated with reduced adiponectin sensitivity and impaired whole-body metabolism (Yamauchi et al. 2007). Adenovirus-mediated expression of AdipoR1 and R2 in the liver of *Lepr−/−* increased AMPK activation and PPARα signaling pathways, respectively (Yamauchi et al. 2007). These expression changes reduced gluconeogenesis and de *novo* lipogenesis, and increased fatty acid oxidation, which led to an amelioration of diabetes in *Lepr−/−* mice (Yamauchi et al. 2007). In humans, AdipoR1 and R2 expression in skeletal muscle is lower in patients with a family

history of type 2 diabetes, and the expression of both receptors positively correlates with insulin sensitivity (Civitarese et al. 2004). In contrast, Debard et al. (2004) did not observe any significant differences in expression of AdipoR1 or R2 in skeletal muscle of insulin-resistant obese patients with type 2 diabetes. AdipoR1 and R2 expression decreased in subcutaneous and visceral adipose tissue from obese subjects (Rasmussen et al. 2006; Nannipieri et al. 2007). Interestingly, weight loss has been associated with an increase in AdipoR1 gene expression, but not AdipoR2 in obese subjects (Rasmussen et al. 2006). Rosiglitazone, a representative TZD, increased AdipoR1 gene expression in adipose tissue but decreased AdipoR1 in skeletal muscle, with no impact on AdipoR2 (Tan et al. 2005). In contrast, Pioglitazone, another representative TZD, increased both AdipoR1 and R2 gene expression in skeletal muscle of type 2 diabetes patients (Coletta et al. 2009).

In 2004, it discovered that the T-cadherin receptor binds adiponectin in C_2C_{12} mouse myoblasts (Hug et al. 2004). Tcadherin is an atypical glycosylphosphatidylinositol (GPI)- anchored cadherin cell surface glycoprotein involved in calcium-mediated cell-cell contacts, and is structurally distinct from AdipoR1 and R2 (Hug et al. 2004). T-cadherin receptor does not have a transmembrane or intracellular domain, but plays a role in binding adiponectin and promoting adiponectin signaling in endothelial and smooth muscle cells, which are the major sites of T-cadherin expression (Hug et al. 2004). Tcadherin receptors bind HMW as well as hexameric forms of adiponectin, but not the trimeric, globular, or bacterially produced multimers of adiponectin, demonstrating that eukaryotic posttranslational modifications are required for adiponectin binding to T-cadherin receptors and proper biological function (Hug et al. 2004). Circulating levels of adiponectin, particularly the HMW form of adiponectin, are elevated in T-cadherin-deficient mice (Denzel et al. 2010). Additional studies have demonstrated that adiponectin levels are dependent on Tcadherin and reverse, levels regulate tissue T-cadherin levels by suppressing GPI phospholipase-mediated release of T-cadherin from the cell surface (Matsuda et al. 2015). T-cadherin plays an important role in adiponectin-mediated revascularization after chronic ischemia by promoting cell migration and proliferation (Parker-Duffen et al. 2013). T-cadherin on cardiac myocytes also

mediates the anti-hypertrophic role of adiponectin by binding adiponectin and activating its cardioprotective functions, including adiponectin-dependent AMPK phosphorylation (Denzel et al. 2010).

In the testis, AMPK, MAPK, and PPAR-a signaling pathways have been shown to be functional and involved in the regulation of steroidogenesis (Li et al. 2011; Brion et al. 2011; Ahn et al. 2012). Therefore, adiponectin might act through these signaling pathways to modulate testicular testosterone production. Metabolic hormones such as leptin, resistin, and adiponectin may act through the AMPK signaling pathway to influence catabolic and anabolic pathways according to energy (ATP) demands. Since the reproductive system tightly coupled to energy balance, the AMPK signaling pathway might be important for adipose-derived hormones actions on reproduction (Tosca et al. 2008). Indeed, AMPK activation reduces progesterone secretion through inhibition of the MAPK extracellular signal-regulated kinase 1/2 (ERK$^{1}/_{2}$) signaling pathway in rat and bovine granulosa cells (Tosca et al. 2005; 2007).

In HUVEC cells, adiponectin counteracts high glucoseinduced ROS production through a mechanism dependent on the cAMP/PKA pathway (Ouedraogo et al. 2006), suggesting that adiponectin might influence testicular steroid production in Leydig cells through actions on the classical LH/cAMP/PKA signaling cascade. In macrophages, globular adiponectin induces IL-10 production by stimulating the phosphorylation of cAMP response element-binding protein (CREB), consequently leading to the transactivation of the IL10 promoter (Park et al. 2008). However, whether or not these adiponectin-mediated signaling cascades also operate in male reproductive function remains to investigate. In addition to local effects at the testicular level, adiponectin may also influence male reproduction through actions on the hypothalamus and pituitary.

2. ADIPOSE TISSUE GLAND

Adipose tissue has long considered a depot for triglycerides during times of metabolic affluence and/or for mobilization during periods of nutrient deprivation. Over the past two decades, adipose tissue has emerged as an endocrine gland that modulates events, including appetite, lipid uptake, and

metabolism, and even the growth of adipose precursor cells. Both the excess of fat mass in obesity and its paucity in anorexia known to result in dysfunctions of reproduction.

Adipose tissue secretes hormones known as adipokines, reflecting their origin and their effects as cytokines on target tissues. Among the best examples of an adipokine that plays a pivotal role in reproductive process in mammals is leptin (Fig. 4). Indeed, leptin-null mice are obese and infertile (Zhang et al. 1994), and administration of recombinant leptin to these animals corrects obesity and restores full fertility (Chehab et al. 1996).

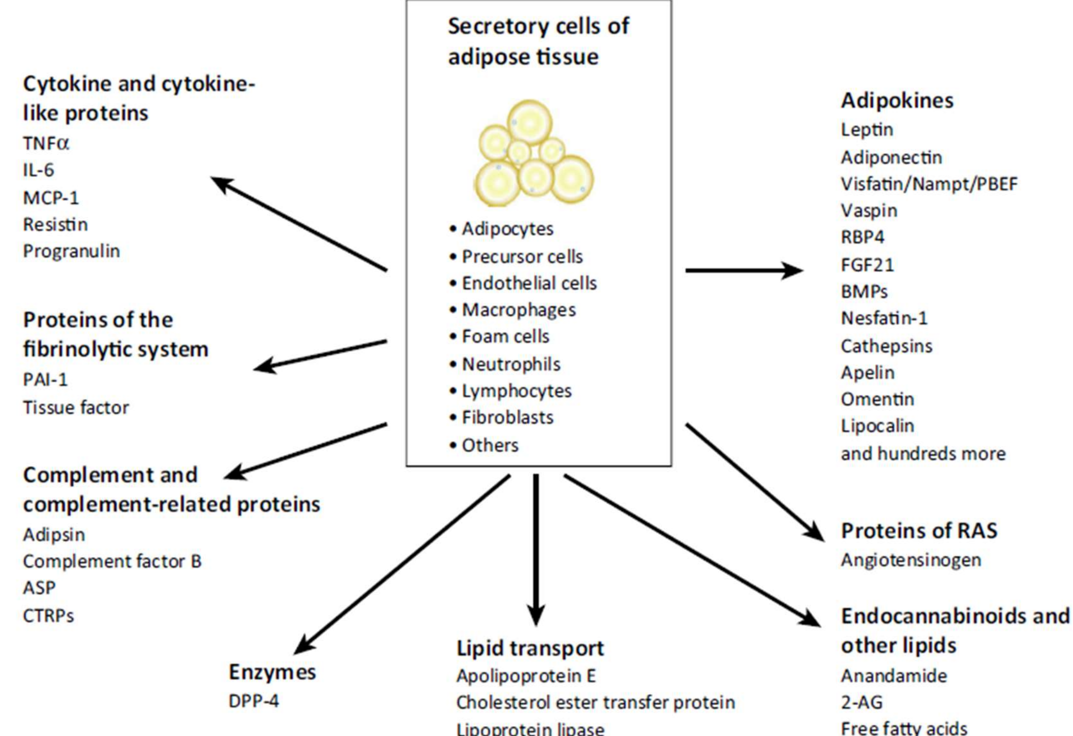

Figure 4. Factors released or secreted by adipose tissue. Adipocytes, immune cells, fibroblasts, endothelial cells, and others contribute to the release of metabolites, lipids, and adipokines. Examples of adipose tissue-derived molecules provided here. Abbreviations: 2-AG, 2-Arachidonoylglycerol; ASP, acylating simulation protein; BMPs, bone morphogenetic proteins; CTRPs, C1q/TNF-related proteins; FGF21, fibroblast growth factor 21; MCP-1, monocyte chemotactic protein-1; PAI-1, plasminogen activator inhibitor-1; RAS, renin angiotensin system; RBP4, retinol binding protein 4 (Fasshauer & Blüher, 2015).

Furthermore, leptin stimulates gonadotropin secretion at the hypothalamic and pituitary levels directly modulate ovarian steroidogenesis, the embryonic implantation process, and placental functions (Cervero et al., 2006; Henson &

Castracane, 2006; Guerre-Millo, 2008). Adiponectin is another adipokine that known for its important role in the regulation of energy homeostasis. More precisely, adiponectin described as a molecule that has antidiabetic, antiatherogenic, anti-inflammatory, and angiogenic properties. Recently, it been proposed to be involved in the control of reproductive functions. Adiponectin may be an important signal indicating the adequacy of nutritional status for reproductive function (Mitchell et al. 2005; Campos et al. 2008). The present review summarizes the structure, expression regulations, transduction signaling, and the implication of adiponectin in the reproductive processes.

3. REGULATION OF REPRODUCTIVE FUNCTIONS IN DIFFERENT REPRODUCTIVE AGES

Adiponectin receptor mRNA expressions in chicken displayed modifications during the puberty. Their expressions were found be increased in adulthood as compared to the levels of expression in the prepubertal phases (Ocón-Grove et al. 2008). In Leydig cells in rodents, the expressions of AdipoR2 protein as well the adiponectin serum concentrations, also showed an increase during puberty (Gui et al. 2004; Pfaehler et al. 2012). These observations may indicate that either physiological changes through the puberty have led to upregulation in expressions of testicular adiponectin and its receptors, or adiponectin may have an essential role in initiating the physiological changes during puberty.

4. PRESENCE AND FUNCTIONS IN MALE REPRODUCTIVE TISSUES

The mechanisms by which testicular functions decline with aging remain largely speculative. Our recent finding showed the importance of adiponectin in the regulation of testicular functions, whereas its concentration declines during male infertility. Choubey et al. (2019), working with mice reported the changes in adiponectin, adiponectin-receptors, and insulin receptor proteins expression in the testis evaluated and compared with the testicular parameters, mass, and

testosterone level in the mice from early post-natal to late senescence period. Further, the current study has examined the effect of exogenous adiponectin treatment on testicular functions in aged mice. The results showed a significant decline in adiponectin/adiponectin-receptors expression simultaneously with a significant decline in testicular mass, insulin receptor expression and testosterone synthesis in the testis of aged mice. Exogenous treatment of adiponectin to aged mice resulted in marked improvements in testicular mass, histological features (cells proliferation), insulin receptor expression, testicular glucose uptake, anti-oxidative enzymes activity and testosterone synthesis as compared with the control. Based on these findings, it may be concluded that a marked decline in adiponectin synthesis and action results in decreased insulin sensitivity (development of insulin resistance) and increased oxidative stress which consequently suppresses testicular functions during aging. This study further showed that treatment with adiponectin ameliorates reduced testicular functions by enhanced expression of insulin receptor in the testis of senescent mice.

5. EXPRESSION AND FUNCTIONS OF ADIPONECTIN IN THE MALE REPRODUCTIVE AXIS (HPG)

The sum of current research findings suggests that reproductive functions closely related to energy homeostasis and that metabolic dysregulation, such as obesity and anorexia nervosa, often leads to reproductive abnormalities. Widespread pattern of expression of AdipoR1 and AdipoR2, which include not only muscle, liver, and adipocytes but also hypothalamus and pituitary (Rodríguez Pacheco et al. 2007). The presence of AdipoR1 and AdipoR2 in the central nervous system (CNS) and the pituitary gland suggests that adiponectin may be involved in the signaling by adipose tissue to the brain to regulate reproductive functions. The first indications that adiponectin might act directly on the CNS came from studies reporting the presence of AdipoR1 and AdipoR2 transcripts in human and mouse whole brain tissues (Yamauchi et al. 2003; Neumeier et al. 2007). Since then, these two receptors have been detected in the human and rat hypothalamus (Hoyda et al. 2007; Kos et al., 2007; Guillod-

Maximin et al. 2009), in the human, rat, and chicken pituitary gland (Rodríguez-Pacheco et al. 2007), in GT1-7 hypothalamic gonadotropinreleasing hormone (GnRH) neurons (Wen et al. 2008), and also in LβT2 pituitary gonadotroph cells (Lu et al. 2008).

The HPG axis is of vital importance in mammalian reproductive system (Schneider, 2004). The secretion and gene transcription of the pituitary gonadotropins LH and follicle-stimulating hormone (FSH) driven by pulsatile release of gonadotropin-releasing hormone (GnRH) from neurons in the hypothalamus. Gonadotropins control gonadal steroid hormones production and sex hormones exert feedback regulation on GnRH, LH and FSH synthesis and secretion (Burger et al. 2004). In response to energy insufficiency, the bodies usually choose to reduce all the dispensable energy expenditure in mammals.

Qi et al. (2004) were the first to detect adiponectin in mice cerebrospinal fluid (CSF), though adiponectin concentrations represented only 1–4% of that found in the mice serum. These findings were then confirmed in human (Ebinuma et al. 2007; Kos et al. 2007; Kusminski et al. 2007; Neumeier et al. 2007) and rats (Caja et al. 2005), where adiponectin concentration in CSF was found to be approximately 1000-fold lower when compared with serum levels. Moreover, it earlier reported that the trimeric LMW adiponectin is the predominant form found in human CSF (Ebinuma et al. 2007; Kusminski et al. 2007) and that HMW adiponectin inefficiently crosses the blood-to-brain barrier. Higher CSF adiponectin levels were found in females than in males (Qi et al. 2004; Neumeier et al. 2007), which is in agreement with gender differences observed in serum (Combs et al. 2003).

Although several studies detected adiponectin in CSF, conflicting results reported with respect to the possible translocation of adiponectin across the blood–brain barrier. Qi et al. (2004) observed that an increase in CSF adiponectin concentrations after intravenous injection of recombinant adiponectin, thus suggesting a blood-to-CSF transport. On the opposite, Spranger et al. (2006) were unable to detect adiponectin in human CSF, and they demonstrated that radiolabeled globular adiponectin was unable to cross the blood-to-brain barrier in mice. Similar results were reported by Pan et al. (2006), reported the absence

of blood-to-brain barrier permeation by adiponectin. The presence of AdipoR1 and AdipoR2 transcripts in brain endothelial cells and the observed reduction in inflammatory cytokine secretion by adiponectin in those cells further suggested that adiponectin does not need to cross the blood-to-brain barrier to exert its effect on the CNS (Spranger et al. 2006). The expression of AdipoR1 and AdipoR2 genes in the rat area postrema (AP), which lacks a blood-to-brain barrier, and the modification of AP neurons membrane properties with the addition of globular adiponectin suggest another possible site of adiponectin action in the CNS (Fry et al. 2006).

In the hypothalamus, AdipoR1 and AdipoR2 colocalize with the leptin receptor Ob-R and adiponectin crosses the blood–brain barrier to activate these receptors (Kubota et al. 2007). Interestingly, the main forms of adiponectin found in cerebrospinal fluid are trimer and hexamer (Kubota et al. 2007), higher molecular weight oligomers being too large to cross the blood–brain barrier (Waki et al. 2003; Pan et al. 2006; Spranger et al. 2006). However, globular adiponectin administered intravenously shown to increase the cerebral spinal fluid levels (Qi et al. 2004). Circulating adiponectin may accumulate in the central nervous system through the ventricular space, as demonstrated with the detection of adiponectin complexes in human cerebrospinal fluid (Kusminski et al. 2007). In addition, adiponectin synthesized locally in the CNS.

In GT1-7 hypothalamic GnRH neuron cells, GnRH secretion is inhibited by adiponectin through activation of AMPK and ERK1/2 (Cheng et al. 2011), possibly leading to *KISS1* transcriptional regulation (Wen et al. 2008). Indeed, adiponectin shown to inhibit *KISS1* gene transcription through activation of AMPK and decreased nuclear translocation of SP-1 in these cells (Wen et al. 2012). In the hypothalamus, *KISS1* expression is the upstream signal of GnRH production and secretion.

In the pituitary, adiponectin regulates hormone secretion and gene expression in two critical endocrine cell types involved in reproduction, somatotrophs and gonadotrophs, by inhibiting GH and LH releases, while no impact was exerted on FSH concentrations (Rodriguez-Pacheco et al. 2007; Psilopanagioti et al. 2009). Adiponectin and its receptors AdipoR1 and AdipoR2

have been described in rat and human hypothalamus (Kos et al. 2007) and pituitary (Rodriguez-Pacheco et al. 2007), suggesting a possible autocrine/paracrine action of adiponectin at the hypothalamic and pituitary levels. AdipoR1 and AdipoR2 expressed in mouse LβT2 immortalized gonadrotropes (Lu et al. 2008). In rat primary pituitary cells, GnRH receptor expression regulated by recombinant adiponectin (Rodríguez-Pacheco et al. 2007). In mouse LβT2 gonadotrope cells and in rat pituitary cells, recombinant adiponectin inhibits both basal and GnRH-stimulated LH secretion (Rodriguez-Pacheco et al. 2007; Lu et al. 2008). This action exerted through increased AMPK phosphorylation, supporting that pituitary AMPK may serve as an energy sensor of the nutritional status and accordingly regulate gonadotropin secretion in order to control reproduction (Tosca et al. 2008). Thus, results from hypothalamic and pituitary cell lines suggest that adiponectin may have inhibitory actions on hypothalamic and pituitary secretions of GnRH and LH, respectively. Overall, adiponectin may serve as a signal that links metabolism and endocrine control to reproduction and growth.

Accumulating evidence suggests that adiponectin may regulate reproductive functions through the modulation of gonadotropic hormone secretion. For example, adiponectin can inhibit LH release and GnRH stimulated LH secretion in rat pituitary cell cultures (Rodriguez-Pacheco et al. 2007). However, these effects only observed after short-term exposures (4 h) to recombinant adiponectin. Interestingly, the GnRH receptor transcript levels reduced by 50% in pituitary cells treated with adiponectin, thus suggesting that adiponectin may reduce LH secretions through reduced gonadotroph sensitivity to GnRH (Rodriguez-Pacheco et al. 2007). Similar results were observed in mouse LβT2 gonadotroph cells, where an acute treatment (30 min) with globular and full-length adiponectin inhibited both the basal and GnRH-stimulated secretions of LH, whereas chronic exposure to adiponectin (48 h) had no effect on LH secretion (Lu et al. 2008). It further demonstrated that adiponectin effect on LβT2 cells LH secretions mediated through an increased activation of AMPK (Lu et al. 2008). In the rat hypothalamus, intracerebroventricular injections of human globular adiponectin also induced AMPK phosphorylation (Guillod-Maximin et al. 2009). It was further demonstrated that globular adiponectin inhibit

GnRH secretion in GT1-7 hypothalamic GnRH neurons through the activation of the AMPK signaling pathway (Wen et al. 2008). Collectively, these results suggest that hypothalamic and pituitary AMPK may act as a whole-body energy sensor mediating adiponectin action on gonadotropin release and reproductive function.

It is well known that reproductive capacity is metabolically gated and growing number of neuropeptides and hormone signals, primarily involved in the control of energy balance and metabolism, have been recently proven as putative regulators of puberty maturation, gonadotropin function, and/or fertility (Fernández-Fernández et al. 2006). Among those, the prominent role of the adipocyte-derived hormone, leptin, in the control of reproduction well characterized over the last decade (Casanueva & Dieguez, 1999; Tena-Sempere & Barreiro, 2002; Tena-Sempere, 2007).

In contrast, the physiological role, if any, of other adipose-born signals in the modulation of reproductive function remains ill defined. Notwithstanding, given its functional profile, the putative reproductive functions of adiponectin have begun to be explored recently. Further evidence for a physiological link between adiponectin and reproductive function came form the observation that adiponectin concentrations are invariantly higher in females than in males and androgens inhibit adiponectin secretion (Nishizawa et al. 2002; Lanfranco et al. 2004). Chabrolle et al. (2007) documented expression of adiponectin and its receptors in the rat ovary, in which adiponectin has been demonstrated to modestly stimulate progesterone and estradiol secretion in response to IGF-I. The same year, Archanco et al. (2007), have documented the presence of adiponectin in rat oviduct has been recently documented. Whether adiponectin is expressed and/or able to conduct direct actions in the testis and/or male reproductive tract remains unexplored to date.

Worth noting, a number of hormonal signals with key roles in energy homeostasis and metabolism expressed and/or conduct biological actions directly at the testicular level. These been reported in a diversity of species (from rodents to humans) and include not only leptin but also ghrelin, the gut-derived orexigenic signal, and resistin (Barreiro & Tena-Sempere, 2004; Nogueiras et al.

2004; Tena-Sempere, 2007). Caminos et al. (2008) worked to evaluate the potential expression of adiponectin and its related receptors, AdipoR1 and AdipoR2, in rat testes, and its hormonal and metabolic regulation, as well as the eventual direct testicular actions of this adipokine, revealing the potential role of adiponectin in the direct control of testicular function.

Adiponectin and its receptors ubiquitously expressed. Indeed, the adiponectin system (adiponectin, AdipoR1, and AdipoR2) described in rat and human hypothalamus (Kos et al., 2007) and pituitary (Rodriguez-Pacheco et al. 2007). Adiponectin and its receptors have also been identified in rat testis (Caminos et al. 2008) and in ovary of various species including pig (Lord et al. 2005; Ledoux et al., 2006), chicken (Ramachandran et al. 2007; Chabrolle et al. 2007), and rat (Pajvani et al. 2003). Interestingly, interstitial Leydig cells from the rat testis, responsible for male testosterone production, express the adiponectin receptor AdipoR2 (Camino et al. 2008). These findings are also of interest for men since AdipoR1 and AdipoR2 characterized in human testis (Civitarese et al. 2004).

Recently Bai et al. (2018) demonstrated, working sheep in the reproductive season, sexual development is implicit in increase the expression of Adiponectin and its receptors AdipoR1 and AdipoR2. However, there was no significant difference in the expression of adiponectin mRNA in sexually immature and sexually mature tests. This study will provide novel evidences for the physiological role of adiponectin and its receptors on testes development. Choubey et al (2019) present similar results been found in mice, where the expression of adiponectin and its receptors (AdipoR1 and AdipoR2) declines significantly in the testis of old animals.

5.1 Adiponectin as anti-inflammatory mediator in testis

Adiponectin been claimed to have regulatory actions over both spermatogenesis and steroidogenesis via its receptors, AdipoR1 and AdipoR2 (Wen et al. 2012; Choubey et al. 2019). *In vitro* experiments demonstrated direct actions of adiponectin on Leydig cells to downregulate androgen secretions, via

inhibiting the steroidogenic acute regulatory protein in Leydig cells (Pfaehlet et al. 2012).

Adiponectin, on binding to its receptors, may trigger the intracellular signaling cascades involving the proteins such as AMPK, peroxisome proliferator-activated receptor-alpha and mitogen-activated protein kinase (Kadowaki & Yamauchi, 2005). This signaling pathway finds relevance in the regulation of testicular functions, essentially steroidogenesis (Ahn et al. 2012). The adiponectin induction of the testicular signaling pathway relevant for steroidogenesis suggests the role of adiponectin in regulating the process of testosterone production.

Another essential aspect of adiponectin action is its capability to sustain insulin sensitivity via induction of testicular glucose uptake (Choubey et al. 2019). It well known that intratesticular glucose level is one of the major regulators of vital testicular functions such as steroidogenesis (Banerjee et al. 2014). Exogenous administration of adiponectin in aged mice has shown to ameliorate testicular mass and functions via elevated expressions of the insulin receptor, inducing the activities of antioxidative enzymes, testosterone biosynthesis along with testicular glucose, lactate uptake by increased tumor of glucose and lactate transporter proteins (Choubey et al. 2019).

Adiponectin also displays some anti-inflammatory properties that render protection to the Leydig cells from inflammatory cytokines and chemokines-mediated cytotoxicity. Thus, adiponectin acts as a testicular defense mechanism to combat the impacts of proinflammatory mediators on steroidogenesis, such as those of the macrophage-derived tumor necrosis factor-α, interleukin 1, and interferon-γ (Wu et al. 2013).

The adiponectin signaling in male gonadal tissue seems to be essential for various testicular functions, but further clarifications are required to establish the exact level of contribution of the adiponectin mediated pathways on male reproduction.

5.2 Sex Hormone and Adiponectin Relation

Adiponectin concentration in rodents and humans is higher in females compared with males possibly a result of inhibition of adiponectin production by androgens (Nishizawa et al., 2002; Combs et al., 2003). Also, adiponectin as well as AdipoR1 and AdipoR2 receptors are expressed in the rat testis (Caminos et al., 2008) and ovary (Chabrolle et al., 2007) suggesting a direct effect of adiponectin in the gonads. In animal models, adiponectin levels dynamically changed with sexual maturation and regulated by estrogens or testosterone: surgical castration induced a rise in plasma adiponectin and testosterone injections reduced plasma adiponectin (Nishizawa et al., 2002; Combs et al., 2003). Nishizawa et al. (2002) observed that in mice and in 3T3-L1 adipocytes, testosterone treatment reduced the production of adiponectin in plasma or media without changing adiponectin mRNA and protein levels of in adipose cells, probably due to intracellular retention of the protein adiponectin (Xu et al. 2005). In adult female mice, ovariectomy induced an increase in plasma adiponectin levels above sham-group, and the use of estrogen implant had a suppressive effect on adiponectin levels. In old female mice, when estrogen level is low due to ovarian cessation, circulant adiponectin was significantly higher than in the young mice (Combs et al. 2003). Addition of estrogen in adipocyte culture cell media, suppressed adiponectin mRNA and protein expression, and this effect was blocked by treatment with estrogen antagonist (Combs et al. 2003). According to Tworoger et al. (2007), between the estrogens, free estradiol is one with strong inverse association with adiponectin levels. Contradicting these results, Nishizawa et al. (2002) found no significant difference in adiponectin plasma concentrations between ovariectomized and sham-operated, indicating no effect of estrogen on plasma adiponectin. In humans, adiponectin levels decreased with the puberty progression in boys, and this decrease related to testosterone and DHEA-S serum concentrations and may account for the gender differences seen in adults (Bottner et al. 2004). Tan et al. (2006) showed that human subcutaneous adipocytes treated with testosterone and the estrogen 17β-oestradiol had increase in AdipoR1 and AdipoR2 gene expression. While Chalvatzas et al. (2009) showed no effect of estrogen treatment on plasma adiponectin of women after ovariectomy. During the menstrual cycle, sex

hormonal changes are accompanied by changes of insulin sensitivity (Bruns & Kemnitz, 2004), and the hormonal fluctuation during the menstrual cycle could affect serum adiponectin levels that in turn could modulate insulin sensitivity. Kleiblova & Haluzík (2006) and Dafopoulos et al. (2009) observed that in women, total circulating adiponectin levels were stable throughout the menstrual cycle, not significantly affected by hormonal changes during the cycle and despite the fact that both androgens and estrogens showed able to modify circulating adiponectin levels. In this sense, in human fat cells SGBS (Simpson-Golabi-Behmel syndrome), incubation with male and female serum led to a downregulation of adiponectin expression, with stronger inhibitory effect of the male serum, but the incubation of these cells with testosterone or estradiol did not influence adiponectin mRNA expression and secretion (Horenburg et al. 2008). This group suggested the existence of serum factor that differently regulated by sex steroids and subsequently causes the sex dimorphism in circulating adiponectin levels (Fig. 5).

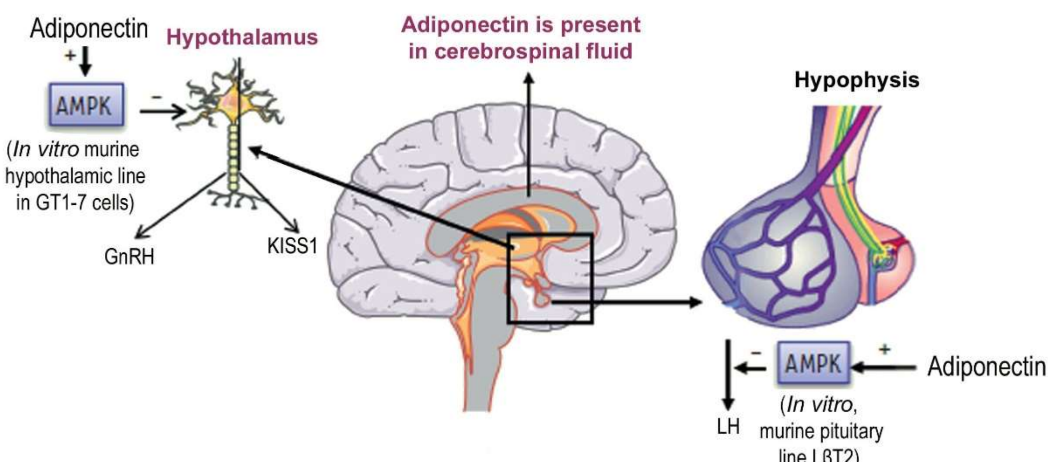

Figure 5. Diagram summarizing the effects of adiponectin on the hypothalamic-pituitary axis. Adiponectin is present in cerebrospinal fluid, as well as in hypothalamic and pituitary cells where AdipoR1 and AdipoR2 receptors also expressed. Adiponectin reduces, via AMPK, hypothalamic GnRH secretions and KISS1 gene transcription in GT1-7 cells. LH: luteinizing hormone; AMPK: adenosine monophosphate activated kinase; GnRH: gonadotropin releasing hormone. Modified from Reverchon et al (2013).

5.3 Adiponectin and Steroidogenesis

5.3.1 Actions of adiponectin on gonadal steroidogenesis

Adiponectin and receptors expressed by different cell types of male gonad, suggesting a possible regulation of testicular function by adiponectin through endocrine and/or paracrine actions. In chicken, adiponectin and AdipoR1 expressed only in peritubular and Leydig cells, whereas AdipoR2 expressed in Sertoli and germ cells as well as Leydig cells (Ocón-Grove et al. 2008). Testicular AdipoR1 and AdipoR2 mRNA were found to be higher in adult compared to prepubertal chickens (Ocón-Grove et al. 2008), suggesting that sexual maturation is likely to be associated with an upregulation of testicular adiponectin receptors genes expressions. AdipoR2 protein expression is also increased in Leydig cells during puberty in rat (Caminos et al. 2008; Pfaehler et al. 2012), rendering these cells more sensitive to changes in plasma adiponectin levels. Related to this, serum concentrations of adiponectin increased in sexually mature compared to immature mice (Combs et al. 2003).

The ability of adiponectin to regulate steroidogenesis been confirmed in different steroidogenic tissues (Caminos et al. 2005; Chabrolle et al. 2007; Caminos et al. 2008; Li et al. 2009). Adiponectin and its receptors been identified in testis (Caminos et al. 2008; Ocón-Grove et al. 2008) and ovary (Chabrolle et al. 2007; 2007a; Toulis et al. 2009). Testicular expression of adiponectin at mRNA and protein levels been shown to be mostly located in interstitial Leydig cells (Caminos et al. 2008). In addition, AdipoR1 and AdipoR2 mRNA been identified in a cDNA library prepared from human testicular RNA (Civitarese et al. 2004; Bjursell et al. 2007). Recombinant adiponectin has shown to have an inhibitory effect on basal and hCG-stimulated testicular testosterone secretion (Caminos et al. 2008; Pfaehler et al. 2012).

Interestingly, adiponectin production at the testicular level is also shown to be inhibited by dexamethasone (Caminos et al. 2008), suggesting that increased cortisol levels in response to stress might downregulate the autocrine/paracrine action of adiponectin in the testis. Unlike its action in adipocytes, the transcription factor PPARc is rather involved in the downregulation of adiponectin expression,

as shown following *in vitro* exposure to its ligand, rosiglitazone, in the rat testis (Caminos et al. 2008).

The expression of a functional AdipoR2 appears to be important for the testicular function in mice as AdipoR2(-/-) mice exhibit reduced testes weight characterized by atrophy of the seminiferous tubules and aspermia, while plasma testosterone levels remained unaffected (Bjursell et al. 2007).

Nonetheless, deletion of the adiponectin gene in mice had no effect on fertility in males and females (Maeda et al. 2002), suggesting the presence of adiponectin-like ligands activating AdipoR2 to compensate the lack of adiponectin. Indeed, aparalog of adiponectin—CTRP3 is a member of the C1q/TNF-related protein superfamily expressed at high levels in adipose tissue. In adult mouse testis, expression of CTRP3 is specific to Leydig cells and contributes to increase testosterone production by upregulating StAR and Cyp11a1 protein expressions (Otani et al. 2012). Specifically, actions on CTRP3 in Leydig cells are dependent on activation of the cAMP/PKA signaling pathway (Otani et al. 2012). However, these results obtained in the TM3 cell line, which are known to have a minimal response to hormonal stimulations and to produce very low levels of testosterone. Furthermore, receptor through which CTRP3 acts on Leydig cells remains characterized and might involve AdipoR2.

Others have shown that high doses and long-term stimulations using recombinant adiponectin were able to activate AMPK in rat, chicken, and porcine granulosa cells (Ledoux et al. 2006; Chabrolle et al. 2007; 2007a). On a basal level, adiponectin also increased ERK1/2, p38, Akt, and AMPK phosphorylations in rat granulosa cells (Chabrolle et al. 2007a). However, such signaling pathways not been characterized regarding adiponectin-dependent regulation of steroidogenesis in Leydig cells. Interestingly, adiponectin has been shown regulate the expression of steroidogenic genes (Cyp11a1, Star, and Cyp19a1) in chicken, rat, swine, and human ovary (Ledoux et al. 2006; Chabrolle et al. 2007; 2007a; Lagaly et al. 2008; Chabrolle et al. 2009; Richards et al. 2012), suggesting that adiponectin might affect steroidogenesis in Leydig cells through regulation of steroidogenic genes expressions as well.

In male rats, low to intermediate doses of adiponectin (10 and 100 ng/mL) shown to inhibit basal testosterone production at the testicular level result of reduced StAR protein expression (Caminos et al. 2008; Pfaehler et al. 2012). Since adiponectin been shown to inhibit *KISS1* gene transcription through decreased nuclear translocation of SP-1 in hypothalamic cells (Wen et al. 2012), is possible that adiponectin may act on Leydig cells to downregulate steroidogenic genes expressions by inhibiting the transcriptional activity of SP-1, a transcription factor known to be involved in steroidogenic genes regulation (Momoi et al. 1992; Pena et al. 1999; Sugawara et al. 2000; Shih et al. 2011). In addition, PPAR transcription factors, being regulated by adiponectin in other cell types (Lin et al. 2010; Liu et al. 2010; Lee et al. 2010), may also be involved in transcriptional regulation of steroidogenic genes in Leydig cells. Indeed, numerous steroidogenic promoters shown to contain consensus regulatory elements for SP-1 and PPARs. However, the relevance of these regulatory elements in regulation of testosterone production from Leydig cells remains confirmed.

The expressions of AdipoR1 and AdipoR2 have been demonstrated in the mouse TM3 and mLTC Leydig cell lines where adiponectin plays a protective role against pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Wu et al. 2013). Such mechanism achieved by inhibiting the nuclear factor κB (NFκB) signaling pathway through AdipoR1-mediated increased AMPK phosphorylation (Wu et al. 2013). Pro-inflammatory cytokines such as TNFα and IL-1β been shown to inhibit Leydig cells steroidogenesis and function (Hong et al. 2004). Therefore, adiponectin might contribute to the protection of Leydig cells from cytokines released from macrophages within the testis during inflammatory promoting conditions. Altogether, adiponectin exerts an inhibitory effect on testosterone production by acting at the testis level, although such regulation may be dependent on hormone concentration and on the gonadal status.

Multiple studies in the literature revealed that adiponectin is able to affect the reproductive system through central effects on the hypothalamus and/or peripheral actions on the ovary (Mitchell et al. 2005; Campos et al. 2008). The adiponectin system (adiponectin, AdipoR1, and AdipoR2) described in rat and human hypothalamy (Kos et al. 2007), in pituitary glands (Rodriguez-Pacheco et

al. 2007), and in ovaries of various species including pigs (Lord et al. 2005), chicken (Chabrolle et al. 2007), rats (Chabrolle et al. 2007), and humans (Chabrolle et al., 2009). AdipoR found abundantly expressed in hypothalamus, and their expression levels were comparable to those in the liver (Yamauchi et al., 2003). Interestingly, unlike in the serum, only trimers and hexamers, and not HMW multimers, found in the central nervous system (Kubota et al., 2007).

Was demonstrated in mouse gonadotropic cells and in rat pituitary cells that adiponectin inhibits basal and GnRH-stimulated LH secretion (Rodriguez-Pacheco et al., 2007; Lu et al. 2008). In rat primary pituitary cells, recombinant adiponectin regulates GnRH receptor expression (Rodriguez-Pacheco et al., 2007). In mouse adrenal cells, adiponectin affects the adrenocortical function as the secretion of corticosterone and aldosterone inhibited by adiponectin (Li et al., 2009). Additionally, *in vitro* studies have shown that physiologically relevant levels of recombinant adiponectin induce ovarian gene expression and steroidogenesis in mammalian ovaries. Indeed, the expression of a cluster of proteins associated with the process of ovulation including cyclooxygenase-2, prostaglandin E2, and vascular endothelial growth factor induced in response to adiponectin (Ledoux et al., 2006). In rat ovaries, the synthesis of the steroid hormones, progesterone and estrogen, regulated by adiponectin (Chabrolle et al. 2007). Responses to adiponectin were additive to gonadotropins, and Ledoux et al. (2006) further demonstrated an interaction with insulin.

Similarly, in rats, adiponectin acts synergically with IGF-1 to induce steroidogenic gene expression and steroidogenesis (Chabrolle et al. 2007). Physiological levels of recombinant adiponectin (5 or 10 mg/mL) are able to increase progesterone and/or estrogen secretions in response to IGF-1 in human cultured granulosa cells (Chabrolle et al., 2009). Bovine granulosa data clearly demonstrate that adiponectin alters cell steroidogenesis, oocyte maturation, and embryo development (Maillard et al., 2010). Thus, while it is important to note that adiponectin can directly induce ovarian gene expression, the interactions between adiponectin and insulin or IGF-1 are consistent with the role of adiponectin in other tissues, that is, the sensibilization to insulin. In support of a role for adiponectin in human folliculogenesis and ovulation, circulating adiponectin positively correlates with the number of oocytes retrieved in women

treated with FSH to induce superovulation for the purpose of *in vitro* fertilization (Liu et al., 2006).

Finally, recombinant human adiponectin decreases LH, insulin-induced progesterone, and androstenedione secretions by bovine theca cells from large follicles (Lagaly et al. 2008).

5.3.2 Adiponectin and regulation of spermatogenesis

In rodents, obesity leads to reduced sperm motility (Fernandez et al. 2011). The resulting lower sperm quality causes a slight reduction in fertility potential (Fernandez et al. 2011), supporting that obesity may lead to impairment of male fertility. Moreover, oxidative stress at the testicular level, common under obesity conditions, may result in decreased spermatogenesis and sperm damage. Indeed, it been proposed that male obesity may alter testicular function leading to subfertility through increased oxidative stress, decreased testosterone levels, and altered spermatogenesis (Erdemir et al. 2012).

In normal-weight men, concentrations of adiponectin in the seminal plasma are higher than in blood serum by three folds (Thomas et al. 2013). In addition, adiponectin concentrations in seminal plasma positively correlated with sperm concentrations, sperm count, and normal morphology of spermatozoa (Thomas et al. 2013). As blood serum concentration, levels of adiponectin in seminal plasma tend to decrease with obesity. However, the origin of adiponectin in seminal plasma remains to identified.

Adiponectin and its receptors play major roles in sperm morphology and function, contributing to increased fertility. Indeed, serum adiponectin concentration and sperm mRNA expressions for adiponectin and its receptors were higher in high-fertility bulls (Kasimanickam et al. 2013). Adiponectin protein was abundant in the tail region of bull sperm, while AdipoR1 localized mainly at the equatorial and acrosome region and AdipoR2 expressed primarily on the sperm head region and on the equatorial line (Kasimanickam et al. 2013). Adiponectin and its receptors were expressed during pre- and post-capacitation

of spermatozoa (Kasimanickam et al. 2013), suggesting that adiponectin might have a role in sperm capacitation. Thus, the production of sperm capable of fertilization supported by local actions of adiponectin at the testis level.

In addition to its role in the regulation of steroidogenesis and sperm capacitation, adiponectin might also be involved in sperm–ovum fusion and fertilization. In ram, adiponectin, AdipoR1, and AdipoR2 transcripts have been detected in the testis, epididymis, (caput, corpus, and cauda), vesicular and bulbourethral glands, while adiponectin could not be found in the vas deferens (Rahmanifar & Tabandeh, 2012). AdipoR1 and AdipoR2 were expressed in all parts of male reproductive tract in ram (Rahmanifar & Tabandeh, 2012), as well as in porcine epididymis (Dai et al. 2006), suggesting that adiponectin produced locally from testes and epithelium of male reproductive tract may influence sperm maturation. Adiponectin production and secretion has been shown in the oviduct of cycling rats (Archanco et al. 2007), while both AdipoR1 and AdipoR2 are highly expressed in the pig endometrium (Lord et al. 2005). Finally, expression of both genes increased in the mid-luteal phase, when the implantation occurs. Therefore, AdipoR1 and AdipoR2 mRNAs expressed along male and female reproductive tract of different species.

Expression of adiponectin and its receptors immensely reported in human testicular cells, especially in the Leydig cells, while the spermatozoa also have been shown to express its receptors (Martin, 2014). AdipoR2 gene knockout murine model demonstrated aspermia atrophy in their seminiferous tubules and enlarged brains, while the testosterone levels remained unchanged (Peng et al. 2007). It also been demonstrated that in the murine model, with the advancement of age, there is a reduction in the testicular expressions of adiponectin and its receptors (Choubey et al. 2019). It may inferred that an adequate adiponectin concentration and expressions of its receptors may be essential for normal testicular functions. Adiponectin therapy may possess potent antiaging characters and may ameliorate and promote normal testicular activities in old aged men. However, their roles in male reproductive function remain investigated.

6. ADIPONECTIN AND LIVESTOCK

6.1 Adiponectin and productive traits in livestock

In livestock, adipose tissue releases numerous adipokines as well as adiponectin that contribute to energy homeostasis, glucose metabolism, and lipid metabolism (An et al. 2017). By phosphorylating and activating AMPK in both skeletal muscle and liver, it increased insulin sensitivity by improving glucose utilization and fat oxidation, thus influencing carcass traits in Nanyang cattle (Zhang et al. 2009). Like in humans, because of combining adiponectin with insulin, glucose tolerance and insulin sensitivity are improved (Ruan et al. 2016). It enhances skeletal muscle glucose uptake and fatty acid oxidation by stimulating AMPK (Natah, 2014). Human skeletal muscle proliferation stimulated by adiponectin via the p38-MAPK pathway (Frankenberg et al. 2017). The adiponectin hormone increases adipocyte lipid storage for the human body as well as inversely correlates with plasma triglycerides levels and positively correlates with HDL cholesterol levels (Miehle et al. 2012). Therefore, it can play a vital role in determining the growth and productive traits of livestock.

In domestic animals, adiponectin polymorphisms are associated with live body weight and productive traits. Adiponectin gene polymorphism is known to affect the fat thickness, growth traits including birth weight, body weight, average daily gain, and body sizes, and carcass traits such as marbling, ribeye muscle area, and fat thickness in Angus cattle (Morsci et al. 2006; Zhang et al. 2009), suggesting that this gene is a potential candidate for animal productivity. The adiponectin gene polymorphism closely related to carcass and meat quality traits, and therefore used as a molecular marker for high-quality meat production (Zhang et al. 2009; An et al. 2017). There is evidence that a C>T mutation within exon3 of the adiponectin gene is associated with carcass traits in Qinchuan beef cattle carrying the CD genotype. Cattle with the CD genotype had greater slaughter weight, fat thickness, back fat thickness, crural girth, and tenderness than cattle with the CC genotype (Yang, 2009). Genetic variants in the ADIPOQ exhibited significant positive effects on marbling score (MAR) in Hanwoo cattle (Choi et al. 2015). For goats, a novel mutation has been reported in the ADIPOQ 3’UTR. This locus did not show a significant statistical relationship with body

weight traits in goats (Lan et al. 2009). Besides, inherited traits such as growth traits (pre-weaning growth rate) and carcass traits (yield of leg-, loin-, and total-lean meat) correlated positively with ADIPOQ haplotypes in New Zealand (NZ) Romney lambs (An et al. 2017). Further, ADIPOQ polymorphisms in the ovine have been associated with sheep fatness (Li et al. 2018). The results of previous studies suggest that this gene plays a fundamental role in several productive traits among livestock.

6.2 Adiponectin and reproductive traits in farm animals

In animal production based on economic principles, reproductive traits are four times more influential than productive traits (Mia et al. 2013). Additionally, sheep's reproductive potential and fertility are also associated with health conditions and biological characteristics (e.g., growth) (Al-Thuwaini 2021). Reproductive traits are complex traits due to genetic factors and endocrine signal transpositions between the pituitary, ovary, and adipose tissues in sheep (Musthafa & Marikar, 2014; Al-Thuwaini 2021b). Adipose tissue and its secreted factors been implicated in all aspects of mammal reproductive functions (Campos et al. 2018). The human adipose tissue contains signaling proteins such as adipocytokines and adipokines (Trayhurn & Wood, 2004). Some domestic animals, like cows, goats, and ewes, express adipokines in their ovarian cells, which modulate ovarian physiology (Kurowska et al. 2018). Recent evidence suggests that adiponectin plays a crucial role in mammal reproductive function. Adiponectin inhibits basal- and GnRH-stimulated LH secretion by gonadotrope cells through phosphorylation of AMPK (Yang et al. 2020). Besides, pituitary AMPK known to act as a sensor of energy, controlling bovine gonadotropin secretion and reproduction (Forny-Germano et al. 2019). It has been shown that animals of all species express adiponectin and its receptors (Maillard et al. 2010), and it affects ovarian steroidogenesis (Lagaly et al. 2008). Additionally, adiponectin regulates mammal oocyte nutrient sensing via AMPK pathways (Mitchell et al. 2005). According to data regarding adiponectin's role in ovarian function, adiponectin exerts its effects through the hypothalamic-pituitary-ovarian axis. These peripheral effects of adiponectin are mediated primarily by AdipoR1

and AdipoR2. These receptors been found in the brain and ovaries. Therefore, adiponectin could affect livestock reproduction (Forny-Germano et al. 2019).

Adiponectin been shown to GnRH in the hypothalamus (Dobrzyn et al. 2018). AMPK activated by adiponectin to inhibit human GnRH release and cause hyperpolarization of plasma membranes as well as calcium influx (Wen et al. 2008). The number of GnRH immunoreactive neurons decreased with adiponectin mutations, suggesting that adiponectin could control GnRH secretion in the mammal hypothalamus (Dutta et al. 2019). As well, human adiponectin is present in pituitary cells that produce the LH, FSH thyroid-stimulating hormone (TSH), and GH (Psilopanagioti et al. 2009). FSH-induced progesterone secretion by the mammal granulosa cells reported to mediated by the MAPK pathway (Moore et al. 2001; Tosca et al. 2005). In addition, there is growing evidence that adiponectin and AMPK work in concert to control mammal ovarian cell function (Dupont et al. 2008).

Adiponectin is a hormone that can be produced by ovarian cells, as demonstrated by AdipoR1 and AdipoR2 mRNA and/or proteins present in the bovine ovary (Maillard et al. 2010; Tabandeh et al. 2020) and cumulus cells and the oocyte (Maillard et al. 2010). Additionally, adiponectin accumulated in mammal follicular fluid from the local circulation and/or the local tissues (Gutman et al. 2009). Dupont et al. (2008) demonstrated that adiponectin is a vital signal for follicles growth and oocytes, suggesting it is involved in follicular dominance and oocyte survival in rodent, bovine, ovine, and human ovaries. In small bovine follicles, adiponectin stimulates the proliferation of cells induced by IGF-1 (Maillard et al. 2010; Reverchon et al. 2014). Besides, increased adiponectin gene expression in granulosa cells in large bovine follicles has been associated with higher levels of estradiol in the follicular fluid (Reverchon et al. 2014; Tabandeh et al. 2020). In sheep, the highest levels of adiponectin expression found in granulosa cells (Ortega et al. 2010). Additionally, mammal antral follicles exhibited significantly greater levels of adiponectin protein than primitive, primary, small, and large preantral follicles (Merhi et al. 2019).

There has been little research regarding the association between genetic polymorphisms in the ADIPOQ gene and reproductive traits in livestock. Only one

study investigated the relationship between ADIPOQ gene polymorphisms and reproductive performance in domestic pigs. A new SNP (c. 1138G > A) is associated positively with litter size in the Wannan Black pig (Zhang et al. 2016. The ADIPOQ/TasI genotypes at nucleotide position 1431C>T also indicated that cows with TT genotypes had a longer calving interval (CI), a prolonged lactation period (LP), and greater milk yield (TMY) than CC or CT cows in Indian Sahiwal cows (Pandey et al. 2020). Except for this, there is little literature on the relationship between this gene's polymorphism and sheep reproductive performance.

7. GENERAL CONCLUSION

Adiponectin, a hormone derived from adipocytes, plays a vital role in the regulation of livestock energy metabolism, productive and reproductive traits. By activating AMPK of skeletal muscle and liver, adiponectin improves insulin sensitivity and increases glucose utilization and fat oxidation, influencing growth, carcass composition, and meat quality traits. In addition, it affects the reproductive system by exerting central effects on the highest branch of the hypothalamic-pituitary-ovarian axis, inhibiting GnRH and GnRH-induced LH secretion. Regulation of phenotypic traits concerning the adiponectin gene suggested that this gene is fundamentally linked to several phenotypic traits among livestock.

The work carried out allowed elucidating the gene expression and histological location of AdipoR1 in all reproductive peripheral tissues in male rams outside the reproductive season, considering that this species, in addition to having an important productive importance, has important hormonal variation, according to the season and the characteristics of breeds.

Daylight hours modify the pattern of LH secretion: short days stimulate its pulsatility and long days decrease it. The photoperiod also influences the secretion of FSH, modifies the pituitary response to GnRH stimulation and the testicular response to LH. There is no doubt that photoperiod acts through GnRH-dependent pathways, and modifies the secretion of this hormone, either by acting directly on the pulsatile generator or by modulating the negative feedback effects that testosterone exerts at the hypothalamic level.

The published antecedents of studies that have tried to elucidate the importance and effects of adiponectin on the development and reproductive function have not, until now, considered the reproductive seasonality of this productive species, a situation that we are trying to begin to discover and report.

Among the results of the study, and that involves the histological location of AdipoR1 and that stands out from the study carried out is that it was not found to be present in Leydig cells, although it was found in other locations of testicular

tissue. This could be explained because outside of reproductive seasonality, HPG axis generates communication pathways that could be involved in the absence of this receptor, which has been reported in steroid-producing interstitial cells testis.

The present results evidenced the possible AdipoQ/AdipoR1 system role in regulating the testicular activity of male ram, during the non-breading season. The study on reproductive activities regulated by the AdipoQ/AdipoQ receptors system is helpful for better knowledge of the physiological mechanisms that link adipose tissue with the mammalian reproductive processes, specifically on how altered energy metabolism can induce reproductive pathologies in humans and animals.

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Article

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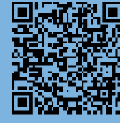

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Article

Presence, Tissue Localization, and Gene Expression of the Adiponectin Receptor 1 in Testis and Accessory Glands of Male Rams during the Non-Breeding Season

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Simple Summary: Adiponectin (ADIPOQ) is the most abundant adipocytokine secreted by adipocytes in white adipose tissue and exerts its action by two receptors, ADIPOQ receptor 1 and -2, respectively (ADIPOR1 and -R2). ADIPOQ has an important role in various physiological mechanisms modulating whole-body energy homeostasis. Besides these metabolic aspects, ADIPOQ has been shown to affect the reproductive system through its actions on the hypothalamic–pituitary–gonadal axis. ADIPOQ and its cognate receptors are expressed in different cell types of the male gonad, indicating that this adipocytokine directly regulates the testicular function. To better understand the role of the ADIPOQ/ADIPOQ receptor system in modulating ovine reproductive processes, we have evaluated the ADIPOR1 presence and gene expression in male ram reproductive tissues during the non-breading season. The reported results support the idea that the mammalian reproductive processes are also modulated by the ADIPOQ/ADIPOR1 system, particularly the testicular activity of male rams, during the non-breading season. The study on reproductive activities regulated by the ADIPOQ/ADIPOQ receptors system is helpful for better knowledge of the physiological mechanisms that link adipose tissue with the mammalian reproductive processes, specifically on how altered energy metabolism can induce reproductive pathologies in humans and animals.

Abstract: Adiponectin (ADIPOQ) is a member adipocytokines, and its actions are supported by two receptors, ADIPOQ receptor 1 and -2, respectively (ADIPOR1 and -R2). Our study was performed to evaluate the ADIPOR1 presence and location and its gene expression in reproductive tissues of the male ram, during its non-breading season. The different portions of the male ram reproductive system (testis, epididymis, seminal vesicle, ampoule vas deferens, bulb-urethral gland) were collected in a slaughterhouse. Immunohistochemistry showed ADIPOR1 positive signals in the cytoplasm of all the glandular epithelial cells, with a location near the nucleus; in the testes, the positive reaction was evidenced in the cytoplasm in the basal portion of the germinal epithelial cells. The immune reaction intensity was highest ($p < 0.001$) in the prostate and seminal vesicles glands than that of other parts of the ram reproductive tract. RT-qPCR detected the *ADIPOR1* transcript in the testes, epididymis, vas deferens, bulbourethral glands, seminal vesicles, and prostate; the expression levels were high ($p < 0.01$) in the prostate and low ($p < 0.01$) in the testis, epididymis, and bulbourethral glands. The present results evidenced the possible ADIPOQ/ADIPOR1 system's role in regulating the testicular activity of male rams during the non-breading season.

Keywords: adiponectin; adiponectin receptors; ovine; ram; testis; sexual glands

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1. Introduction

Adiponectin (ADIPOQ) is a member of the adipose-secreted proteins, called adipocytokines. The initial report on ADIPOQ in 1995, just one year after the discovery of leptin,

was published by Scherer et al. [1]. This molecule is a 244-amino acids protein with a molecular weight of 30 kDa that belongs to the superfamily C1q/TNF-α (tumor necrosis factor-α) [2]. It comprises an N-terminal signal peptide, a collagenous domain, and a globular C1q-like domain at its C-terminus [3]. In sheep, this hormone is encoded by the *ADIPOQ* gene, located on chromosome 1q27 and comprising three exons and two introns [4]. These authors suggested that the *ADIPOQ* gene regulates several productive traits and that sheep with the AA genotype have heavier and larger body dimensions, thereby improving their productivity and reproducibility [4].

ADIPOQ is composed of four distinct domains, which include a signal peptide at the N-terminus, followed by a short variable region, a collagenous domain, and a C-terminal globular domain [5]. ADIPOQ has been found in human and mouse sera as trimeric and hexameric oligomers, although heavy molecular weight forms as well as small proteolytic cleavage products have also been detected [6,7].

ADIPOQ is the most abundant adipose-derived hormone secreted by adipocytes in white adipose tissue, with an important role in the regulation of whole-body energy homeostasis, insulin sensitivity and lipid/carbohydrate metabolism in human and animals [8]. ADIPOQ also plays a role in the stimulation of fatty acid oxidation in the liver and skeletal muscle, suppression of hepatic gluconeogenesis, stimulation of glucose uptake in the skeletal muscle, and increasing insulin secretion [9]. The ADIPOQ actions are supported by two distinct, structurally related, receptors, ADIPOQ receptor 1 and -2, respectively (ADIPOR1 and -R2). These two receptors have been identified in different species, including human [10], rodents [10], chicken [11], pig [12,13], and cow [14].

In addition to its well-known metabolic effects, ADIPOQ has been shown to affect the reproductive system, partially, through central actions on the hypothalamic–pituitary axis [15]. Hypothalamic neurons secrete a gonadotropin-releasing hormone (GnRH) in a pulsatile pattern, stimulating the release of pituitary gonadotropins. These gonadotropins regulate testicular steroidogenesis and spermatogenesis [16]. ADIPOQ receptor R1 and -R2 are generally expressed in the human hypothalamus and pituitary [17], thus suggesting that ADIPOQ could participate in the modulation of the endocrine reproductive axis. ADIPOQ and its cognate receptors are also expressed in different cell types of the male gonad, indicating that this adipocytokine directly regulates the testicular function ADIPOQ through an endocrine and/or paracrine way. In chicken, the presence of the ADIPOQ/ADIPOR1 and -R2 system was evidenced in the seminiferous and peritubular tubule cells [18].

Functional differences and signaling pathways were demonstrated through the generation of ADIPOR1 and -R2 knockout mice: ADIPOR1 related to the activation of AMP-activated and mitogen-activated protein kinase (AMPK) and its pathways [19] and regulates adipose metabolism throughout the regulation of the hormone-sensitive lipase and the peroxisome proliferator-activated receptor (PPAR) γ expression, during adipocyte differentiation [2]. Conversely, ADIPOR2 appears to be associated with the activation of pathways of PPARα [19]. Simultaneous disruption of both ADIPOR1 and -R2 abolished ADIPOQ binding and actions, resulting in increased tissue triglyceride content, inflammation, and oxidative stress, and thus leading to insulin resistance and marked glucose intolerance [19]. Therefore, ADIPOR1 and -R2 serve as the predominant receptors for ADIPOQ in vivo and play important roles in the regulation of glucose and lipid metabolism, inflammation, and oxidative stress in vivo [19].

Recent evidence suggests that ADIPOQ plays a crucial role in mammal reproductive function: ADIPOQ-induced AMPK activation repressed the promoter activity of the kisspeptin1 gene via inhibition of the translocation of specificity protein-1 from the cytoplasm to the nucleus and subsequently influenced GnRH secretion [20]; this AMPK activation by ADIPOQ reduced GnRH-stimulated LH secretion, and this repression was mimicked by 5-aminoimidazole-4-carboxamide riboside, an activator of AMPK [20]

In ovine, the expression of ADIPOQ and ADIPORs has been reported in the male reproductive tract [21] and sperm cells [22]. The latter study also reported that some sperm motility indices (curvilinear velocity, straight-line velocity, average path velocity, linearity,

wobble, and straightness) were also significantly correlated with ADIPOQ and ADIPOR1 relative expression, whereas the correlation of ADIPOR2 was also significant with the mentioned parameters, although this correlation was not comparable with ADIPOQ and ADIPOR1 [22]. To better understand the role of the ADIPOQ/ADIPOQ receptor system in modulating ovine reproductive processes, the purpose of this work was to evaluate the ADIPOR1 presence and location and its gene expression in the reproductive tissues of the male ram during the non-breading season.

2. Materials and Methods

2.1. Collection of Ram Reproductive Tissues

Male reproductive tissues were collected during the non-breading season (May 2021) at Viterbo (Lazio, Italy) slaughterhouse from 12 healthy adult rams (aged 3–8 years, weigh 118–135 kg). The different portions of the reproductive system (testis, epididymis, seminal vesicle, ampoule vas deferens, bulb-urethral gland) of each animal were promptly removed, identified and divided into two fractions, one immediately frozen at −80 °C, and the other fixed by immersion in 4% (*w*/*v*) formaldehyde solution in phosphate buffered solution (PBS) (0.1 M, pH 7.4) for 24 h at room temperature and subsequently processed for embedding in paraffin, following routine tissue preparation procedures.

2.2. Immunohistochemistry

The immunohistochemistry method followed that previously reported [23]: 5 µm thick serial sections, mounted on poly-L-lysine coated glass slides using the avidin-biotin complex (ABC, Vector Laboratories, Burlingame, CA, USA) and the chromogen 3,3′diaminobenzidine-4-HCl (DAB, Vector Laboratories). First of all, the sections were dewaxed in xylene and then rehydrated by alcohols in descending percentage. Then, the sections were microwaved three times (5 min at 750 W) in 10 mM citric acid (pH 6.0) for antigen retrieval and cooled at room temperature (15 min). All subsequent steps were performed in a humid chamber at room temperature. Non-specific binding of the primary antibody was prevented by sections' pre-incubation with the goat normal serum (30 min). The excess liquid was removed, and the sections were incubated (overnight) in the presence of the primary antibody, rabbit polyclonal anti-ADIPOR1 (LS-C151518/55035, 1:100, LSBio, Seattle, WA, USA). The next day, the sections were rinsed in PBS (5 min) and incubated (30 min) with the secondary biotin-conjugated antibody, a goat anti rabbit IgG (BA-1000-1.5, 1:200, Vector Laboratories, Burlingame, CA, USA). Subsequently, they were rinsed (5 min) in PBS and then processed (30 min) with the Vectastain ABC kit (PK-4000, Vector Laboratories) at the manufacturer dilution. The sections were rinsed in PBS, and the reaction was developed with the chromogen solution. After several rinses in PBS, they were counterstained with hematoxylin, dehydrated and mounted in Canada Balsam (BDH, Poole, Dorset, UK). The immunoreaction and the reagents used were validated by positive and negative controls: sections of tissues with the testified presence of the same primary antibody were the positive control [23]; and sections without the presence of the primary antibody and/or replaced with pre-immune mouse-globulin were the negative control of unspecific staining. The intensity of immune reactions was evaluated with the image analysis system (IAAS 2000 image analyzer, Delta Sistemi, Rome, Italy) as described in a previous work [24] through optical density, using five microscope fields of each sample, evaluating the absorbance of the treated tissue in relation to the same without immunohistochemistry treatment.

2.3. RNA Extraction and RT-qPCR

Total RNA was purified from the different portions of the genital tract (testis, epididymis, seminal vesicle, ampoule vas deferens, bulbourethral gland) of each ram as previously described [25]. Five micrograms of total RNA were reverse transcribed in 20 µL of iSCRIPT cDNA using a random hexamer method according to the protocol provided by the company. Genomic DNA contamination prevention was realized by an RT-qPCR without reverse transcriptase. Serial experiments were carried out to optimize

the quantitative reaction, efficiency, and Ct values. In 25 µL RT-qPCR reaction volume were added 12.5 µL of iQ SYBR Green SuperMix (Bio-Rad Laboratories, Hercules, CA, USA), 1 µL forward and 1 µL re-verse primers (stock concentration 10 µM) and 8.5 µL of water. The primers used are listed in Table 1. The final master mix was distributed into a 96-well RT-qPCR plate before adding 2 µL of cDNA for each gene (diluted 10-fold with water). To avoid genomic DNA contamination, for every PCR run, negative reaction controls without reverse transcriptase in RT were performed. Samples' amplification fidelity was also confirmed by agarose gel electrophoresis. RT-qPCR was carried out in an iCycler iQ (Bio-Rad Laboratories) with an initial incubation at 95 °C for 1.5 min, followed by 40 cycles at 95 °C for 15 s, and 53 °C for 30 s, during which fluorescence data were evaluated. The cycle threshold (CT) value was automatically computed for each trace. The beta-actin Ct housekeeping gene (*ACTB*) was determined to normalize sample variations in the amount of starting cDNA. Standard curves were generated by plotting the Ct against the log cDNA standard dilution (1/5 dilution) in nuclease-free water, and the graph slope was used to determine reaction efficiency. Quantification of the standard curve was evaluated using iCycler system software (Bio-Rad Laboratories), while mRNA gene expression was quantified with the $2^{-\Delta\Delta Ct}$ method [26,27]. The melting curve analysis, performed immediately after the RT-qPCR end cycle, was used to determine the specificity of each primer set. A melt curve protocol was performed by repeating 80 heating cycles for 10 s, from 55 °C with 0.5 °C increments, during which fluorescence data were collected.

Table 1. Primers for *ADIPOR1* and *ACTB* [28] housekeeping gene used for RT-qPCR quantification.

Gene	NCBI Seq. Ref.	Primers		Bp
ADIPOR1	NM_001306110.1	F	GGTGGTGTTCGGGATGTTCT	128
		R	CGATCCCCGAATAGTCCAGC	
ACTB	U39357.2	F	CCTTAGCAACCATGCTGTGA	130
		R	AAGCTGGTGCAGGTAGAGGA	

2.4. Statistical Analysis

Data were analyzed by one-way ANOVA, and multiple comparisons were performed with a Student–Newman–Keuls post hoc t-test. Differences with a probability level of $p < 0.01$ were considered statistically significant. Equality of variances was checked by Levene's test.

3. Results

This is the first publication that reports the histological localization (immunolocalization) of ADIPOR1 in testis and accessory glands of the ram, outside of its reproductive seasonality.

3.1. ADIPOR1 Immunolocalization

The immunohistochemical studies revealed a positive signal for ADIPOR1 and evidenced its presence and localization in the cytoplasm of all the glandular epithelial cells. The positive reaction seems to be localized near the nucleus, while the rest of the cytoplasm appeared faintly colored or even negative.

In the testes, the positive reaction was evidenced and localized in the cytoplasm of cells placed in the basal portion of the germinal epithelial cells (arrows) and, also in this case, the localization was peculiarly perinuclear. The particular positivity localization within the cells is typical of many receptors and is an expression of their internationalization after binding to the molecule [29].

The intensity of immune reactions was higher ($p < 0.001$) in the prostate and seminal vesicles glands compared with other parts of the ram reproductive tract (Figures 1 and 2).

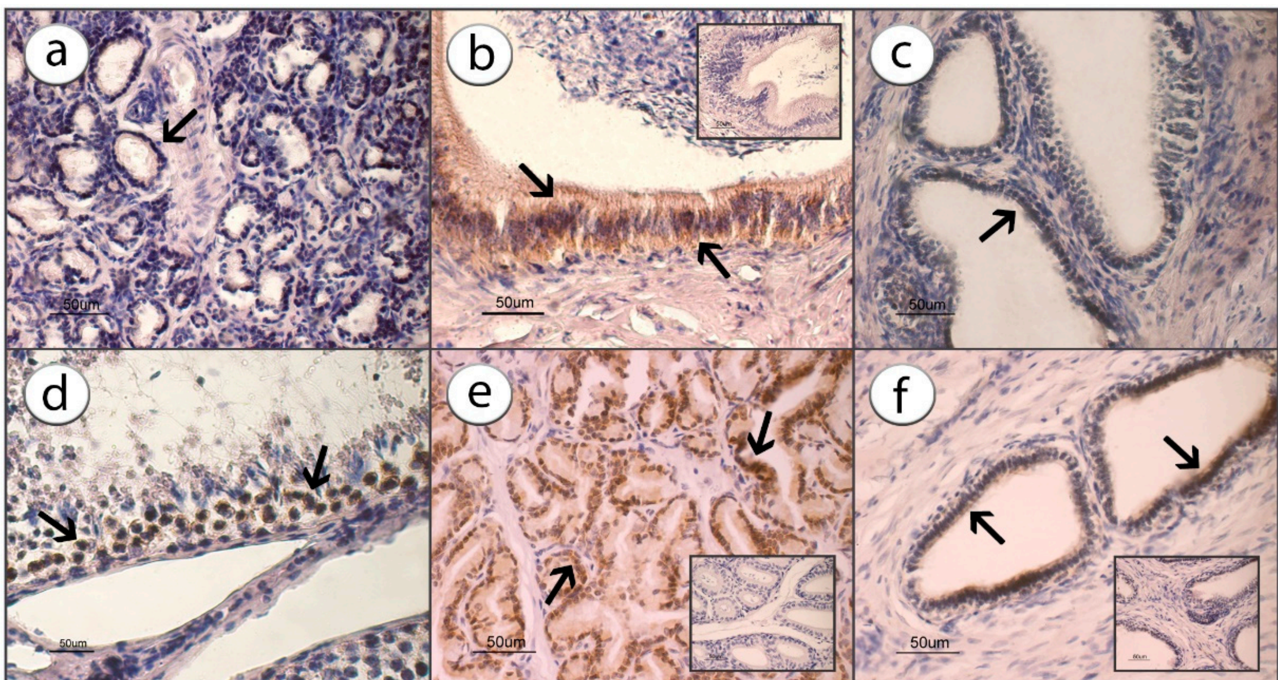

Figure 1. Immunostaining for ADIPOR1 in bulbourethral gland (**a**), epididymis (**b**), seminal vesicle (**c**), testicle (**d**), prostate (**e**) and vas deferens (**f**) counterstained with hematoxylin. The arrows indicate the positive localization of the immunoreaction, while the inserts in (**b**,**e**,**f**) are examples of the negative reactions.

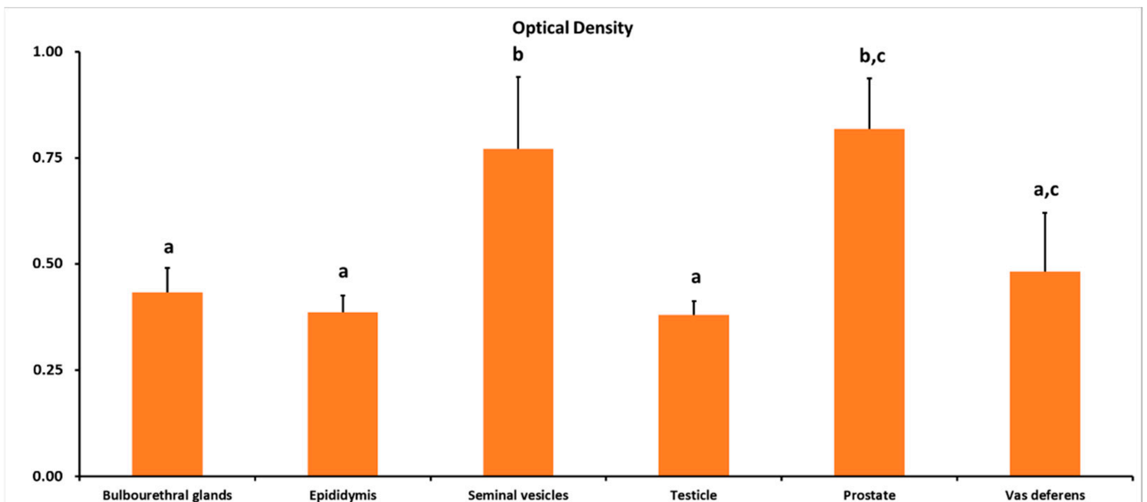

Figure 2. Immunoreaction intensity of ADIPOR1, performed on adult ram male reproductive tissues. Different letters above bars indicate significantly different values (ANOVA $p < 0.001$, Levene's test $p > 0.05$).

3.2. Gene Expression

ADIPOR1 transcripts were detected in the testes, epididymis, vas deferens, bulbourethral glands, seminal vesicles, and prostate (Figure 3). The *ADIPOR1* mRNA expression level was higher ($p < 0.01$) in the prostate and lower ($p < 0.01$) in the testis, epididymis, and bulbourethral glands (Figure 3).

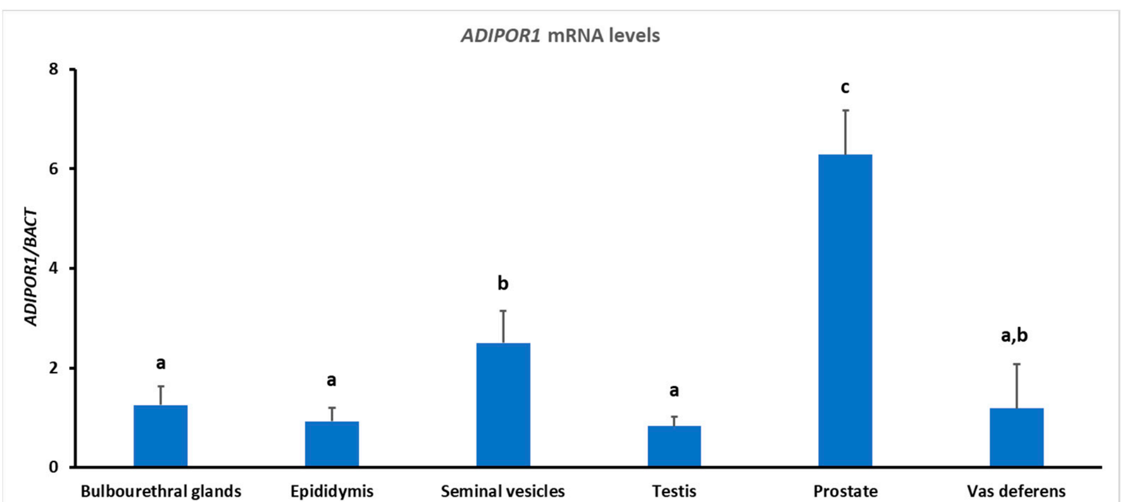

Figure 3. RT-qPCR analysis of *ADIPOR1* gene expressions performed on adult ram male reproductive tissues. Different letters above bars indicate significantly different values (ANOVA $p < 0.001$, Levene's test $p > 0.05$).

4. Discussion

In the present study, we have analyzed the gene and protein expressions and the location of ADIPOR1 in the reproductive tissues of adult male rams. First, our results establish that, for the adult rams, Leydig cells did not express ADIPOR1 differently from other species, where the presence of this receptor and its possible function was found [18,30,31]. Caminos et al. [30], working with rats, found the ADIPOQ IHC presence in adult Leydig cells. Ocón-Grove et al. [18] and Ramachandran et al. [31], also by IHC, established the ADIPOQ presence in chicken Leydig cells. These authors also described the presence of these receptors in Sertoli cells, spermatids, and sperm cells [18,31].

ADIPOR1 was found located in seminiferous tubules of rats [30]. Wu et al. [32] found it in TM3 and mLTC Leydig cell lines in mice, using Western blot, and Ocón-Grove et al. [18] and Ramachandran et al. [31] found it in peritubular locations in chickens.

ADIPOQ and its cognate receptors are also expressed in male reproductive tracts in different species [8,9,14,15,18,21,30]. In particular, they are present in human testes (seminiferous tubules and interstitial tissue), epididymis, Leydig cells and spermatozoa [33]. In mice, the loss of ADIPOR2 induced seminiferous tubular atrophy associated with aspermia and reduction of testes weight [33].

In recent years, Choubey and coworkers [34–38] have extensively studied the role of ADIPOQ on mice testicular activity; in particular, that of the ADIPOQ/ADIPORs system in the prevention of aging and obesity-associated testicular reproductive dysfunctions. In an initial study, these authors [35] reported that ADIPOR1 and -R2 are localized in adult mice Leydig cells and seminiferous tubules. The in vitro study showed the ADIPOQ direct action on spermatogenesis by stimulating cell proliferation (proliferating cell nuclear antigen) and survival and by suppressing cell apoptosis (anti-apoptosis gene Bcl2) [35], thus suggesting an ADIPOQ role in cell survival and proliferation during mice spermatogenesis [35]. Another study [36] reported that, in aged mice testis, the decline in ADIPOQ/ADIPORs system expression is concomitant with that of testicular mass, insulin receptor expression, and testosterone synthesis. In addition, aged mice treated with ADIPOQ showed improvements in testicular mass, cell proliferation, insulin receptor expression, testicular glucose uptake, anti-oxidative enzymes activity and testosterone synthesis [36]. ADIPOQ exogenous administration to type 2 diabetes-induced mice showed an increase of testicular steroidogenic activities, insulin receptor and glucose transporter 8 proteins, and glucose and lactate intra-testicular concentrations [37], thus supporting the idea that ADIPOQ

improves testicular functions also through the increase of intra-cellular energy substrate transport and the reduction of oxidative stress [37].

As far as the avians are concerned, in male chickens, the ADIPOQ/ADIPOR1 and -R2 system was expressed in the testes [31]. More precisely, ADIPOQ and ADIPOR1 were localized in the peritubular and Leydig cells, and ADIPOR2 was mainly observed in the Sertoli cells, spermatids, and spermatozoa, suggesting that ADIPOQ can affect the maturation and differentiation of spermatocytes [31].

According to Ocón-Grove et al. [18], in chicken, ADIPOQ and ADIPOR1 immunolocalization and gene expression were evidenced exclusively in the peritubular cells as well as in Leydig cells. Conversely, ADIPOR2 positive cells were found in the ad luminal and luminal compartments of the seminiferous tubules as well as in interstitial cells. In particular, Sertoli cell syncytia, round spermatids, elongating spermatids, spermatozoa, and Leydig cells showed strong ADIPOR2 immunoreactivity.

In agreement with Tabandeh et al. [14], we found that ADIPOR1 immunopositivity was in the cells of the basal portion of germinal epithelium surrounding the seminiferous tubules. Caminos et al. [30] have suggested that the ADIPOQ presence is exclusively in Leydig cells and macrophages in the rat testis interstitium [30]. In addition, these authors [30] reported that *ADIPOR1* mRNA, but not *ADIPOR2*, is present in the seminiferous tubular epithelium isolated from rat testis. In the chicken testis, based on the distinguishable flattened cell morphology of the peritubular cells, *ADIPOQ* and *ADIPOR1* were expressed in peritubular myoid cells [18]. The localization of both *ADIPOQ* and *ADIPOR1* in peritubular cells indicate that ADIPOQ could influence myoid cell function [18]. Peritubular myoid cells are involved in the transport of spermatozoa and testicular fluid from the seminiferous tubule [39], secretion of extracellular matrix proteins such as fibronectin [40], and regulation of Sertoli cell function [39,41]. In addition to myoid cells, the peritubular space also contains immune cells such as macrophages [42]. *ADIPOR1* was shown to be expressed in the epithelium of the seminiferous tubules of rams, where it is involved in the regulation of spermatogenesis, as previously reported in rats [19,30]. Since ADIPOQ has a fundamental role in the male HPG axis and regulation of steroidogenesis [43,44], the effects of circadian disruption on testicular *ADIPOQ, ADIPOR1* and *ADIPOR2* mRNA expressions were examined in some seasonal species [43], finding an inverse relationship between light hours and their gene expression.

Rahmanifar and Tabandeh [21] reported that *ADIPOQ*, *ADIPOR1* and *ADIPOR2* transcripts are present in the testes, epididymitis, and vesicular and bulbourethral glands. Our results demonstrated the location *ADIPOR1* in cells of glandular epithelium in adult rams. Additionally, the *ADIPOR2* expression level in different parts of the male reproductive tract was more than that of *ADIPOR1* [21]. Unfortunately, this study lacks the date of sampling, and these data would have been important to understand the possible seasonality of the ADIPOQ/cognate receptors system. In mammals, testicular growth and regression are photoperiod-dependent, meaning they are mainly determined by the endogenous circadian secretion of melatonin [45]. In mammals, endogenous biological rhythms regulate multiple physiological and behavioral processes that are essential for successful reproduction, so much that their misalignment provokes reproductive disorders [45]. Within this context, in rats, a species that shows a circadian rhythm, Moustafa [46] demonstrated that *ADIPOR1* is expressed in the epithelium of the seminiferous tubules, where it is involved in the regulation of spermatogenesis [30]. Since ADIPOQ has a fundamental role in the male HPG axis and regulation of steroidogenesis, the effects of circadian disruption on testicular *ADIPOQ*, *ADIPOR1* mRNA expressions in the ram should be examined, as has been demonstrated in other species.

Ovine are known as mammals with a marked seasonality of breeding activity. In fact, in these species, the daily photoperiod is the determinant factor for this activity, whereas environmental temperature, nutritional status, and social interactions are modulators, as evidenced by the correlation between AMPK and variable gonadotropins [19] during the ovine cycle. Taibi et al. [47] indicated that AMPK is expressed in the ovine testis and regu-

lates steroidogenesis in male sheep. Additionally, pituitary AMPK is known to act as an energy sensor, thus controlling gonadotropin secretion and reproduction in bovine [20]. In this context, it is important to emphasize that AMPK is activated by ADIPOQ to inhibit human GnRH release, through the hyperpolarization of plasma membranes as well as calcium influx [48]. In addition, Dutta et al. [49] reported that the amount of GnRH immunoreactive neurons decreased with ADIPOQ mutations, suggesting that this cytokine controls GnRH secretion in mammal hypothalamus. The important role of ADIPOQ in reproductive mechanisms is also suggested by its effects on prostaglandins secretion [50]. In particular, it is well known that the semen of mammalian species contains high amounts of different prostaglandins. These findings support the idea that the ADIPOQ/cognate receptor system may be associated with the secretion of these factors in the male reproductive tract.

5. Conclusions

The present results strengthen the evidence of the ADIPOQ/ADIPOR1 system's role in regulating the mammalian reproductive processes, particularly in the testicular activity of male rams, during the non-breading season. Despite this, our knowledge is still underdeveloped; therefore, future studies are needed to better elucidate the fine mechanisms of the ADIPOQ/ADIPOQ cognate receptors system in modulating reproductive processes. This future research on reproductive activities regulated by the ADIPOQ/ADIPOQ receptors system will enable us to better understand the physiological mechanisms that link adipose tissue with the mammalian reproductive processes, specifically on how an altered energy status can induce reproductive pathologies in humans and animals.

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