International Journal of Endocrinology

Energy Sensors in Female and Male Reproduction and Fertility

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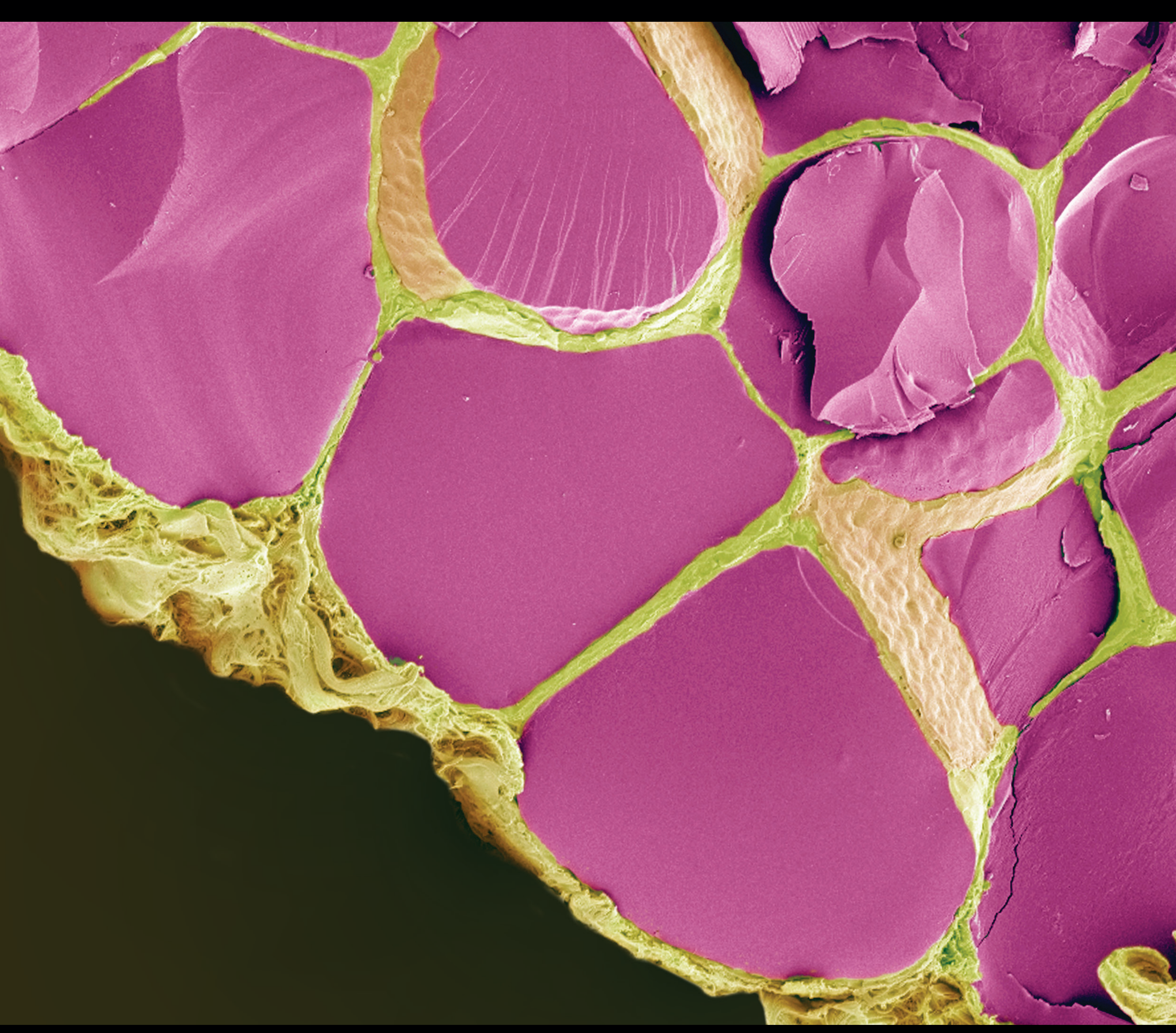

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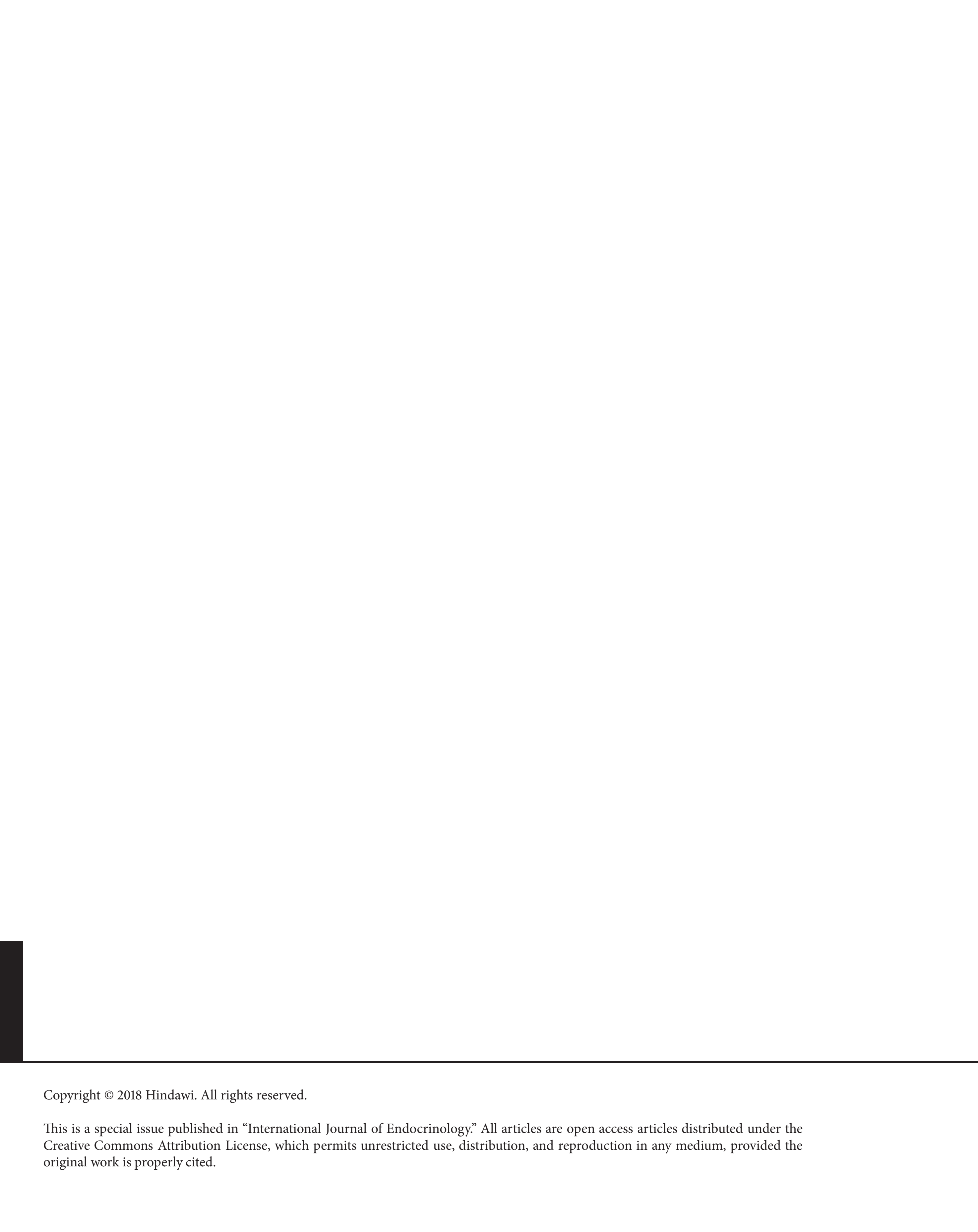

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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 8285793, 3 pages
https://doi.org/10.1155/2018/8285793

Editorial

Energy Sensors in Female and Male Reproduction and Fertility

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Received 6 August 2018; Accepted 6 August 2018; Published 27 September 2018

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1. Introduction

In mammals and birds, the reproductive system is very sensitive to states of energy deficit. For example, chronic food restriction in prepubertal female rats prevents the normal onset of puberty [1]. In chickens, food restriction decreases body weight, the numbers of yellow preovulatory follicles, and the proportion of atretic yellow follicles and increases the age at onset of lay [2]. In pigs, a negative energy balance and a decrease in body fat result in a reduction in litter size and viability of piglets [3]. In sheep, it is well known that an increase in availability of energy substrates is associated with an increase in prolificacy [4]. Furthermore in human, both clinical and experimental studies reveal the negative consequences of obesity on male and female reproductive function [5]. Obese women also have higher rates of many complications in pregnancy, including gestational diabetes and rates of cesarean delivery [6]. Although the clinical impact of obesity on male and female infertility has been well described, the mechanisms involved that could lead to effective treatment are still being elucidated.

The hypothalamic pituitary gonadal (HPG) axis is central to the reproductive system. Pulsatile gonadotropin-releasing hormone (GnRH) released from the hypothalamus stimulates the secretion of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. In females, FSH promotes follicle maturation and LH regulates ovulation whereas in males, FSH is mainly involved in spermatogenesis and LH stimulates the synthesis and the secretion of testosterone by the testis.

Compelling evidence indicates that common regulatory signals involving energy sensors are implicated in the integrated control of energy balance and reproduction. At the whole animal level, energy sensors play a crucial role to indicate whether energy reserves are abundant (obesity) or poor (in case of food restriction and sports training). These energy sensors can be hormones, kinases, or nutrients.

Some fertility disorders related to abnormal metabolism have highlighted the importance of the role of hormones produced by white adipose tissue (adipokines) in the regulation of mechanisms involved in human and animal reproductive functions [7]. It is well known that white adipose tissue provides energy in the form of fatty acids and glycerol to other organs, especially when dietary intake does not meet requirements. However, adipokines produced by white adipose tissue play an important role in the regulation of the reproductive axis [8]. The most studied and described adipokine in the literature is leptin [9]. For example, leptin has been proved to cooperate with other regulatory signals such as ghrelin (the endogenous ligand of the growth hormone (GH)) in the integrated control of energy balance and reproduction [10]. The major involvement of leptin in the regulation of food intake, adiposity, insulin sensitivity, and reproduction has paved the way for the discovery of new adipokines such as adiponectin, visfatin, chemerin, and apelin [11]. Deregulation of plasma levels of these adipokines has been linked to the onset of pathologies such as obesity,

insulin resistance, diabetes (type 2 or gestational), and polycystic ovary syndrome (PCOS) [12–15]. Furthermore, the removal of adipose tissue has been demonstrated in mice to inhibit reproductive functions [20]. Thus, an adequate amount and distribution of white adipose tissue may be essential for the completion of normal gametogenesis and fertility.

Given the tight link between energy metabolism and reproduction, in this special issue, we propose to discuss the role of some energy sensors such as adipokines on the fertility in different species including mammals and birds. Birds have some metabolic and reproductive peculiarities interesting to study the relationship between adipokines, metabolism, and reproduction.

This special issue includes one novel research article and 4 reviews summarized as follows:

2. Adipokines in Female Reproductive Tractus

2.1. Review Article: "Apelin in Reproductive Physiology and Pathology of Different Species: A Critical Review." In this review article, P. Kurowska et al. described the structure, expression, and function of apelin and its receptor, APJ. They also reported data concerning ELABELA, an endogenous ligand for APJ recently discovered. The authors summarized the physiological and pathological role of apelin in the hypothalamus-pituitary-gonadal axis. For example, this article summarizes the results of a series of recent studies on the effect of apelin on reproduction pathologies, like polycystic ovary syndrome, endometriosis, and ovarian cancer. Many of these pathologies are still in critical need of therapeutic intervention, and the authors report data showing that apelin could be a target in various reproductive pathologies.

2.2. Review Article: "Adiponectin: A New Regulator of Female Reproductive System." K. Dobrzyn et al. focused their attention on adiponectin that is the most abundant adipokine in plasma. After a brief description of the structure of the hormone and its main receptors, AdipoR1 and AdipoR2, the authors described the role of adiponectin in the hypothalamus-pituitary-ovary axis. Furthermore, they reported new data about the involvement of adiponectin in the embryo development and in the physiology of the placenta in different species including humans.

3. Adipokines in Gestational Diabetes Mellitus

3.1. Research Article: "A Randomised, Controlled Study of Different Glycaemic Targets during Gestational Diabetes Treatment: Effect on the Level of Adipokines in Cord Blood and ANGPTL4 Expression in Human Umbilical Vein Endothelial Cells." P. Popova et al. investigated the gene expression of adipokines (leptin, adiponectin, and angiopoietin-like protein 4 (ANGPTL4)) in human umbilical vein endothelial cells (HUVECs) and adipokine concentration in cord blood from women with gestational diabetes mellitus (GDM) depending on glycaemic targets. Three groups of patients were studied: control ($n = 25$) and two groups of GDM—GDM1 (tight glycaemic targets, fasting blood glucose < 5.1 mmol/L and <7.0 mmol/L postprandial, $N = 20$) and GDM2 (less tight glycaemic targets, <5.3 mmol/L and <7.8 mmol/L, respectively, $N = 21$). In HUVECs, they showed that ANGPTL4 expression was decreased in GDM patients (with no difference between GDM1 and GDM2) whereas adiponectin gene expression was similar between control and GDM patients and leptin expression was undetectable. In cord blood, the leptin/adiponectin ratio (LAR) was increased in GDM2 compared to controls and GDM1 and did not differ between GDM1 and controls.

4. Adipokines in Male Reproductive Function

4.1. Review Article: "Adipokines in Semen: Physiopathology and Effects on Spermatozoa." Y. Elfassy et al. summarized the current data on the localization in the male genital tract and the role of seven adipokines (leptin, adiponectin, resistin, chemerin, visfatin, vaspin, and progranulin) and other cytokines in male reproductive fertility. This review is interesting because most of the published reviews about the link between adipokines and fertility are focused on females but not on males. More precisely, the authors described the regulation of these adipokines in blood and seminal plasmas in different conditions (infertilities associated or not with obesity) based mainly on *in vivo* studies. Furthermore, they summarized some data obtained mainly from *in vitro* studies about the role of the adipokines in semen parameters (sperm concentration, motility, and morphology). Several human polymorphisms of these adipokines are also reported. A list of transgenic animals for different adipokines or adipokine receptors is presented with a description of phenotypes.

5. Peculiarities of Adipokines during the Interaction between Reproduction and Metabolism in Birds

5.1. "Chicken Is a Useful Model to Investigate the Role of Adipokines in Metabolic and Reproductive Diseases." N. Mellouk et al. provided an overview of the structure and function related to metabolic and reproductive mechanisms of four adipokines (leptin, adiponectin, visfatin, and chemerin) in avian species as compared to mammals. They emphasize and discuss why avian species are an interesting model to study the adipokines.

6. Conclusions

In animal and human species, the optimum body condition level is necessary for normal physiological activities including reproductive efficiency. Inadequate nutrition impairs human fertility and reproductive potential in livestock. This special issue reports how the adipokines convey the body metabolic status to the hypothalamic-pituitary-gonadal axis to regulate fertility. It shows that the molecular mechanisms of these adipokines in reproductive tissues are still unclear. Thus, further research investigations need to be undertaken to unveil the exact mechanism of actions and signalling pathway of adipokines in gonadal dynamics and functions which might improve reproductive efficiency.

Conflicts of Interest

I and the other guest editors declare to have no conflict of interest.

Joëlle Dupont
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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 4579734, 19 pages
https://doi.org/10.1155/2018/4579734

Review Article

Chicken Is a Useful Model to Investigate the Role of Adipokines in Metabolic and Reproductive Diseases

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Received 15 March 2018; Accepted 16 May 2018; Published 19 June 2018

Academic Editor: Michaela Luconi

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Reproduction is a complex and essential physiological process required by all species to produce a new generation. This process involves strict hormonal regulation, depending on a connection between the hypothalamus-pituitary-gonadal axis and peripheral organs. Metabolic homeostasis influences the reproductive functions, and its alteration leads to disturbances in the reproductive functions of humans as well as animals. For a long time, adipose tissue has been recognised as an endocrine organ but its ability to secrete and release hormones called adipokines is now emerging. Adipokines have been found to play a major role in the regulation of metabolic and reproductive processes at both central and peripheral levels. Leptin was initially the first adipokine that has been described to be the most involved in the metabolism/reproduction interrelation in mammals. In avian species, the role of leptin is still under debate. Recently, three novel adipokines have been discovered: adiponectin (ADIPOQ, ACRP30), visfatin (NAMPT, PBEF), and chemerin (RARRES2, TIG2). However, their mode of action between mammalian and nonmammalian species is different due to the different reproductive and metabolic systems. Herein, we will provide an overview of the structure and function related to metabolic and reproductive mechanisms of the latter three adipokines with emphasis on avian species.

1. Introduction

Adipose tissue was initially recognised only as an energy storage organ. Since the discovery of leptin, an increasing number of studies have reported that adipose tissue may also play a role as a dynamic endocrine organ by synthesising and secreting numerous bioactive factors termed adipokines [1–4]. In mammals, these molecules are involved in the regulation of multiple biological processes such as metabolism (glucose and fatty acid) and reproduction (steroidogenesis, gonadal development, and gametogenesis).

Energy homeostasis is mostly dependent on lifestyle, including physical activity and a healthy diet (food variety and intake), and also on hormonal regulation and a genetic predisposition to metabolic diseases like obesity. Globally, the number of obesity cases has almost tripled since 1975 and has become a major public human health problem. In 2016, the number of overweight (body mass index (BMI) $\geq 25\,kg/m^2$) or obese (BMI $\geq 30\,kg/m^2$) people reached 1.9 billion people in the world (World Health Organisation 2016). People that suffer from this pathology have a high risk of developing type 2 diabetes, insulin resistance, cardiovascular disease, and infertility [5]. One of the female reproductive pathologies that may be associated with obesity and insulin resistance is polycystic ovary syndrome (PCOS). PCOS is characterised by the consensus of Rotterdam as a syndrome of ovarian dysfunction presenting 2 of the following 3 criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries without any sign of other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumours, and Cushing's syndrome) [6]. Approximately 75% of PCOS patients are overweight and central obesity is observed in both normal and overweight PCOS women [7, 8]. One of the potential biochemical tools that can be used to give an overview of the state of reproductive health is the measurement of serum adipokines. Indeed,

adipokines have recently been shown to be increased in the serum of overweight/obese PCOS patients compared to normal-weight patients [9–14]. In males, obesity has been linked to hypogonadism as well as to a reduction of sperm quantity and quality [15, 16]. These impairments appear when the endocrine system is altered. In fact, in obese men, there is excessive activity of cytochrome P450-alpha, leading to an increase in the conversion of androgen into oestrogen and a decrease of testosterone levels [17]. Consequently, testosterone and FSH plasma levels are negatively correlated with BMI [18] and testosterone levels increase after bariatric surgery [19]. In addition, rising plasma levels of leptin and chemerin are observed, while those of adiponectin are decreased in obese men [20–22]. The relationship with other adipokines is still obscure, even though some studies have focused on their molecular role [23]. Metabolic diseases also affect farm animals, especially chickens, because of the genetic and nutritional practices used to optimise meat and for egg production. The domestic chicken represents both a widely used biomedical model and an important source of high-quality protein in the human diet. Despite decades of intensive genetic selection, the remarkable growth rate of commercial broiler chickens is still improving but is also accompanied by deleterious increases in body fat and skeletal muscle and disorders in metabolism and reproduction.

In this review, we report several traits that make chicken a viable model for studies of adipose biology, obesity, and insulin resistance. Most metabolic genes are conserved in humans, and a number of quantitative trait loci (QTLs) that have been linked to fatness in chickens contain genes implicated in human susceptibility to obesity or diabetes [24]. In addition, a recent study described the differential expression of adipokines in adipose tissue of two lines of meat-type chickens that have been genetically selected for either high (FL) or low (LL) visceral abdominal fatness [25]. In addition, overfeeding of hens led to reproductive deficiencies linked to the anarchic follicular hierarchy for females and a delay in sexual maturation in males [26, 27]. Finally, the egg presents an opportunity to directly manipulate the developmental milieu and study the consequences on adipose metabolism via in ovo injection. These peculiarities make chickens a good animal model to understand the relationship between adipokines, metabolism, and reproduction and their associated mechanisms.

The most studied adipokine in mammals was leptin, but its existence in avian species faced extensive controversies for a long time. Nowadays, the long list of adipokines reached more than a hundred and included adiponectin, visfatin, and chemerin, which control glycaemia, energy, and fertility homeostasis [23, 28]. Their structures and physiological functions were largely described in mammals, particularly in humans and rodents, but less is known about their involvement in avian species. Furthermore, several adipokines found in mammals like TNFα, resistin, and omentin have not been mapped to the chicken genome [29]. Recently, the chicken genes of three novel adipokines (adiponectin, visfatin, and chemerin) were cloned and evidence showed their potential role as key regulators of food intake, muscle growth, and reproduction [30–32] in avian species; however, knowledge of their functional activity needs to be expanded.

In the current review, after a brief description of the metabolic and reproductive peculiarities of avian species and the impact of metabolism on reproduction in this species, we will focus on the structure and function of three adipokines (adiponectin, visfatin, and chemerin) with regard to chicken metabolism and reproduction.

2. Metabolic Peculiarities in Avian Species

The metabolic system of chicken is closely related to that in mammals. Glucose is stored as glycogen in tissues and used for energy production through glycolysis. Glucose is the exclusive source of energy for the brain. However, chickens constitutively exhibit "hyperglycaemia" (>200 mg/dL), despite rather normal levels of a hyperactive endogenous insulin. Large doses of exogenous insulin are required to induce hypoglycaemia; furthermore, chickens tolerate doses of exogenous insulin that would be lethal to mammals [33, 34]. The release of insulin by the perfused chicken pancreas also appears unusual in response to metabolites, which are insulinotropic in nondiabetic mammals [35, 36]. Therefore, chickens are constitutively hyperglycaemic and insulin resistant, which makes chickens mimic the condition of type 2 diabetes in mammals [37]. Glycaemia levels depend on the line, age, and sex of the animals [38], and enhanced adiposity in chickens is associated with lower fasting plasma glucose, which is in contrast to the situation in mammals [39]. This has been observed in four experimental chicken lines that were genetically selected for fatness (FL) versus leanness (LL) [40] or high growth (HG) versus low growth (LG) [41], where the HG chickens are also fatter than LG chickens. Interestingly, FL chickens are clearly not hyperphagic, since they eat the same amount of feed as LL chickens. Furthermore, FL chickens are not resistant to exogenous insulin; in fact, the FL birds are more sensitive to the hypoglycaemic effect of insulin than LL chickens [42]. Reciprocally, divergent selection for high or low fasting plasma glucose levels induces an associated change in adiposity, where chickens with low fasting plasma glucose are also fatter [39, 43].

In mammals, insulin sensitivity of the various tissues is an important factor controlling nutrient partitioning. Any alteration of the insulin signalling cascade in one of the major metabolic tissues (liver, muscle, or fat tissues) will alter nutrient utilisation and storage and ultimately body composition. The peculiarities exhibited by chickens for plasma glucose levels and insulin action compared with mammals have been described in different reviews [34, 37]. Insulin exerts pleiotropic effects in chicken [44]. To date, insulin receptors, two receptor substrates (IRS-1 and Shc), and major downstream components of insulin signalling have been characterised in chicken liver, muscle, and adipose tissue in different experimental models [45–47]. Insulin signalling appears to proceed through tissue-specific cascades in chicken metabolic tissues. In the liver, insulin elicits a signalling cascade with a similar response to those observed in mammals, including tyrosine phosphorylation of the insulin receptor

β-subunit (IRβ), insulin receptor substrate-1 (IRS-1), and Src homology 2 domain-containing substrate (Shc) and activation of phosphatidylinositol 3-kinase (PI3K) [45, 46]. The situation in skeletal muscle is very different. Tyrosine phosphorylation of IRβ and IRS-1 and PI3K activity are not regulated by insulin, whereas event downstream of PI3K (e.g., Akt and P70S6K activation) is accordingly sensitive [46]. Furthermore, in several skeletal muscles, chickens and ducks are not totally insensitive to exogenous insulin, which enhances the uptake of glucose [48, 49]. Moreover, immunoneutralisation of insulin rapidly induces considerable increases in plasma levels of glucose in young chickens [44]. Insulin induces a rapid although modest increase in glucose uptake by chicken myotubes, an uptake that is inhibited by phloretin, an inhibitor of glucose transporters [50]. These findings support the existence of functional glucose transporters in avian muscle. Nevertheless, the mechanism of the control of plasma glucose in chickens remains to be elucidated as immunoreactive GLUT1, but no GLUT4 has been detected in chicken tissues. Recently, Coudert et al. suggested that the facilitative glucose transporter protein GLUT12 could act in chicken muscle as an insulin-sensitive transporter that is qualitatively similar to GLUT4 in mammals [51, 52]. In chicken adipose tissue, as in muscle, we reported that insulin also does not elicit a classical IRβ-initiated cascade, including the downstream steps of Akt and P70S6K activation [47].

The chicken metabolic system was submitted to large changes since their body weight and fat are approximately four times heavier than 50 years ago [53]. The increase in adipose tissue mass was needed to assume the huge requirement of meat and egg production [54]. The abdominal (visceral) fat pad is the major fat tissue in chickens. Adipose tissue growth is a combination of hyperplasia during young age and hypertrophia in adult chickens mostly, which contributes to fat deposition [55]. It expands rapidly during post hatch. Chicken adipocytes increase volume by storing fatty acids that come primarily from the liver. In both chickens and humans, the liver serves as the primary site of de novo lipogenesis, whereas the rate of lipogenesis in adipose tissue is about 100 times lower. Hormonal and nutritional control of hepatic lipogenesis is comparable between birds and mammals. In chicken, lipogenesis is low in adipose tissue as compared to liver. Furthermore, the regulatory mechanisms of lipid metabolism can be different in these two tissues. As previously described, the existence of the insulin-dependent glucose transporter (GLUT4) has not been established in chickens. No direct effect of insulin on glucose transport has been shown in chicken adipocytes, although an increase in glucose disappearance from the incubation medium of cultured chicken adipocytes has been taken as indirect evidence of an effect of insulin on glucose transport [56]. In isolated chicken adipose tissue or adipocytes, insulin slightly stimulates glucose oxidation and the incorporation of acetate-U-^{14}C into lipids in the presence of glucose. Compared to rat adipocytes, the insulin stimulation of lipogenesis is slow (~3 hours), is low in magnitude (30–40%), and requires very high insulin concentrations [36].

3. Reproductive Peculiarities in Chicken Species

In all birds, the female is the heterogametic sex (ZW), while the male is homogametic (ZZ). In contrast to mammals, female chickens maintain only the left reproductive tract (ovary plus oviduct). The ovary is typically organised in a strict follicular hierarchy consisting of 2 to approximately 6 preovulatory follicles and ovulates at most a single follicle per day. Physiologically, only the largest preovulatory follicle ovulates every 26–28 h. The characteristics of ovarian asymmetry and preovulatory follicle hierarchy are generally believed to be at least in part reflections of weight reduction for flight [57]. In ad libitum (free access to food) fed hens, the ovarian follicular hierarchy is disorganised by multiple ovulations resulting in fertility deficiency and ovarian cancer [58, 59]. As in mammals, steroidogenesis in preovulatory follicles occurs within multiple layers of the theca. In birds, theca cells express aromatase and synthesise oestrogens from androgen precursors that are localised to the externa while pregnenolone, progesterone, and androgen precursors are produced almost exclusively within the theca interna [60]. The granulosa cells produce progesterone, de novo, from cholesterol and pregnenolone and has the capacity to convert progesterone to testosterone but not to oestrogen. In contrast to mammals, ovulation in birds is induced by the stimulatory action of ovarian progesterone derived predominantly from the granulosa layer of the largest preovulatory follicle and pituitary LH.

Interestingly, the ovary of the aging domestic hen has been utilised as a model for human reproductive cancers. This is based upon observations that the hen develops spontaneous ovarian/oviductal tumours with high incidence (estimated in 30–35% of hens by 3.5 years of age); the tumours are associated with the accumulation of ascites fluid; plus, they biochemically and histologically resemble human tumours of epithelial origin [61]. Furthermore, birds offer excellent models to study the mechanism and function of hormone-mediated maternal effects since the embryo develops outside the mother's body, facilitating the measurement and manipulation of early hormone exposure. Finally, another peculiarity in female birds is that the oviduct is able to store sperm for a prolonged period. The sperm storage tubules (SST) are located in the uterovaginal junction of the oviduct, where sperm can be stored and survive for a few weeks after insemination or natural mating [62]. Nowadays, the advantage of prolonged sperm storage and survival in the oviduct of laying hens is utilised in practical poultry production systems. Indeed, this peculiarity enables laying hens to produce a series of fertile eggs following a single copulation event or artificial insemination. In the male chicken prepubertal layer, anaerobic glycolysis in the testis may participate in sertoli cell proliferation, which may improve meiotic processes and consequently sperm production [63]. Finally, unlike mammals, birds do not possess a pampiniform complex (venous and arterial complex which makes it possible to maintain the intratesticular temperature constant).

4. The Metabolic Impact on Reproductive Performances in Chicken Species

In birds, like all other species, nutrition, more particularly energy metabolism, influences the reproductive function. Models of hyperphagic birds have shown the negative effect of overfeeding in both sexes, while a dietary restriction during their growth increases oviposition rates and the duration of the fertile period.

4.1. Relationship between Growth, Restriction, and Fertility. In chicken selected for meat production, the rapid growth of selected individuals is almost always accompanied by an impairment of maximum reproductive capacity in both sexes [64]. The case of broiler lines is typical: the selection of fast-growing lines for more than 60 years was accompanied, in the males of these lines, by a very high sexual precocity. This great "spontaneous" precocity results in the appearance of testicular spermatozoa from the age of 11-12 weeks in roosters. It also results in relatively low maximum testicular development and testicular regression from the age of 43–45 weeks. In addition, an increasing proportion of these cocks (around 40–60% of the total) showed a shorter breeding season, which leads to replacing them well before the end of the laying period in females, sometimes causing severe problems in social behaviour. In 1990, Reddy and Sadjadi estimated that males had a decreased ability to fertilise eggs by about 0.5% for each new generation [65]. However, the excessive growth of males is usually accompanied by hyperphagic behaviour due to the overconsumption of food in relation to their needs. In females, the increase in weight induces the anarchic development of follicles, which can lead to the coexistence of several follicular hierarchies that disrupt ovulation [66]. A relationship between the weight of chickens at sexual maturity and the number of large follicles growing on the ovary has been shown [67].

For both sexes, the maintenance of reproductive performance (e.g., spawning and fertility) according to the standard of the strain can be assured only if strict food restriction is applied at a very young age (2-3 sem after hatching). Thus, the control of body weight via food restriction makes it possible to preserve in males (a) a morphology and reduction of locomotor disorders induced by overweightness, compatible with mating [68]) and (b) an acceptable fertility at least during the first part of the sexual season [69–71]. However, it appears that the application of restrictions will have side effects on behaviour, such as pecking [72].

4.2. Overfeeding-Fertility Relationship. In birds, as previously described, liver function has some specificities compared to mammals. During food intake, the lipids absorbed in the intestine will first cross the liver, where they can be collected and used before reaching the bloodstream [73]. In addition, the intake of dietary carbohydrates will stimulate lipid synthesis. In birds, the liver is also the main site of de novo lipogenesis, including triglyceride synthesis and also, as in mammals, phospholipids and cholesterol [74]. These triglycerides produced by the liver are either incorporated in VLDL (very low-density lipoprotein) and then transported by the blood to growing oocytes, adipocytes, and muscle tissue or can be stored in the liver. In birds, the liver also produces vitellogenin, which acts on ovarian function. The ovary can also interact with liver activity, since both vitellogenin and hepatic VLDL production are stimulated by oestrogen [75].

Although there is no real obesity in poultry, unlike mammals, the overweightness observed is rather associated with fast-growing lines (strain meat) that can have a behaviour of hyperphagia. Models of overweight birds (ad libitum food or gavage) have provided a better understanding of the consequences of overfeeding on reproductive function in both sexes. Thus, in roosters, gavage-induced obesity decreases sperm production by 50% and reduces egg production in chicken. In males, this drop in fertility is the result of a decrease in testicular weight (approximately 30% in 4 weeks), which is accompanied by a decrease in testosterone and an increase in intratesticular cholesterol and an internal temperature of 0.3°C [76]. Thus, the increase in temperature following the increase in energy resulting from gavage would lead to an alteration in the functional state of the spermatogonia stem, thereby causing a decrease in the production of spermatozoa.

In the immature female, significant body weight is often a consequence of excessive consumption in comparison to their needs, which causes the accelerated development of the reproductive system at the time of sexual maturity [77, 78] and ovarian hyperactivity. Although the production of an egg is a process requiring a lot of energy, an excess of energy decreases the production of functional oocytes by causing dysregulation of the follicular hierarchy [79]. An increase in the frequency of multiple ovulations or close ovulation leads to a higher incidence of abnormal eggs (deformed, soft, etc.). Multiple ovulations lead to the appearance of "double eggs" (eggs with two egg yolks), whereas close ovulations usually lead to the appearance of a first "normal" egg, with the second being smaller, deformed, and presenting calcification defects [80]. Walzem et al. studied the effect of overfeeding on hepatic lipoprotein production using the laying hen as a model. They observed an increase in the diameter of lipid vesicles of VLDL type, which have the peculiarity in hens of having an identical size of approximately 30 nm. This alteration in physicochemical properties modifies the blood transport to the follicle under development [80]. Eventually, this lack of transport leads to a cessation of yolk deposition in growing follicles. In such females, moderate quantitative restriction or a limitation of dietary energy intake ("qualitative" restriction) is usually sufficient to restore the optimal ovulation rate [78, 81, 82].

All together, these findings show that chickens, in the same way as mammals, do not escape reproductive disorders in the case of metabolic dysfunction. Various hormones, including growth hormone, insulin-like growth factors (IGFs), and insulin, have been proposed as potential mediators affecting reproductive function. However, the interactions between the reproductive endocrine axis and the metabolic axis have not been clearly determined. Adipokines represent good candidates for such reproductive-metabolic interactions.

5. Leptin Controversy in Avian Species

In mammals, leptin was discovered as the first obesogenic gene in 1994 by Zhang et al. [4]. Clinical investigations as well as mice in vivo studies proved that leptin is a key regulator of energy homeostasis and mediates satiety signals to the central nervous system [83]. Leptin secreted from adipocytes is clearly positively related to adipose tissue masses and is secreted more by subcutaneous than visceral adipose tissue [84]. In accordance with this finding, circulating leptin remains elevated in obese patients and is associated with reproductive functions [85, 86]. The leptin gene has also been cloned in other mammalian species such as primates, rodents, and porcine, ovine, bovine, and canine species and shares a close homology with the mouse leptin gene [87–92]. However, in chicken, subsequent studies have brought conflicting results regarding leptin gene cloning. First, Taouis et al. and Ashwell et al. reported avian leptin sequences, after which Friedman-Einat et al. contradicted their findings [93–95]. Thus, the leptin gene was considered for a decade to be missing from the avian genome. However, 2 or 3 years ago, leptin genes were discovered in several bird species: in zebra finch (*Taeniopygia guttata*), rock dove (*Columba livia*), falcon (*Falco peregrinus*), and quail (*Coturnix japonica*) [96–99]. More than 20 years after the characterisation of leptin in mammals, Seroussi et al. identified the leptin (LEP) genes of chicken (*Gallus gallus*) and duck (*Anas platyrhynchos*) [100, 101]. These newly identified avian LEP proteins share only 26–30% identity with human LEP. This group suggests an autocrine/paracrine mode of action for bird leptin instead of it being a circulating hormone, as in mammals. Chicken leptin mRNA was highly correlated with leptin receptor (LEPR) expression (except in the pituitary) and was reported to be mostly expressed in the brain, with LEPR expressed mostly in the pituitary. Similar to other avian species and conversely to humans, chicken leptin mRNA is not highly expressed in adipose tissue and, similar to zebra finch, is not expressed in the liver [97, 100]. The intramuscular administration of leptin antibodies induces feed intake and increases glycaemia and lipaemia, which mimic the effect of leptin depletion in the ob/ob mouse model, and increases the expression of the leptin receptor in adipose tissue, the liver, and muscle [102]. Moreover, a recent study showed no effect of a chicken leptin peptide on food intake or behaviour, suggesting that chicken leptin is not sufficient to mediate effects on appetite in the brain [103]. The in vivo injection of leptin also improved the negative effects of fasting on ovarian function by attenuating follicular apoptosis, delaying the cessation of egg laying and influencing ovarian steroidogenesis [104]. Thus, the role of leptin in avian species is still unclear and chicken leptin likely has a different physiological role in birds than in mammals. Two independent studies report that approximately 274 to 640 protein-encoding genes that are present in the genomes of most vertebrate lineages including humans are missing from 60 bird genomes [105, 106]. A recent study based on the phylogenic evolution of genome supported the hypothesis that other adipokines, including TNFα, resistin, and omentin, might be missing from the chicken genome [29]. However, Lovell et al. brought new arguments to contradict the absence of some genes in the bird genome, especially due to their location in GC-rich regions and the technical limitations to identifying them [107]. Based on this hypothesis, Bornelöv et al. conducted a de novo transcriptome assembly and identified 191 new GC-rich genes in chickens, including TNFα [108]. One year later, Rohde et al. reported the identification and functional characterisation of the avian orthologue of TNFα [109]. An additional study also indicated that TNFα mRNA was poorly expressed in the visceral fat of female broilers and layer chickens and was not affected by feed deprivation [110]. These recent data open new debates on the inexistence of other adipokines considered missing from the chicken genome; if they are identified, more investigations will be needed to determine their potential involvement in the endocrine control of metabolic and reproductive functions in chicken.

6. Adiponectin

6.1. Structure and Expression of Adiponectin and Its Receptors. Adiponectin cDNA was isolated from human adipose tissue in 1996 by Maeda et al. as the adipose most abundant gene transcript 1 (apM1) [111] and in parallel from murine fibroblast cell lines (AdipoQ) by Hu et al. [112]. The 15.8 kb adiponectin gene encodes a 26 kDa protein that was described for the first time by Scherer et al. and designated as adipocyte complement-related protein (ACRP30) [113]. The adiponectin protein was also extracted from human plasma [114], where it was considered the most abundant adipokine, ranging between 5 and 30 mg/L. Adiponectin is secreted into the blood from adipocytes with a higher serum level associated with the female gender and inversely related to body weight. It is found in cells and plasma in three major forms: trimers, hexamers, and high-molecular weight (HMW) [115]. In addition, a smaller fragment generated by the proteolytic cleavage of full-length adiponectin gives rise to a globular domain of protein gAd which is secreted in the plasma. Among them, the HMW form plays important roles in the regulation of insulin signalling and is closely associated with peripheral insulin sensitivity [116]. In patients with obesity or type 2 diabetes, plasma levels of HMW adiponectin are decreased [117, 118] and a reduction in HMW adiponectin levels, rather than total adiponectin levels, contributes to the aetiology of obesity-associated diseases [119]. Adiponectin is able to bind three kinds of receptors: AdipoR1, AdipoR2, and T-cadherin. The first two consist of seven transmembrane domains, with the opposite topology to G-protein-coupled receptors in which the N-terminal region is cytoplasmic, while the C-terminal region is extracellular [120]. The binding of adiponectin to AdipoR1 preferentially results in the activation of AMPK pathways, whereas the adiponectin/AdipoR2 interaction induces the stimulation of the PPARα (peroxisome proliferator-activated receptor alpha) signal. These receptors, although expressed ubiquitously, have different tissue distributions. AdipoR1 has a predominant location in skeletal muscle and endothelial cells, while AdipoR2 is mainly expressed in the liver. The third receptor is a glycosyl-

Table 1: Expression of adipokines (visfatin, adiponectin, and chemerin) and adipokine receptors (ADIPOR1, ADIPOR2, CMKLR1, GPR1, and CCRL2) in the main metabolic and reproductive tissues in chicken.

		Adipose tissue	Liver	Muscle	Brain	Ovary	Testis	References
Visfatin	mRNA	+	+	+	+	+	+	[161, 162, 206, 207]
	Protein	nd	nd	+	nd	+	+	[180,185]
Adiponectin	mRNA	+	+	+	+	+	+	[119, 144, 145, 206, 207]
	Protein	+	+	+	nd	+	+	[117, 118, 121, 145]
Chemerin	mRNA	+	+	+	nd	+	nd	[206, 207]
	Protein	nd	nd	nd	nd	nd	nd	
ADIPOR1	mRNA	+	+	+	+	+	+	[119, 143, 144, 145, 206, 207]
	Protein	nd	nd	nd	nd	+	+	[118, 145]
ADIPOR2	mRNA	+	+	+	+	+	+	[119, 144, 145, 206, 207]
	Protein	nd	nd	nd	nd	+	+	[118, 145]
CMKLR1	mRNA	+	+	+		+		[206, 207]
	Protein	nd	nd	nd	nd	nd	nd	
GPR1	mRNA	+	+	+		+	nd	[206, 207]
	Protein	nd	nd	nd	nd	nd	nd	
CCRL2	mRNA	+	+	+		+	nd	[206, 207]
	Protein	nd	nd	nd	nd	nd	nd	

+: detected; nd: not determined.

phosphatidylinositol receptor, belonging to the cadherin family, which lacks a transmembrane domain. The intracellular signalling connected to this receptor seems to require other unidentified coreceptors or AdipoR1/AdipoR2.

In chicken, the coding region of chicken adiponectin shares 67% and 65% identity with human and mouse, respectively [121]. In addition, the chicken ADIPOR1 cDNA was found to be 80–83% homologous to human, mouse, rat, or pig ADIPOR1 cDNA, while the deduced protein sequence was 91% similar to mammalian ADIPOR1. Similarly, the chicken ADIPOR2 cDNA was 76–78% homologous to human, mouse, or pig ADIPOR2 cDNA, while the deduced protein sequence was 82% similar to mammalian ADIPOR2 [122]. Adiponectin and adiponectin receptor genes are ubiquitously expressed in various tissues (Table 1) [123, 124], and the expression of the adiponectin system (adiponectin, ADIPOR1, and ADIPOR2) in adipose tissue and muscle depends on the gender and age of the animals [125]. In adipose tissue, adiponectin mRNA was higher in 154-day-old females than in males and ADIPOR1 mRNA was higher in 154-day-old males than in females. Adiponectin and ADIPOR2 mRNA were higher, and ADIPOR1 mRNA was lower, in thigh muscle in female compared with male chickens. Furthermore, the adiponectin plasma levels are lower in 8-week-old chickens which have more abdominal fat pad mass relative to body weight than 4-week-old chickens, suggesting that adiposity or age influence the adiponectin plasma levels in chickens [126]. In addition, the adiponectin gene may be associated with the initiation and growth processes of adipose tissue deposition in chickens [127, 128]. Chicken fed ad libitum develop more abdominal adipose tissue which is accompanied by an increase in adiponectin mRNA expression in adipose tissue [128].

6.2. Role of Adiponectin. In mammals, basic science studies have shown the beneficial effects of adiponectin on various physiological functions, including glucose homeostasis, food intake, apoptosis, oxidative stress, and atherosclerotic processes; so, this molecule usually has been considered a beneficial adipokine [129, 130]. For example, adiponectin is known to play key roles as an insulin sensitiser and an anti-inflammatory regulator, in addition to the regulation of glucose metabolism and fatty acid breakdown [130]. In wild-type and diabetic mice, a two- to five-fold increase in circulating adiponectin levels can reduce plasma glucose levels [131]. The injection of adiponectin in obese and type 1-diabetes mice models displaying hyperglycaemia and severe hyperinsulinaemia restored normal circulating levels of glucose [131]. Furthermore, adiponectin knockout mice fed with a high-fat diet develop glucose intolerance and severe hepatic insulin resistance [132]. Chronic treatment with globular adiponectin resulted in decreased body weight and adipocyte areas in high-fat diet-fed rats accompanied by an increase in PPARγ expression in adipose tissue that prevents the dysregulation of lipolysis [133]. There were strong inverse associations between circulating HMW adiponectin and intramyocellular lipid content in human skeletal muscle [134]. Adiponectin also enhances AMPK activity in the arcuate hypothalamus (ARH) via its receptor AdipoR1 to stimulate food intake in mice [135]. In addition, several reports have indicated an association between low adiponectin levels and an elevated risk of various cancers (breast, endometrial, and gastric). Concerning the reproductive functions, plasma adiponectin levels were found to be 4-fold higher in sexually mature versus sexually immature mice [136]. In the ovary, adiponectin and its receptors appear to be involved in steroidogenesis in a different manner

depending on the species [137]. In human granulosa cells, both FSH and hCG (as a surrogate for LH) treatment increased AdipoR2 mRNA by more than 2-fold and stimulation with adiponectin improved hCG-induced progesterone production 3-fold [138]. In porcine follicular cells, adiponectin increases steroidogenic acute regulatory protein (StAR) transcript abundance but reduces cytochrome P450 aromatase expression [139]. Similarly, adiponectin inhibits insulin-induced progesterone and androstenedione production in bovine theca cells [140]. In our lab, we showed that adiponectin decreases insulin-induced steroidogenesis and increases IGF1-induced proliferation of cultured bovine granulosa cells [141]. In males, the expression of AdipoR2 appears to be critical for testicular function since AdipoR2-deficient knockout mice exhibit reduced testis weight characterised by atrophy of the seminiferous tubules and aspermia, while plasma testosterone levels remained unaffected [142]. Stimulation with recombinant adiponectin also inhibited basal and human hCG-stimulated testosterone secretion in rat-cultured Leydig cells [143]. The role of adiponectin in the hypothalamic-pituitary-gonadal axis and in the PCOS pathology has been recently reported by Rak et al. [137].

In broiler chickens, Tahmoorespur et al. showed that adiponectin mRNA expression in adipose tissue was inversely related to chicken belly fat deposition levels [128]. Adiponectin has a remarkable effect on the impairment of adipocyte differentiation, which contributes to the negative regulation of fat deposition in chicken [144]. Yan et al. observed that adiponectin inhibited lipid deposition and the differentiation of chicken preadipocytes through the p38 MAPK/ATF-2 and TOR/p70 S6 kinase signalling pathways [145]. Chicken globular adiponectin inhibits lipid deposition in adipocytes by suppressing the expression of CEBP and FAS, while increasing the expression of ATGL. The mechanism is explained by the observations that globular adiponectin stimulates p38 MAPK/ATF-2 activation and suppresses the TOR/p70 S6 kinase pathway [146]. More precisely, the ADIPOR1 gene is implicated in metabolism and/or fat deposition in broilers [147]. In chicken adipocytes, adiponectin also regulates mitochondrial biogenesis by inhibiting lipid accumulation and activating the AMPK/ACC signalling pathway [148]. In the muscle of broilers, rosiglitazone (antidiabetic drug) increases circulating adiponectin levels while dexamethasone (glucocorticoid anti-inflammatory drugs) has opposite effects and adiponectin has an antilipogenic effect through the p38 MAPK/ATF2 signalling pathway [145]. For the same body weight and egg production, the high residual feed intake chicken line (R^+: fat line) consumes 40% more food than their counterpart low residual feed intake chicken line (R^-: lean line). In the hypothalamus, ADIPOR1 expression is increased in R^+ as compared to R^- chickens, suggesting a role for this receptor in food intake regulation in chicken [149]. In reproduction, the adiponectin gene was found in the chicken ovary to be mainly expressed in theca cells and is suggested to exert a paracrine or autocrine effect on ovarian steroidogenesis. Adiponectin increased IGF-1-induced progesterone secretion in F2 and F3/4 follicles, whereas it halved progesterone production in response to LH and FSH in F3/4 follicles [150]. In male broiler breeder chickens, the expression of adiponectin and its receptors has been studied in testes [151]. A significant elevation of ADIPO1 and ADIPOR2 gene expression is observed in sexually mature chickens, which could be a result of the higher metabolic activity related to spermatogenesis, testicular steroid hormone production, and the transportation of spermatozoa and testicular fluid [151].

Globally, adiponectin limits lipid deposition in adipose tissue and induces food intake through AdipoR1/AMPK signalling in the human and chicken adipose hypothalamus (Figure 1(a)). Also, adiponectin as an insulin sensitiser could be tested in chicken, especially for their natural insulin resistance. However, the effects of adiponectin on steroidogenesis are dependent on the species, suggesting different physiological regulations (Figure 2).

7. Visfatin

7.1. Structure and Expression of Visfatin. Visfatin was first discovered as a growth factor called pre-B cell colony-enhancing factor (PBEF) in 1994 from human peripheral blood lymphocytes that are able to initiate the maturation of B-cell precursors (135). Visfatin has also been considered a type II nicotinamide phosphoribosyltransferase (NAMPT) due to its ability to synthesise nicotinamide mononucleotide (NMN) from nicotinamide and 5′-phosphoribosyl-1′-pyrophosphate. NMN is a therapeutic target for treating metabolic disorders by improving glucose clearance in obese and diabetic mice models [152–154]. The identification of visfatin as an adipokine has been controversial since an active binding site of the insulin receptor was discovered. The adipogenic and insulin mimetic action of visfatin depends on the preparation of recombinant visfatin. To date, only four different recombinant visfatin forms were validated [155–157]. Recently, the crystal structure of rat [158, 159], mouse [160], and human [161, 162] visfatin has been solved and revealed a dimer organisation separated by an active site. In humans, the visfatin gene is on the long arm of chromosome 7 and encodes a 52 kDa secreted protein [163]. Visfatin expression has been studied to a large extent in humans and also in animal models [164–166] including chicken [167].

The full length of the chicken visfatin gene has been cloned from adult liver. The chicken visfatin protein had high amino acid sequence similarities with those of humans (94%), rodents (94%) [167] and other agronomic species (94%) [168]. The chicken visfatin mRNA was detected in many tissues such as the brain, heart, intestine, kidney, liver, lung, muscle, spleen and gonads (Table 1) [167, 169]. Not surprisingly, visfatin was also expressed in adipose tissue without any difference between subcutaneous and visceral fat tissues in humans [170] and chicken [167]. However, Li et al. showed that visfatin was differentially expressed in adipose tissue depending on the chicken species, with higher mRNA levels in broiler chicken (fast growing) than in silky flow (low growing), suggesting a potential role as a marker of fat accumulation [168]. In addition, visfatin expression is sexually dimorphic and depends on tissue types. In chicken, it was described more as a myokine than an adipokine, because of its main expression in muscle and its ability to

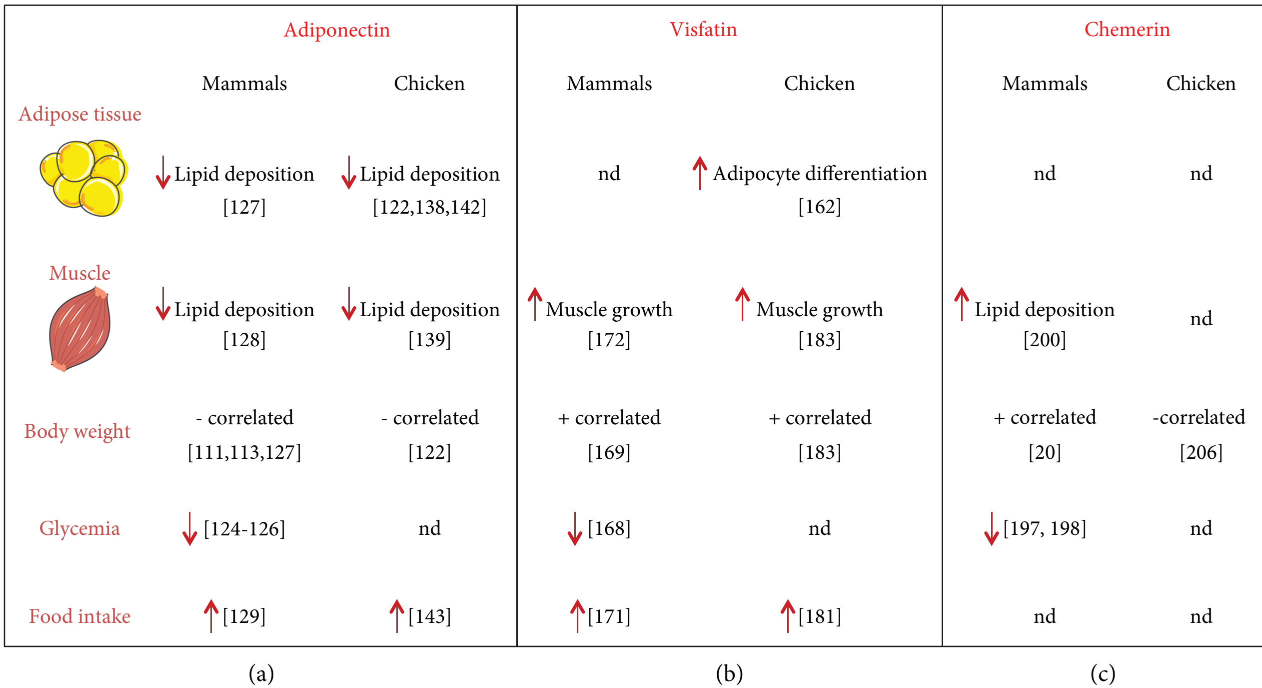

Figure 1: Comparison of adiponectin (a), visfatin (b), and chemerin (c) effects on main metabolic functions in mammal versus chicken. nd: not determined; + correlated: positively correlated; − correlated: negatively correlated; ↑: increase; ↓: decrease.

decrease the expression of MYF5 expression (a myogenic factor) in myoblasts [31]. One of our recent studies also demonstrated that visfatin was more expressed in the theca than in granulosa cells in turkeys [32] and that its plasma level was higher at the end of the laying period compared to the beginning. We also described its expression in the ovarian cortex, granulosa, and theca cells of chicken hierarchical follicles. To date, no visfatin receptor has been identified.

7.2. Role of Visfatin. Visfatin is a pleiotropic protein involved in a large spectrum of physiological processes from aging to atherosclerosis [171]; here, however, we will focus only on metabolic and reproductive functions. Physiological studies have revealed a strong role of visfatin on glucose, fatty acid metabolism, and muscle growth. A loss of visfatin in mice adipose tissue impaired adipose tissue functions such as inflammation, severe insulin resistance via the synthesis of nicotinamide that is one of the oldest drugs known for its antilipolytic effects [172], mediated by its interaction with GPR109A, a receptor on the adipocyte plasma membrane [173]. Visfatin also improves glucose-stimulated insulin secretion in pancreatic β-cells by increasing nicotinamide adenine dinucleotide biosynthesis, while visfatin haplodeficiency causes impaired glucose tolerance in mice, which was rescued after NMN administration [174]. Visfatin has become an emerging adipokine due to subsequent studies that have brought proof regarding it positive association with obesity and type 2 diabetes [175, 176]. In rats, the injection of visfatin in the arcuate nucleus of the hypothalamus plays an orexigenic role via the modulation of dopamine, CART, and CRH peptide activity [177]. In addition, the depletion of visfatin in mice leads to a decrease in intramuscular NAD synthesis and consequently induced fibre degeneration and progressive loss of strength and treadmill endurance muscle [178]. On the other hand, the visfatin concentration profile in follicular fluid is a potential indicator for ovarian reserve for woman undergoing ovarian stimulation regarding to the positive correlation between its expression in follicular fluid and the number of oocyte retrieved [179]. The visfatin expression in ovarian mice increased with advancing follicular development [180]. Choi et al. [180] also reported that the administration of low concentrations of visfatin during superovulation improved the fertility of aged female mice. Furthermore, visfatin increases IGF-1-induced progesterone and oestradiol production in human and bovine-cultured granulosa cells [181, 182]. Visfatin protein expression was detected in human sertoli cells and Leydig cells and in the tail and the connecting piece of spermatozoa. Visfatin protein expression and release are higher in immature than in mature ejaculated spermatozoa leading to the increased production of nicotinamide without any effect on sperm mobility and viability [183]. Visfatin also seems to be involved in the regulation of rat testicular activity since its testicular decreasing expression is positively correlated with serum testosterone levels and testis weight in a diabetic rat model [184]. In addition, visfatin increases testicular steroidogenesis from purified rat Leydig cells [185].

In chickens, there is increasing evidence that visfatin is involved in the regulation of muscle growth [186], metabolism [31], food intake, and reproductive functions [187, 188]. For instance, a polymorphism in exon 7 of the visfatin gene was positively associated with the body weight of 4- and 6-week-old chickens, as well as the body slanting length, fat bandwidth, breast muscle water loss rate, and

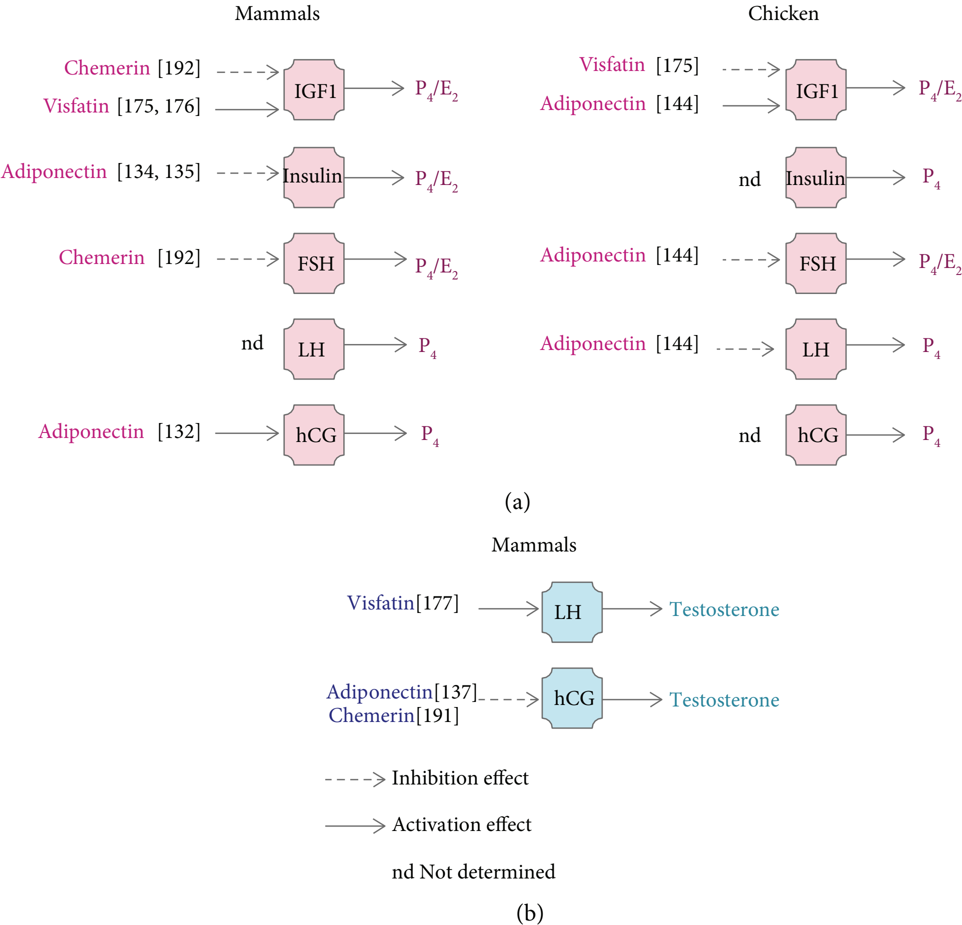

Figure 2: Comparison of adiponectin, visfatin, and chemerin effects on female (a) and male (b) steroidogenesis in mammal (a, b) versus chicken (a). IGF1: insulin like growth factor 1, FSH: follicle-stimulating hormone, LH: luteinizing hormone, hCG: human chorionic gonadotropin.

breast muscle fibre density and breastbone length of 4-week-old chickens [189]. The central injection of visfatin in chicks induced an increase in their food intake, suggesting that visfatin is a potent orexigenic factor [187]. In addition, supplementing the chicken diet with chenodeoxycholic acid induced a decrease in feed intake and body weight associated with a reduction of the expression of visfatin in the liver. This suggests a potential role of visfatin in hepatic lipogenesis [190]. Similarly to insulin, recombinant chicken visfatin may induce the differentiation of 3T3-L1 cell lines by increasing the mRNA expression of adipocyte differentiation markers (PPARγ, aP2, FAS, and C/EBPα) [168]. Visfatin also acts in reproductive tissues such as ovarian (theca and granulosa cells) and testicular cells (sertoli cells, Leydig cells, and spermatozoa). More precisely, visfatin inhibits IGF1-induced progesterone production in hen granulosa cells and its protein levels in the testis and plasma increase in adults compared to prepubertal chickens, suggesting a potential role in regulating testosterone production [188, 191].

Thus, chicken visfatin and mammalian visfatin act as an orexigenic factor, regulating muscle growth, and their expression is positively correlated with body weight (Figure 1(b)). However, chicken visfatin plays an opposite role on male and female steroidogenesis compared to mammals (Figure 2). This makes chicken a good model to deepen our knowledge on the regulatory mechanisms induced by visfatin in food intake, adipogenesis, and myogenesis. On the other hand, the insulin mimetic activity of visfatin could be interesting to confirm in chicken.

8. Chemerin

8.1. Structure and Expression of Chemerin. Chemerin is an adipose cytokine which was previously known as tazarotene-induced gene 2 (TIG2) and retinoic acid receptor responder protein 2 (RARRES2) [192]. Chemerin is a recently identified adipokine that is closely related to the pathogenesis of metabolic syndrome [193]. It is secreted as a 143-amino acid inactive prochemerin, which is then hydrolysed by the enzymatic cleavage of 5 to 7 amino acids from its carboxyl terminus in the extracellular compartment. Two neutrophil serine proteases, elastase and cathepsin G, remove 6 and 7 amino acids, respectively, to generate an active form. Plasmin and tryptase are also able to cleave 5 amino acids from the carboxyl terminus followed by cleavage of the carboxyl-terminal lysine by carboxypeptidases N and B that

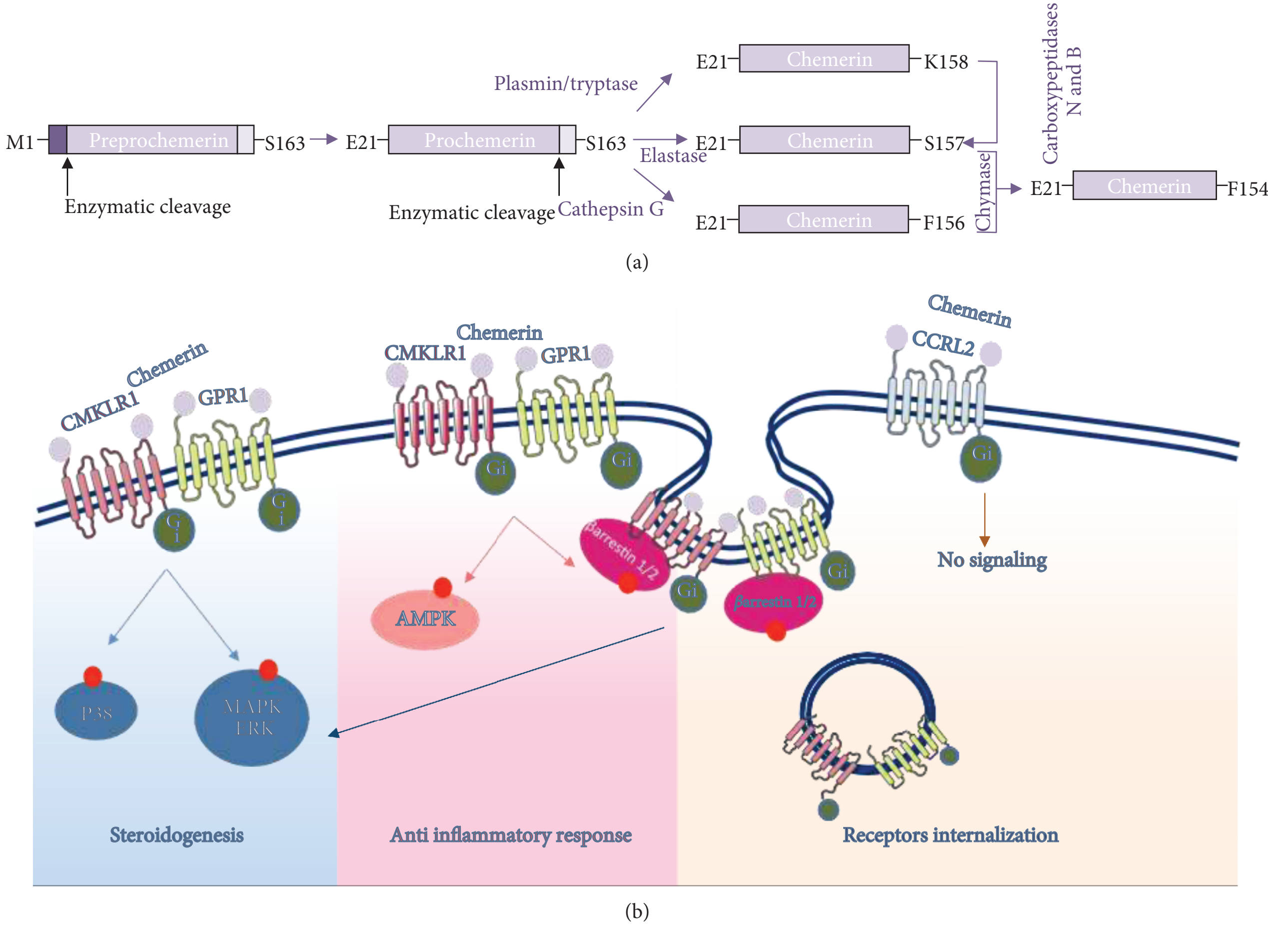

Figure 3: Structure of chemerin (a) and representation of chemerin system signalling (b). CMKLR1 (chemokine-like receptor); GPR1 (G protein-coupled receptor 1); and CCRL2 (chemokine (C-C motif) receptor-like 2).

also result in the active chemerin [194] (Figure 3(a)). Chemerin is secreted from white adipocytes and expressed in several tissues, mainly white and brown adipose tissue and the liver, pancreas, placenta, skin, kidney, adrenal gland, lung, intestine, ovary, and testis [195–198]. Chemerin exerts its physiological functions through the binding of three G protein-coupled receptors: the chemokine-like receptor 1 (CMKLR1), G protein-coupled receptor 1 (GPR1), and chemokine (C-C motif) receptor-like 2 (CCRL2) [195, 199]. CMKLR1 is coupled to the Gi/o family of G proteins and inhibits the cAMP signalling pathway while promoting phospholipase C, PI3K, MAPK, calcium mobilisation [194], and β-arrestin recruitment, which activate MAPK (ERK1/2) [200]. The GPR1 sequence is closely related to CMKLR1 with more than 40% identity and activates the same pathway [201]. In contrast, CCRL2 does not seem to promote any signalling pathway and does not induce receptor internalisation [200] (Figure 3(b)).

In avian species, chemerin and their receptors are expressed in peripheral tissues and ovarian cells (Table 1). In turkeys, chemerin mRNA was mainly present in the liver compared to the heart, adipose tissue, and muscles, while CMKLR1 and GPR1 mRNAs were ubiquitous. CCRL2 mRNA was highly expressed in pectoral muscle and adipose tissue compared to the liver, heart, and leg muscle. In addition, chemerin and its receptors were more expressed in theca cells compared to granulosa cells in both preovulatory follicle 1 and 3/4 hierarchical follicles [32].

8.2. Role of Chemerin. Chemerin is involved in the regulation of blood pressure, inflammation, immune responses, adipocytes differentiation, and carbohydrate metabolism and plays a key role in metabolic diseases, such as obesity and diabetes [202]. GPR1 knockout mice fed with a high-fat diet developed serious glucose intolerance and a test of pyruvic acid tolerance suppressed glucose-stimulated insulin levels that consequently increased glycaemia [203]. Similar results were observed in CMKLR1-knockout mice which developed exacerbated glucose tolerance and insulin sensitivity with no effects on high-fat diet-induced glucose intolerance after cold exposure [204]. In overweight/obese patients, the chemerin concentration is rising and is positively correlated with BMI and waist circumference [20]. Chemerin levels are reduced after bariatric surgery [205]. In bovine intramuscular adipocytes, chemerin also promotes lipolysis in mature adipocytes and adipogenesis during adipocyte differentiation [206]. These results suggest that the chemerin system could act on glucose and fat metabolism linked to obesity. Subsequent

studies have described chemerin to inhibit gonad steroidogenesis from the testis and ovary and be involved in follicular development [196, 207]. We showed that chemerin decreased IGF- or FSH-induced progesterone and oestradiol secretion in cultured granulosa cells [198]. In addition, the level of chemerin is increased in the plasma and adipose tissue of patients with PCOS [208], as well as in the plasma of patients affected by preeclampsia [209]. Chemerin expression is also increased in the ovaries of rats treated with 5alpha-dehydrotestosterone (DHT) (mimicking PCOS) that is associated with a decrease in oestradiol secretion in granulosa cells and induces apoptosis [210]. In mice with CMKLR1 gene deletions, the effects of chronic DHT treatment on ovarian function in experimental PCOS are largely reduced, suggesting a role of the chemerin system in PCOS pathology [211]. As already mentioned, PCOS syndrome is, in some cases, associated with insulin resistance, which can both be treated with antidiabetic drugs. Metformin, an antidiabetic agent, restores physiological plasma chemerin concentrations (around 2 ng/mL) and decreases chemerin protein expression in the adipose tissue of women with PCOS, while insulin increases them, confirming the interrelation between chemerin, insulin, and reproductive homeostasis [208]. Chemerin also exerts an important role in male reproductive functions, including gametogenesis and steroidogenesis. In humans, chemerin levels in seminal plasma are negatively correlated with sperm quantity, maturation, and motility [20]. Chemerin also inhibits in vitro hCG-induced testosterone secretion in primary cultured Leydig cells [197]. These findings suggest that chemerin can regulate steroid secretion in reproductive organs and may act as a key regulator of metabolic diseases such as obesity and PCOS.

Recently, we described that chemerin and its receptors are expressed in chicken adipose tissue, liver, muscle, and ovarian cells. Our results indicated that plasma chemerin levels are negatively correlated with the fattening state of broiler hens. We also found that a restricted diet applied from 3 to 39 weeks begins to increase the plasma chemerin levels in hens during the laying period (18–39 weeks) and decreases the mRNA expression of chemerin in the liver and adipose tissue compared to ad libitum hens at 39 weeks. Furthermore, fish oil supplied (1% of the diet) from 9 to 39 weeks decreased the plasma levels of chemerin from the beginning of the treatment to the end of the prepubertal period (21 weeks) in broiler hens and decreased the mRNA expression of CCRL2 in adipose tissue and muscle and those of CMKLR1 only in adipose tissue [212]. In addition, we found that chemerin was negatively correlated with the percentage of hatchability of fertile eggs in broiler hens and the weight of preovulatory follicle 1 was positively correlated with the expression of chemerin in granulosa cells and that the production of progesterone by granulosa cells was negatively correlated with the expression of chemerin in theca cells. Restrictedly fed hens expressed lower chemerin mRNA levels in theca cells from preovulatory follicles 1 and 3 than ad libitum-fed hens. Fish oil supplement (1% of the diet) increased the mRNA expression of CMKLR1 in theca cells of preovulatory follicle F1 and decreases those of chemerin in theca cells of preovulatory follicle F3 [213]. The chicken chemerin gene sequence shares 81% identity with the turkey chemerin sequence. In turkeys, the plasma concentration of chemerin decreases at the end of the laying period and is negatively correlated with levels of plasma cholesterol, triglycerides, and phospholipid levels during the entire laying period [32]. The literature on chicken chemerin is poorly enriched and further experiments are needed to understand its promising role in metabolism and reproduction.

Finally, not enough studies were conducted in chicken to draw conclusions. However, regarding our discoveries, chemerin seems to be involved in the regulation of chicken metabolism and reproduction but in an opposite way as in those of mammals (Figure 1(c)).

9. Conclusions

Reproductive dysfunction arising from metabolic dysregulation is mostly associated with obesity and other metabolic and reproductive syndromes in humans and farm animals. In this review, we reported that many researches have linked food intake, body and fat weight, and reproductive function to plasma adipokines levels or tissue expression, especially those of leptin, visfatin, and chemerin levels. We particularly focused on chickens that were submitted to various nutritional, metabolic, and reproductive changes due to their rapid growth and high production. Chicken is an atypical species in view of their natural hyperglycaemia, insulin resistance, hepatic fatty acid synthesis, and reproductive system. Studies on chicken adipokines are emerging, and, regarding physiological features, chicken appears as an interesting model for in vivo studies that may provide critical information on the roles of adipokines on lipid and carbohydrate metabolism and the link to reproductive physiology.

Conflicts of Interest

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

Acknowledgments

This work was financially supported by the “Prevadi” grant (Grant no. 32000820) from Région Centre.

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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 9170480, 12 pages
https://doi.org/10.1155/2018/9170480

Review Article

Apelin in Reproductive Physiology and Pathology of Different Species: A Critical Review

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Received 4 January 2018; Accepted 2 April 2018; Published 6 June 2018

Academic Editor: Ludwik K. Malendowicz

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Apelin has been isolated from the bovine stomach extracts as an endogenous ligand of the previously orphan receptor APJ. Expression of the apelinergic system (apelin and APJ) was described in many organs where pleiotropic effects like regulation of food intake, body weight, or cardiovascular and immune function were described. Recent studies have shown that apelin also plays an important role in the regulation of female and male reproduction. Some data showed that the gene and protein of apelin/APJ are expressed in the hypothalamic-pituitary-gonad (HPG) axis tissue. Thus, apelin is synthesized locally in the hypothalamus, pituitary, ovaries, and testis of many species and has autocrine and/or paracrine effects. Most research indicates that apelin has an inhibitory effect on gonadotropin secretion and participates in the direct regulation of steroidogenesis, cell proliferation, and apoptosis in gonads. The article summarizes also results of a series of recent studies on the effect of apelin on reproduction pathology, like polycystic ovarian syndrome, endometriosis, and ovarian cancer. Many of these pathologies are still in critical need of therapeutic intervention, and recent studies have found that apelin can be targets in reproductive pathological states.

1. Introduction

The hormonal interactions of the hypothalamic–pituitary–gonadal (HPG) axis are accountable for a proper physiology of both female and male reproduction. It is of importance to have knowledge of new regulators/hormones controlling reproduction. It is well known that adipose tissue is implicated in the secretion of several hormones such as adiponectin, resistin, leptin, visfatin, and apelin called adipokines "adipose tissue-derived hormones." There is evidence that the increased production of adipokines might have a strong link to insulin resistance, metabolic syndrome, and obesity [1]. Apelin is a regulatory peptide, identified as an endogenous ligand of the apelin receptor named APJ [2]. Recently, the apelinergic (apelin and APJ) system was found in the HPG axis and apelin has been extensively described as a beneficial factor controlling reproduction both in females and in males. The intention of this paper is to review current knowledge concerning the expression of apelin/APJ in tissue of the HPG axis and physiological aspects of apelin on the physiology of both female and male reproduction. It will also describe apelin linked with reproduction dysfunctions like infertility, polycystic ovarian syndrome (PCOS), endometriosis, and ovarian cancer. Many of these pathologies are still in critical need of therapeutic intervention, and recent studies have found that apelin can be targets in pathological states. Therefore, apelin activity could be applied in the future in the treatment of many diseases of the reproductive system.

2. Apelin: Structure, Expression, and Function

2.1. Structure of Apelin. Apelin has been isolated from the bovine stomach extracts as an endogenous ligand of the previously orphan receptor APJ (putative receptor protein

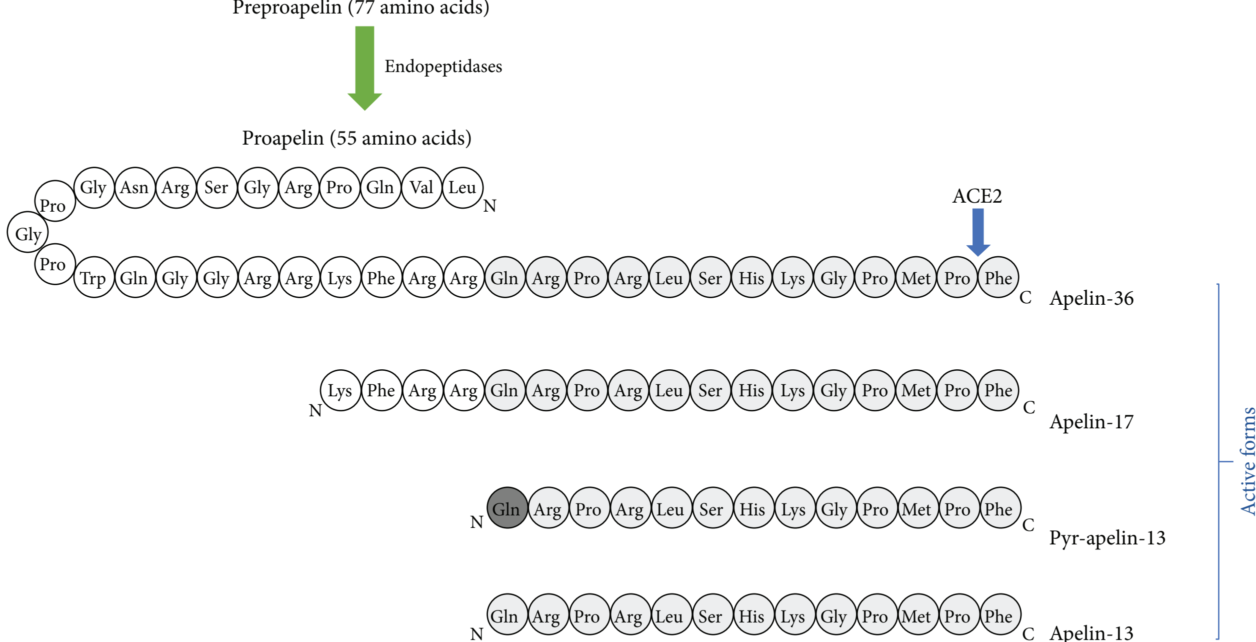

Figure 1: Amino acid sequence of native apelin and apelin isoform structure. ACE2: angiotensin I-converting enzyme 2.

related to the angiotensin receptor ATl), which is a G protein-coupled receptor [2]. Human apelin is encoded by the *APLN* gene located on chromosome Xq 25-26 [2]. This peptide has a 77-amino-acid preproapelin precursor and exists in multiple molecular forms with different biological activities. Native preproapelin, as a result of enzymatic hydrolysis, is transformed into active forms: apelin-36 (preproapelin-42–7), -17 (preproapelin 61–77), and -13 (preproapelin-65–77) and pyroglutamate-apelin-13 (pyr-apelin-13) (Figure 1) [2, 3]. Shorter forms of apelin (apelin-13) show much higher biological potency than longer forms do (apelin-36); thus, apelin-13 has been used for many different *in vitro* and *in vivo* experiments to investigate several physiological functions of apelin [2]. Additionally, pyr-apelin-13 and apelin-17 show a conserved binding to the angiotensin-converting enzyme 2 (ACE2) catalytic site and human ACE2 can cleave pyr-apelin-13 and apelin-17 [4]. Pyr-apelin-13 is a major isoform in human tissues, for example, in cardiac tissue from patients with coronary artery disease [5], and the plasma ranges from 7.7 to 23.3 pg/ml [6]. Moreover, pyr-apelin-13, apelin-13, and apelin-36 have similar efficacy and potency in cardiovascular tissues of humans [5].

The N-terminal region of apelin is rich in hydrophobic amino acids, indicating that these represent secretory signal sequences, while the C-terminal region has a sequence of 23 amino acids. It is conserved and critical for biological activity [2, 7]. Bovine, human, rat, and mouse preproapelin precursors have 76–95% homology. The endogenous form of these proteins is a dimer linked by a disulfide bond [6]. Mature forms of apelin do not have cysteine residues, and they are probably only monomeric proteins [7]. In order to bind apelin to its receptor, it is necessary to have a 13-amino-acid C terminus, which is observed in the in apelin-36 and pyr-apelin-13 [8].

2.2. Expression of Apelin. Apelin expression (mRNA and protein) was detected in various tissues and organs such as stomach, brain, heart, lung, uterus, and ovary (Figure 2) [8–10]. Additionally, literature data also documented apelin localization in the endothelia of small arteries in many organs such as lung, spleen, liver, pancreas, and adipose tissues in rats [3, 11]. Expression of apelin increases during adipocyte differentiation, and its production is regulated by several factors such growth hormone (GH) or tumor necrosis factor (TNF-α) and insulin which increased apelin production by adipocytes [12].

2.3. Function of Apelin. The apelin signaling pathway plays a role in the central and peripheral regulation of the cardiovascular system, such as blood pressure and blood flow, in water and food intake, energy metabolism, and possibly immune function (Figure 2) [10, 13]. Apelin causes endothelium-dependent vasorelaxation by triggering the release of nitric oxide (NO), and it increases myocardial contractility [3, 14]. Moreover, it is reported that apelin is a potent angiogenic factor inducing endothelial cell proliferation, migration, and the development of blood vessels in an *in vivo* study [14, 15]. APJ mRNA expression was detected in areas of the brain critical for the control of fluid homeostasis, so apelin may play a role in the regulation of water balance [7]. Levels of apelin and APJ mRNA increase in white adipose tissue and plasma with obesity than in control subjects. However, obesity has to be associated with hyperinsulinemia [12, 16], so it may be the main cause for the rise in the expression of apelin. On the other hand, apelin inhibits

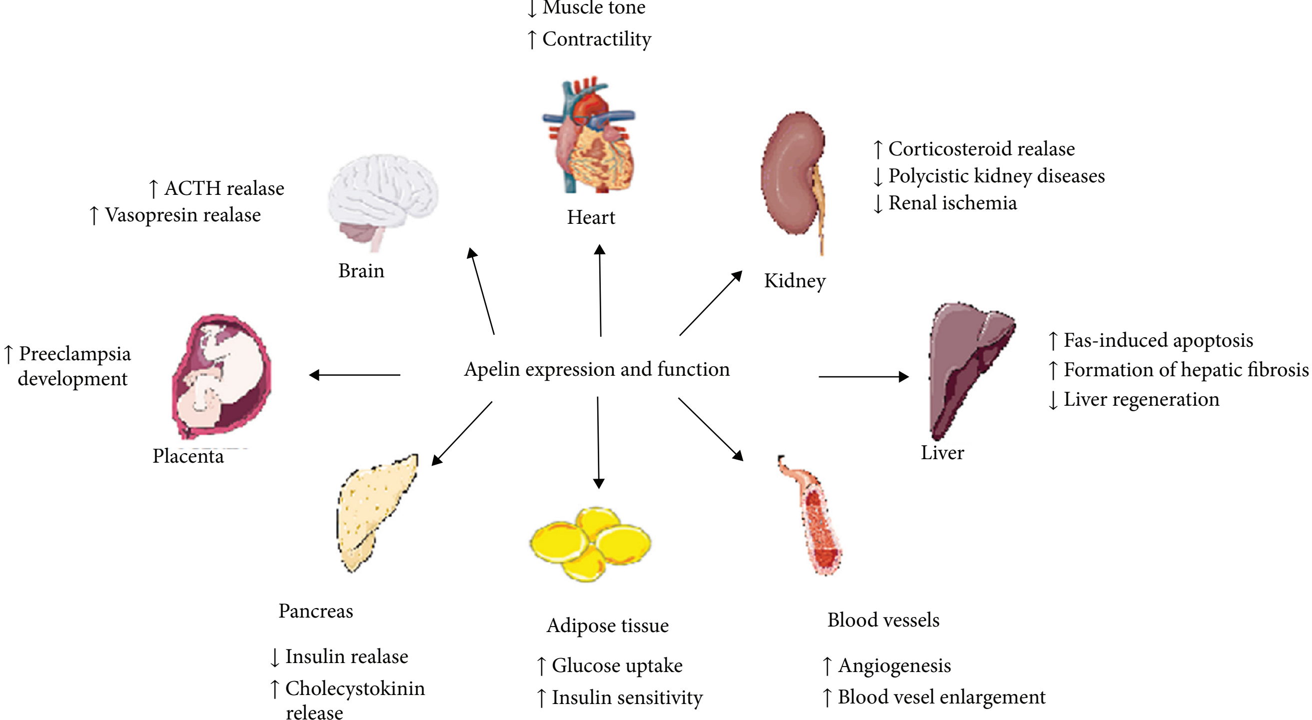

Figure 2: Apelin expression and function in the organism. ACTH: adrenocorticotropic hormone; PRL: prolactin; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

insulin release [17]. Data of Heinonen et al. [16] showed a positive correlation between the level of apelin in plasma and the body mass index (BMI). Furthermore, research studies based on young females with eating disorders showed the highest level of apelin in the group of obese patients [17]. Apelin serum levels are related to the nutritional status and parallel insulin plasma levels in mice and humans [12, 18]. Furthermore, apelin plasma concentrations are increased in obese [16] and type 2 diabetic subjects [19] as well as in hyperinsulinemic obese mice [12]. In mice, apelin inhibited glucose-stimulated insulin secretion in pancreatic islets [20], suggesting a link with glucose homeostasis. Recently, a 14-day apelin treatment in mice was shown to regulate adiposity and to increase uncoupling protein expression [21], suggesting a role of apelin in energy metabolism. Literature data documented also that apelin has anti-inflammatory effects on the release of inflammatory mediators [22]. It also inhibits release of reactive oxygen species (ROS) in adipocytes and promotes an expression of antioxidative enzymes [23]. Additionally, apelin may play an important role in lymphatic tumor progression, because its overexpression was proved in rat malignant cells [24].

3. Characteristic of Apelin Receptor: APJ

3.1. Apelin Receptor (APJ). This receptor is encoded by the APLNR (also known as AGTRL1, APJR, APJ, and FLJ90771) gene [25]. APJ is a class G protein-coupled receptor (GPCR) identified in 1993, and its structure shows high homology (40–50% in the transmembrane region) with angiotensin II receptor type AT1, but angiotensin II is unable to attach to this receptor [26]. The exact location of this gene was also determined for mice on chromosome 2E1 and for rats on chromosome 3q24 [27]. Both the structure and functioning of the human gene promoter APJ have not been fully understood [7]. APJ has a high (90%) similarity between human, rat, and mouse [28, 29] and about 50% between man and royal macaque, cow, frog, and zebrafish *Danio rerio* [7].

APJ, due to the different affinity for various forms of apelin and cointeraction with different G (Gα, Gβ, and Gγ) proteins, interacts with activation of many signaling pathways [2] (Figure 3), thereby causing various effects in the body. In early experiments, apelin-13 has been observed to inhibit forskolin's stimulating effect on 3′,5′-cyclic adenosine monophosphate (cAMP) by binding APJ to the Gi/o protein [2]. These studies have been confirmed by Habata and coauthors [6], who proved that both apelin-13 and apelin-36 are not capable of generating calcium (Ca^{2+}) mobilization in Chinese hamster ovary (CHO) cells. The different effects of both of these apelin isoforms are observed in neurons and in the human embryonic kidney cell line (HEK-293), where both isoforms increase Ca^{2+} levels [30]. APJ can also act via Gαi1 and Gαi2 proteins to inhibit adenylate cyclase in rats [31]. In turn, the CHO and the HEK-293 cell lines bind apelin with the APJ receptor via Gαi2 and then consequently activate the extracellular signal-regulated kinase (ERK 1/2) pathway [32]. Additionally, apelin binding APJ activated phosphorylation of phosphoinositide 3-kinase (PI3K) and

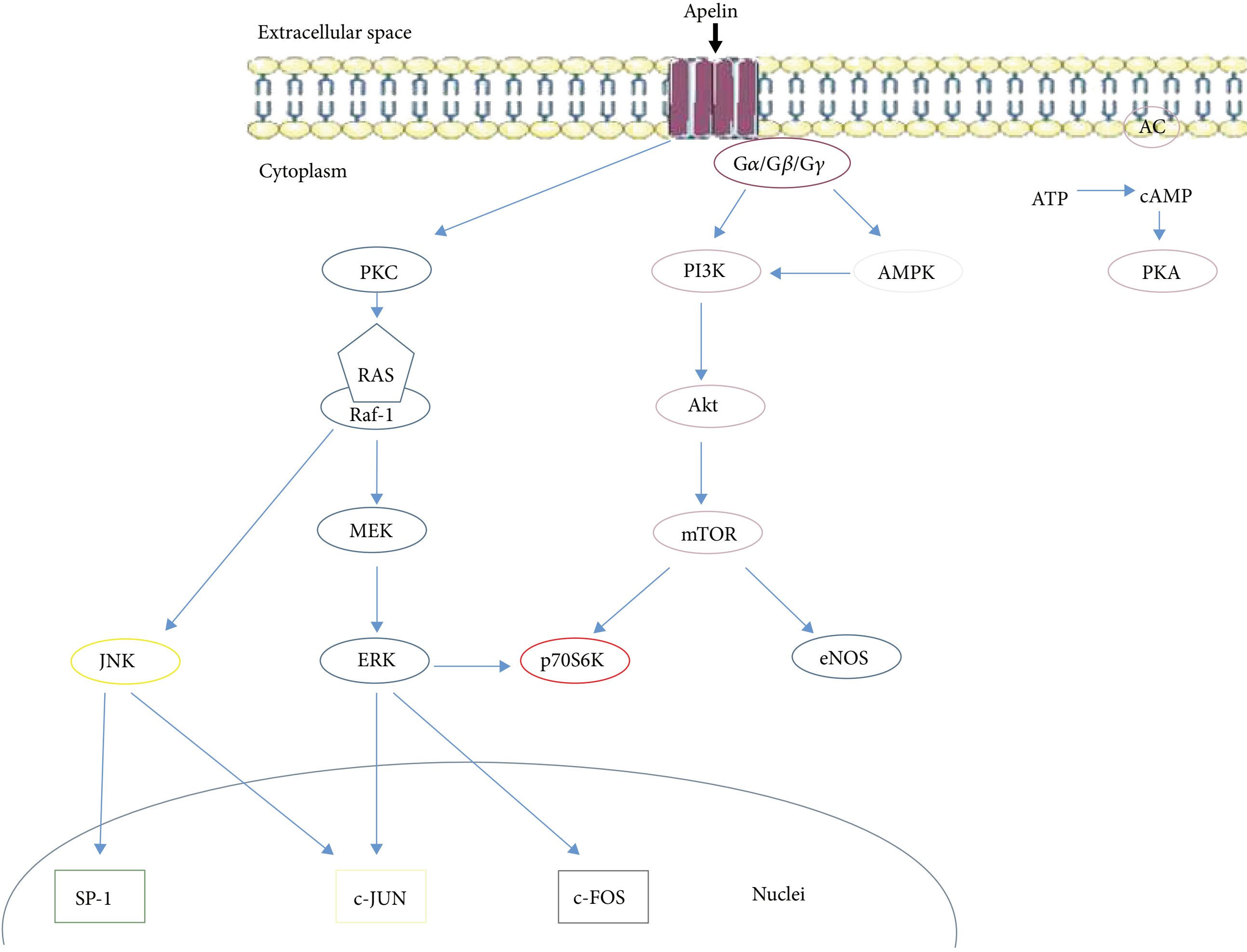

Figure 3: Pathways of apelin signaling after connection with APJ. PKC: protein kinase C: MEK-ERK activator kinase; ERK: extracellular signal-regulated kinase; JNK: c-Jun N-terminal kinases; SP-1: specificity protein 1; PI3K: phosphoinositide 3-kinase; Akt: protein kinase B; mTOR: mammalian target of rapamycin kinase; p70S6K: ribosomal S6 kinase; eNOS: endothelial NOS; AMPK: 5′-AMP-activated kinase: AC: adenyl cyclase; PKA: protein kinase A; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; c-JUN: transcription factor c-JUN; c-FOS: transcription factor c-FOS.

protein kinase B (Akt), which play an important role in cell proliferation or apoptosis. Apelin phosphorylates also the ribosomal S6 kinase (p70S6K) in human umbilical vein cells (HUVEC), thereby promoting the proliferation of these cells [31]. APJ signaling changes the level of ROS, so that apelin with APJ can stimulate catalase production and inhibit the production of hydrogen peroxide, thus protecting against cardiac hypertrophy [33]. In addition, apelin, by reducing ROS production and activating the actin kinase, protects mouse neurons from cell death [34]. One form of apelin, apelin-13, through kinase 5′AMP-activated kinase (AMPK) phosphorylation, lowers the process of mouse neuronal apoptosis after stroke. Studies on APJKO knockout mice have shown that apelin-13 by binding with APJ negatively regulates AMPK, which lowers the lipolysis process, the hydrolytic degradation of triglyceride in adipose tissue to fatty acids and glycerol [35, 36].

Gene and protein expression of APJ has been demonstrated in several tissues including the brain, ovary, kidney, pancreas, breast, and heart. Moreover, in humans, expression of APJ was high in the human brain and spleen and slightly lower in the ovary and placenta. In contrast, in the case of rat and mouse, the highest APJ expression was observed in the heart cells [7]. APJ expressions are regulated by many factors, for example, estrogens, insulin, cAMP, and CCAAT-(C/EBP-) binding protein, and strong brain stress significantly stimulates APJ secretion by adipose tissue cells [37].

3.2. ELABELA/Toddler as a Ligand of APJ. The recent discovery of a new endogenous peptide ligand for APJ, currently known as both Toddler [38] and ELABELA [39], followed screens to discover signals regulating early development. Although characterized in zebrafish, a high degree of conservation of the ELABELA/Toddler gene in vertebrate species including humans implies likelihood of similar importance in human development, but this has yet to be shown. Like apelin, this peptide exists in multiple endogenous isoforms [40]. ELABELA/Toddler signaling is motogenic, and its

absence or overproduction reduces the movement of mesendodermal zebrafish cells during gastrulation, inhibiting proper development [38]. Moreover, in ELABELA/Toddler KO knockout zebrafish, the cells of the endoderm have impaired differentiation potential and embryos exhibit stunted or completely absent heart development. This mirrors the phenotype observed in targeted deletion of APJ (APJKO) embryos [39]. Apelin KO embryos, on the other hand, do not have this phenotype. Systemic administration of ELABELA/Toddler in ELABELA/Toddler KO zebrafish rescues the otherwise aberrant phenotype [38].

Receptor activation studies revealed that the zebrafish Toddler-21 peptide acts by binding APJ and inducing receptor internalization [38]. Moreover, the expression profiles of ELABELA/Toddler and apelin differ during zebrafish development [38]. In particular, during gastrulation ELABELA/Toddler is highly expressed, whereas apelin expression remains low. Following this period, however, ELABELA/Toddler expression drops sharply and apelin levels begin to rise steadily. All these findings indicate that ELABELA/Toddler is a developmentally critical APJ ligand whose signaling behavior differs significantly from that of apelin. The exact intracellular signaling mechanism(s) of ELABELA/Toddler remains unknown. ELABELA/Toddler by activated G protein- and β-arrestin-dependent pathways acts in the human heart. Moreover, apelin acting on cardiac contractility and vasodilatation in *in vitro* experiments in rat heart [41]. Another team discovered that ELABELA/Toddler increases cardiac contractility in an ERK1/2-dependent manner in adult rat hearts [42].

4. Physiology and Pathology of Apelin in the Hypothalamus–Pituitary Axis

4.1. Expression and Effect of Apelin on the Hypothalamus–Pituitary–Axis. The central nervous system, especially in the hypothalamus and pituitary, contains primary sites of apelin action. The apelinergic neurons were firstly observed in the central nervous system of rats using the immunohistochemistry method [43], indicating the topographical distribution of apelinergic neurons suggesting multiple roles for apelin in the control of behaviors, pituitary hormone release, and circadian rhythms. Apelin and APJ gene expression was observed in the hypothalamic supraoptic nucleus and in the magnocellular and parvocellular parts of the paraventricular nucleus (PVN) in rats [43]. In the hypothalamus, apelinergic nerve fibers were detected in the periventricular, suprachiasmatic, ventromedial, dorsomedial, nucleic, and retrochiasmatic areas. The immunofluorescence method shows that apelin-immunoreactive neuronal cell bodies were localized throughout the rostrocaudal extent of the mouse activity-regulated cytoskeleton-associated protein (Arc). Moreover, apelin localized with proopiomelanocortin (POMC) and weakly with neuropeptide Y (NPY). By immunohistochemistry using in situ hybridization, APJ is present in Arc POMC neurons. Apelin/APJ mRNA was also detected in the anterior and posterior pituitary and in intermediate lobes of the rat pituitary [29]. Moreover, Reaux et al. [43] using immunofluorescence staining discovered that apelin is coexpressed in the anterior pituitary with corticotrophs and somatotrophs using rats as model.

The hypothalamic localization of apelin fibers and receptors suggests an involvement of apelin in the control of hormone release [44]. In an ex vivo perifusion system of rat anterior pituitaries, apelin-17 significantly increased basal adrenocorticotropic hormone (ACTH) release [45]. Moreover, results of the perifusion technique for hypothalamic explants have been demonstrated that apelin-17 increased α-melanocyte-stimulating hormone (α-MSH) release, suggesting that apelin released somatodendritically or axonally from POMC neurons may stimulate α-MSH release in an autocrine manner [46]. In the hypothalamus, apelin may be involved also in food intake; in rats, apelin-13 intracerebroventricular (icv) injection increased food intake by inhibited cocaine- and amphetamine-regulated transcript (CART) mRNA expression and serotonin secretion and by increased orexin mRNA expression in the hypothalamus [47]. Chronic icv infusion of apelin in the mouse hypothalamus increased also the expression of proinflammatory factors, associated with higher levels of interleukin-1 beta in plasma [48]. Apelin-13 in the PVN increased c-*Fos* expression [49] and secretion of both plasma ACTH and corticosterone (CORT) [50, 51]. Moreover, icv administration of pyr-apelin-13 was used to indicate where the postranslation modification occurs and showed apelin-13 decreasing prolactin (PRL), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels [50]. An *in vitro* study documented that apelin-13 increased the release of corticotropin-releasing hormone (CRH) and vasopressin (AVP) from hypothalamic explants, with no effect on NPY release [44, 50], suggesting that apelin may play an important role in the hypothalamic regulation of water intake and endocrine axes. Newson et al. [52] using APJ KO mice had established a role for APJ in the integration of neuroendocrine responses to acute stress and had demonstrated a gender-specific function of apelin in peripheral immune activation of the hypothalamus–pituitary–adrenal axis [52]. Moreover, Tobin et al. [53] documented that apelin-13 administration onto the hypothalamic supraoptic nucleus increased the firing rates of vasopressin cells but had no effect on the firing rate of oxytocin neurons, suggesting a local autocrine feedback action of apelin on magnocellular vasopressin neurons.

An icv administration of apelin-13 produced a dose- and time-related antinociceptive effect; this effect was significantly antagonized by the APJ receptor antagonist apelin-13, indicating an APJ receptor-mediated mechanism [54]. Apelin-13 is also involved in the autophagy suppression of neural cells; thus, it attenuates traumatic brain injury [55]. In lactating rats, apelin modulates the activity of oxytocin neurons; the activity is inhibited by a direct action of the apelin on its receptor, expressed by these neurons [56].

5. Physiology and Pathology of Apelin in the Ovary

5.1. Expression and Function of Apelin/APJ in the Ovary. The apelinergic system was found in the ovary of many species like bovine, rhesus monkey, porcine, and human (Table 1)

[13, 57–61]. Shimizu et al. [62] demonstrated that in bovine follicles the expression of apelin mRNA was not found in granulosa cells (Gc), while the APJ gene was increased in Gc of estrogen-inactive dominant follicles. Additionally, the expression of apelin mRNA increased in theca cells (Tc) of estrogen-inactive dominant follicles but APJ expression in Tc increased with follicle growth [62]. *In vitro* experiments of bovine ovarian cells showed that several factors regulated apelin/APJ expression; for example, progesterone (P4) and FSH stimulated the expression of APJ mRNA in the cultured Gc, while LH induced the expression of apelin and APJ in cultured Tc [62]. In the next study, Schilffarth et al. [13] observed that in the bovine ovary, the expression level of apelin during the oestrous cycle was significantly higher compared to the one during pregnancy. Moreover, apelin mRNA was high during the cycle and decreased after corpus luteum (CL) regression, while in ovarian follicles the expression of apelin/APJ was significantly upregulated in follicles with an estradiol (E2) concentration of more than 5 ng/ml, suggesting that the apelin/APJ system is involved in the mechanism regulating angiogenesis during follicle maturation as well as during CL formation and function in the bovine ovary [13]. Our last data demonstrated that the expression of both apelin and APJ in bovine granulosa and oocytes significantly increased with ovarian follicle size whereas it was similar in theca interstitial cells [59]. Furthermore, *in vitro* experiments showed that insulin-like factor I (IGF1) increased apelin expression, whereas it decreased the mRNA expression of APJ [59]. In the porcine ovary, apelin concentration in the follicular fluid and expression of both apelin and APJ increased with follicular growth; the greatest values were found in large follicles [61]. Immunohistochemistry revealed the positive staining for apelin and APJ in membranes of porcine Gc, than in Tc; additionally, a strong expression of apelin in oocytes and APJ in the zona pellucida was observed [61]. Similar as in bovine CL, our data also documented that in porcine CL, apelin/APJ is dependent on the CL growth and development phase; apelin expression was similar in early and middle CL and then decreased in regressing CL [63]. Moreover, localization of apelin was found in the cytoplasm of luteal cells in all stages of CL development, while the strongest APJ staining was found in middle cells [63]. Roche et al. [58] demonstrated apelin and APJ at the gene and protein levels also in human ovarian cells and granulosa cell lines (KGN). These authors demonstrated higher immunolocalization of APJ in human Gc, cumulus, and oocyte as compared to Tc. The high expression is also demonstrated in primary, medium, and mature follicles; apelin/APJ is expressed in the cytoplasm and nuclei of Gc [58].

The presence of apelin/APJ (Table 1) in various ovarian cells and its change during ovarian follicles and CL development suggests a potential role of apelin in the control of several aspects of ovarian cell function such as folliculogenesis, steroid hormone secretion, proliferation, or apoptosis. *In vitro* studies indicate that apelin may directly regulate steroidogenesis in ovarian cells. Apelin by activation of the APJ receptor causes a statistically significant increase in P4 and E2 secretion and 3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase (3βHSD) protein level both in primary cell cultures and in IGFI-induced human and porcine ovarian cells [58, 59, 61]. As a molecular mechanism of apelin action on the steroid synthesis process authors considered activation of the serine–threonine kinase, mitogen activated protein kinase (MAPK3) and AMPK kinase pathways [58]. Similar results have been obtained in *in vitro* studies of bovine ovarian cells, which show that apelin stimulates P4 production and proliferation of these cells by activating Akt kinase [59]. In addition, the authors demonstrated an inhibitory effect of apelin on the *in vitro* maturation of bovine oocytes and the release of P4 by cumulus cells, indicating the direct role of this adipokine in the maturation of oocytes. Shuang et al. [64] showed that apelin stimulates proliferation and inhibits the process of apoptosis in rat Gc by activating the Akt kinase pathway. In addition, Shimizu et al. [62] suggest involvement of apelin in follicular atresia induced by Gc apoptosis during bovine follicular because they have demonstrated high expression of the APJ receptor in atretic bovine follicles.

Apelin is also a regulator of the CL luteolysis process [57]. In the middle CL (sensitive to PGF2α), a transient increase in blood flow associated with the stimulation of endothelium nitric oxide (eNOS) was observed, which is the first signal that initiates luteolysis [65]. Apelin activates the eNOS pathway through stimulation of nitric oxide production, resulting in the expansion of blood vessels [3]. Another mechanism to explain the luteolytic effect of apelin is CL apoptosis. Apelin is one of the factors that slow down the process of ovarian apoptosis. On the other hand, apelin induces the expression of the antiapoptotic B-cell lymphoma 2 (Bcl-2) protein, while decreasing proapoptotic Bax production further blocks the release of cytochrome c and activates the caspase-3 apoptosis executive enzyme resulting in apoptosis suppression in osteoblast cells [66].

5.2. Apelin and Ovarian Pathology. PCOS is the most common cause of infertility due to lack of ovulation. This syndrome was first described by Stein and Leventhal in 1935. They described women with excessive hair, obesity, and ovaries covered with cysts. It is the main endocrinopathy of reproductive-age women. PCOS also binds to insulin resistance, which results in hyperinsulinism, which affects the production of androgens by the ovaries and adrenal glands. There are also changes in the lipid and carbohydrate economy, which in turn leads to diabetes type 2 and cardiovascular and biliary tract diseases. Increased risk of endometrial cancer and diabetic pregnancies, preeclampsia during pregnancy, or venous thrombosis are also symptoms of this condition [67]. Genetic factors responsible for PCOS pathogenesis are mutations in the genes responsible for steroid hormone synthesis, regulation of gonadotropins, and those associated with the pathway for weight regulation. Environmental factors can also be classified as obesity, occurring in 50% of patients, resulting in disorders of implantation, cycle, ovulation, and miscarriage [68]. The results of Roche's et al. [58] data compared the expression of apelin and APJ in Gc from obese or nonobese patients with or without PCOS. They

Table 1: Apelin/APJ expression in the ovary of many species and direct effects of apelin on ovarian function.

Species	Apelin/APJ expression				References	Effect of apelin on ovarian function			Apelin doses	References
	Granulosa	Theca	Oocyte	CL		Steroidogenesis	Proliferation	Apoptosis		
Human	+/+	+/+	+/+	ns	[58]	↑P4, ↑E2	ns	↓	10^{-9} M	[58]
Pig	+/+	+/+	+/+	+/+	[61]	↑P4, ↑E2	ns	ns	0.02, 0.2, 2, and 20 ng/ml	[61]
Bovine	−/+	+/+	+/+	+/+	[13, 57, 59, 62]	↑P4	↑	ns	10^{-9} M, 10^{-8} M, and 10^{-6} M	[59]
Rat	ns	ns	ns	ns	—	ns	↑	↓	10^{-8} mol/l	[64]
Rhesus monkey	+/+	+/+	ns	+/+	[60]	ns	ns	ns	ns	—

+: present; − does not exist; ns: no study; ↑: increase; ↓: decrease; P4: progesterone; E2: estradiol.

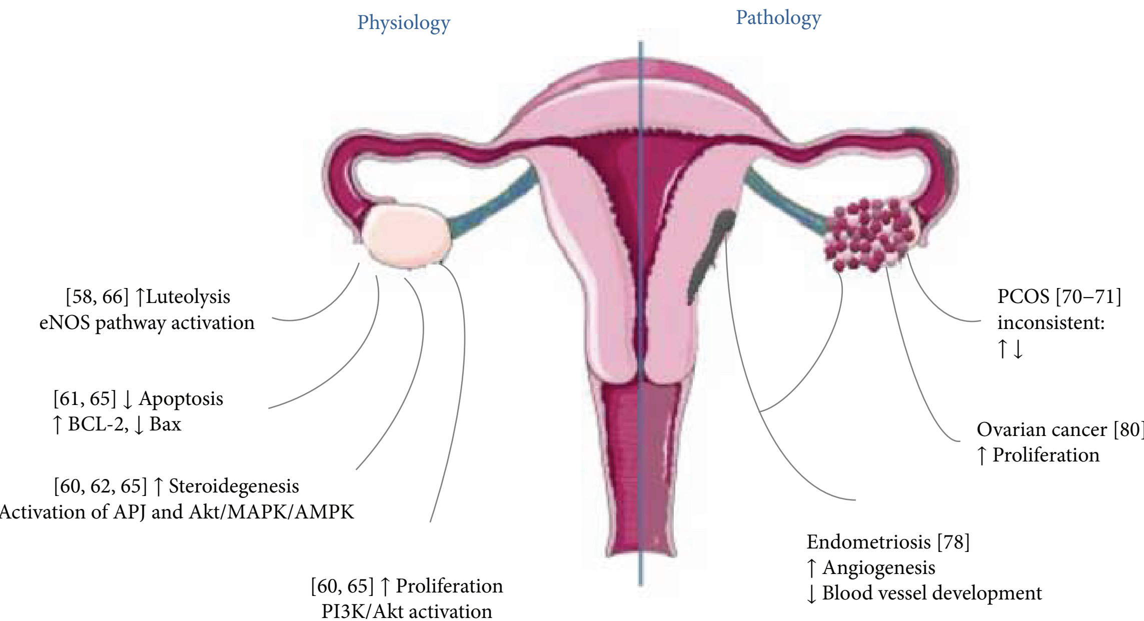

Figure 4: Apelin effect on ovarian physiology and pathology. eNOS: endothelium nitric oxide; Bcl-2: B-cell lymphoma 2; APJ: apelin receptor; Akt: protein kinase B; MAPK: mitogen-activated protein kinases; AMPK: 5′AMP-activated kinase; PI3K: phosphoinositide 3-kinase; PCOS: polycystic ovary syndrome.

observed that apelin and APJ mRNA expression is increased in PCOS patients, and it was higher in obese patients [58], suggesting the role of apelin as a marker of PCOS pathogenesis (Figure 4). Moreover, higher levels of apelin-13 in follicular fluid in obese women compared to nonobese women in both the PCOS and non-PCOS groups was observed [58]. However, the published data comparison of serum apelin levels in PCOS and non-PCOS women is inconclusive. Some authors point to its considerable elevation in serum PCOS [69–74]. Data of Sun et al. [72] indicated a weight-dependent increase in the concentration of apelin in obese women with PCOS compared to PCOS-deficient women. Apelin was found to be higher in PCOS patients by Gören et al. [70] but without a significant correlation with homeostatic model assessment (HOMA-IR). Olszanecka-Glinianowicz et al. [73] reported an inverse association between apelin and glucose, insulin, and HOMA-IR values, supporting the role of apelin in the regulation of insulin sensitivity. Apelin levels were higher in nonobese PCOS patients, suggesting a compensatory mechanism for metabolic consequences of insulin resistance. Comparative results of studies showing lower serum apelin levels in PCOS have been obtained by several authors [73–76]. Different from Cekmez et al.'s study [69], lower serum concentrations of apelin were found in PCOS subjects by Altinkaya et al. [75] with a positive correlation with BMI, insulin, HOMA-IR, triglyceride, and free testosterone, speculating that apelin can be used as a marker for insulin sensitivity. Conversely, Sun et al. [72] observed a significantly enhanced apelin concentration in PCOS patients with a positive association with BMI and HOMA-IR; treatment with drospirenone–ethinylestradiol plus metformin improved insulin resistance and apelin levels. Discrepant findings among the published studies may be attributed to the differences in ethnicity, age, study design, sample size, genetic characteristics of populations, and assessment methodology, defining PCOS definitions; the difference in the test was used to analyze the concentration of different apelin isoforms.

Another ovarian pathology that has been recently linked to apelin action is endometriosis. Endometriosis is a disease which is characterized by the survival and growth of endometrial tissue outside the uterus primarily in the pelvic area. It is one of the most common gynecological diseases with up to 10% of women in the USA suffering from its symptoms which include infertility and severe pelvic pain [77]. This disease is highly estrogen-dependent and is accompanied by a major inflammatory response. Apart from surgical removal of endometriotic lesions, the main therapeutic approach is continuous treatment with progestins to inhibit the proliferation of this ectopic tissue which is not always effective [77]. Therefore, investigation of steroid hormone signaling in this disease is critical to identifying new therapeutic targets. Apelin might be a factor playing a role in the endometrial regeneration via angiogenesis. Ozkan et al. [77] using the immunohistochemistry method and immunoassay detected apelin in the eutopic and ectopic endometrium of women with or/and without endometriosis. Apelin concentrations increased during the secretory phase and decreased during proliferative phases of eutopic and ectopic endometrial tissue. Moreover, the higher immunoreactivity of apelin was observed in the endometrium in the secretory phase and in glandular cells of both eutopic and ectopic endometrial tissues, suggesting that increased local apelin concentration may indicate a paracrine function on the endometrium [77]. Additionally, apelin causes endothelium-dependent vasorelaxation by triggering the release of nitric oxide and

is a potent angiogenic factor inducing endothelial cell (EC) migration, proliferation, and blood vessel *in vivo* development, indicating its effects as a chemoattractant for endothelial cell growth [77].

Recent data indicate the relationship between apelin and ovarian cancer. Ovarian tumors, the second most common type of gynecological malignancy [78], are heterogeneous neoplasms classified into three major categories, namely, epithelial ovarian tumors, sex cord-stromal tumors (e.g., granulosa cell tumors), and germ cell tumors. Epithelial tumors account for 80% to 90% of ovarian malignancies, whereas Gc tumors account for 1% to 2% of ovarian malignancies in the USA and Europe. Data of Hoffmann et al. [79] documented the expression of apelin/APJ in different ovarian cell lines; they observed that the APJ expression level was higher in epithelial cancer cells than in Gc tumor, whereas the reverse was true for apelin expression and secretion. Additionally, these data indicate that apelin stimulated OVCAR-3 cell proliferation and suggest its mitogenic action in ovarian epithelial cancer cells. Furthermore, recent studies report that apelin stimulates cancer cell migration in the lung, oral cavity, and colon [80, 81].

6. Physiology and Pathology of Apelin in the Testis

To our knowledge, there is one published data demonstrating the effect of apelin on male reproduction. Infusion of apelin-13 in male rats significantly suppressed LH release compared with the vehicle values, while levels of FSH did not significantly differ among the groups [82]. Furthermore, serum testosterone levels in the apelin-13 group were statistically lower than in the control group; histological examination showed that infusion of apelin-13 significantly decreased the number of Leydig cells, suggesting that apelin may play a role in the central regulation and decrease testosterone release by suppressing LH secretion. Finally, these authors concluded that the agonist of APJ may be a useful drug for pharmaceuticals in the treatment of male infertility [82].

7. Summary and Conclusion

In summary, the apelinergic (apelin and APJ) system was found in the hypothalamus, pituitary, ovaries, and testis of many species and has autocrine and/or paracrine effects on control reproduction both in female and in male regulation of their physiology. Most research indicates that apelin has an inhibitory effect on gonadotropin and PRL secretion in females, while in male rats, an inhibitory effect of apelin on LH and testosterone was observed in *in vivo* experiments. Apelin also participates in the direct regulation of ovarian physiology; it was clearly documented that apelin has a stimulatory effect on steroidogenesis and proliferation but an inhibitory action on cell apoptosis by activation on several kinase pathways such as AMPK, ERK, and Akt. Based on available data, we speculated that apelin has a connection with such dysfunctions like PCOS, endometriosis, and mitogenic action in ovarian cancer. Many of these pathologies are still in critical need of therapeutic intervention, and recent studies have found that apelin can be a target in pathological states. Therefore, apelin activity could be applied in the future in the treatment of many diseases of the reproductive system.

Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this article. Patrycja Kurowska and Agnieszka Rak were BGF scholars of the French Embassy in Poland 2017.

Acknowledgments

This work was supported by the Ministry of Science and Higher Education for the PHC project under the bilateral Polish-France Agreement “POLONIUM” (2016–2017) between Agnieszka Rak and Joelle Dupont, as well as the French Government and the French Embassy in Poland and Region Centre in France (PREVADI Project no. 32000820).

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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 3906490, 11 pages
https://doi.org/10.1155/2018/3906490

Review Article

Adipokines in Semen: Physiopathology and Effects on Spermatozoas

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Received 5 January 2018; Revised 7 March 2018; Accepted 19 March 2018; Published 5 June 2018

Academic Editor: Rosaria Meccariello

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Adipokines are secreted by adipose tissue and could be the link between obesity and infertility. Different studies investigated the involvement of adipokines in reproductive functions but only a few have looked into the male part. This review assesses adipokine functions on male reproductive parameters. Adiponectin seems to have a positive effect on sperm parameters, whereas other adipokines such as resistin or chemerin would have a rather deleterious effect on spermatogenesis. Semen parameters seem to be impacted when resistin and chemerin are increased: indeed, there is a decrease of sperm motility. Sperm morphology is improved when adiponectin is increased. The most studied adipokine, leptin, has a dual effect with a positive effect on sperm at physiological levels and a negative one for high seminal concentrations. Many semen parameters and fertility itself are disturbed according to semen adipokine levels, even if it is not the only interfering element. Taken together, adipokines are found in human and animal semen and most of them or their receptors are expressed in male genital tract. Although the pathophysiological role of adipokines in semen is not clearly elucidated, the adipokines could influence sperm functionality and could be potential biomarkers of male fertility.

1. Introduction

It is well known that adipose tissue is an endocrine organ. It secretes adipokines, which act at endocrine, paracrine, and autocrine levels [1]. 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 [2, 3]. Moreover, proinflammatory cytokines are secreted mainly by nonadipose cells in adipose tissue [3]. The prevalence of obesity has tripled in the last 30 years [4] in men of childbearing age, which coincides with an increase in infertility that affects currently one in six couples in France (according to the report annual report of the ABM in 2012). Indeed, the Institute of Public Health Surveillance (InVS) found a secular decline in spermatic concentration in the past decades in Western Europe. The link between these two public health problems has been widely described. Studies carried out on large cohorts (1558 men [5] and 526 men [6]) showed a significant correlation between a drop in sperm parameters and an increase in body mass index (BMI) higher than 25 kg/m^2. The study by Jensen et al. [5] carried out on 1558 men showed a decrease in sperm concentration and count of 21.6% (95% CI: 4.0–39.4%) and 23.9% (95% CI: 4.7–43.2%), respectively, when the BMI was higher than 25 kg/m^2. In addition, a decrease in sperm motility was

observed by an Argentinian team in obese patients (51.4% in the normal BMI group versus 46.6% when BMI was higher than 30, $p < 0.007$) [7]. In 2007, a Chinese study found in the same way a decrease in spermatic parameters (count, concentration, and morphology) in overweight subjects, regardless of circulating concentrations of LH, FSH, estradiol, and testosterone [8]. This suggests that these hormones alone do not explain the association between BMI and sperm parameters. Moreover, obesity is promoted by a positive energy balance, which impacts on the function of the cells involved in spermatogenesis [9]. This hypothesis is reinforced by the results obtained in animal experiments, which showed the existence of a direct relationship between epididymal adipose tissue and fertility, since in rats, the removal of this tissue caused a significant decrease in sperm count [10]. Relationships between circulating concentrations of adipokines and BMI have been widely studied. Indeed, different studies showed a variation of these factors associated with overweightness. Thus, obesity is associated with hyperleptinemia and leptin resistance [11]. In contrast, adiponectinemia decreases in overweight cases [2].

Interestingly, these variations are not definitive since they are reversible after weight loss [12], especially after bariatric surgery. Nevertheless, an association has set up evidence between circulating concentrations of adipokines and sperm quality. Thus, comparing two groups (obese fertile versus infertile men), an Egyptian team observed circulating concentrations of leptin higher in the infertile group compared to the fertile group [13]. It has also been shown that leptinemia was positively correlated with abnormal sperm morphology and negatively correlated with the concentration and sperm motility [13, 14]. This correlation could be the result of the higher circulating leptin levels observed in obese or overweight men leading to a decreased testosterone production by Leydig cells, which is able to interfere with the normal cycle of spermatogenesis [15]. Although it is not an adipokine, ghrelin, a peptide hormone secreted by the stomach which is increased in obesity, is also present in the whole human testis and more particularly in Leydig and Sertoli cells. Its receptors (growth hormone secretagogue receptor (GHS-R)) have been identified in germ cells [15]. In vivo studies demonstrated that ghrelin inhibits the proliferative activity of immature Leydig cells and regulates stem cell factor mRNA expression in rat testis [15]. This hormone in link with fasting is also involved in male fertility. Thus, sperm quality is related to the circulating concentrations of adipokines, but the link with fertility is not currently established.

In addition, the concentrations of adipokines in blood and in seminal plasma are not in the same range. Indeed, adiponectin is 1000 times lower in seminal plasma than in blood, whereas progranulin and visfatin are 100 times more concentrated [2]. The varying concentrations between these two biological fluids suggest a difference in production and a potential action on the surrounding cells (germ cells for sperm). Indeed, several studies carried out in humans and animals showed that most of the adipokines and their receptors are expressed in testis especially in seminiferous tubes and more specifically in Leydig and Sertoli cells and on spermatozoa themselves [16].

Thus, the adipokines of seminal plasma could be privileged actors involved in the relationship between obesity and fertility. Obesity is characterized by an increased number of adipose cells and an excessive storage of triglycerides in the adipose cells. The hormonal interaction between the adipose tissue and other endocrine organs including the gonads is complex and not fully understood. Some endocrine changes involving adipokines could contribute to understand the negative effects of obesity on reproductive function. Many studies have shown the presence and the role of adipokines and their receptors in the female reproductive tract of different species. However, fewer studies have investigated the role of adipokines on male fertility in case of obesity or not, whereas it has consistently been shown that high BMI reduces male fertility. The present review will highlight the location of adipokines in the male genital tract, the molecular mechanisms of action of these molecules, and their potential effect on spermatic parameters in human and animal models when this information is available. Indeed, several adipokines (leptin, adiponectin, resistin, chemerin, visfatin, vaspin, and progranulin) and certain cytokines have already been detected in semen. For adipokines that have been studied thoroughly, we will also report their effects on spermatozoa.

1.1. Leptin

1.1.1. Topography in Male Genital Tract and Mechanism of Action. Leptin is an adipokine mainly secreted by adipose tissue. This hormone of 167 amino acids is encoded by the obese gene (*ob* gene) [17], and its tertiary structure consists of four alpha helices connected by two long and one short loop [18]. This molecule has been widely studied in animals and in humans. Leptin signaling via STAT3 suggests a role in the proliferation of undifferentiated germ cells. Leptin activation of prosurvival pathways may lead to the activation of ERK1/2 signaling, representing capacitation signaling crosstalk (Figure 1). It is intriguing to speculate that acrosomal leptin receptor expression is associated with cholesterol efflux and acrosome reaction, whereas tail leptin receptor expression in human sperm may reflect leptin's modulation of hyperactivated sperm motility. Leptin STAT3 signaling may enable undifferentiated germ cells to replicate without loss of potency while triggering late-stage spermatocytes to undergo development and differentiation [19]. Moreover, leptin modulates the nutritional support of spermatogenesis by human Sertoli cells [15]. Indeed, a Portuguese team demonstrated that acetate production by human Sertoli cells, a central metabolite for spermatogenesis, is severely decreased after exposure to leptin (5 to 50 ng/mL) [9].

Leptin is present in testis and particularly in seminiferous tubules [20]. In animals, studies demonstrate that leptin is expressed differentially between species. Indeed, in pigs, leptin and its receptors are expressed in Leydig cells, whereas in mice, no leptin is present in interstitial cells. In rats, leptin receptor (LepR) mRNA is present in Leydig cells, in Sertoli cells, and possibly in germ cells [21]. Concerning dogs, LepR is absent from Leydig cells and Sertoli cells but present in spermatocytes and spermatids [22]. Aquila [23] has demonstrated the presence of leptin in human sperm at different

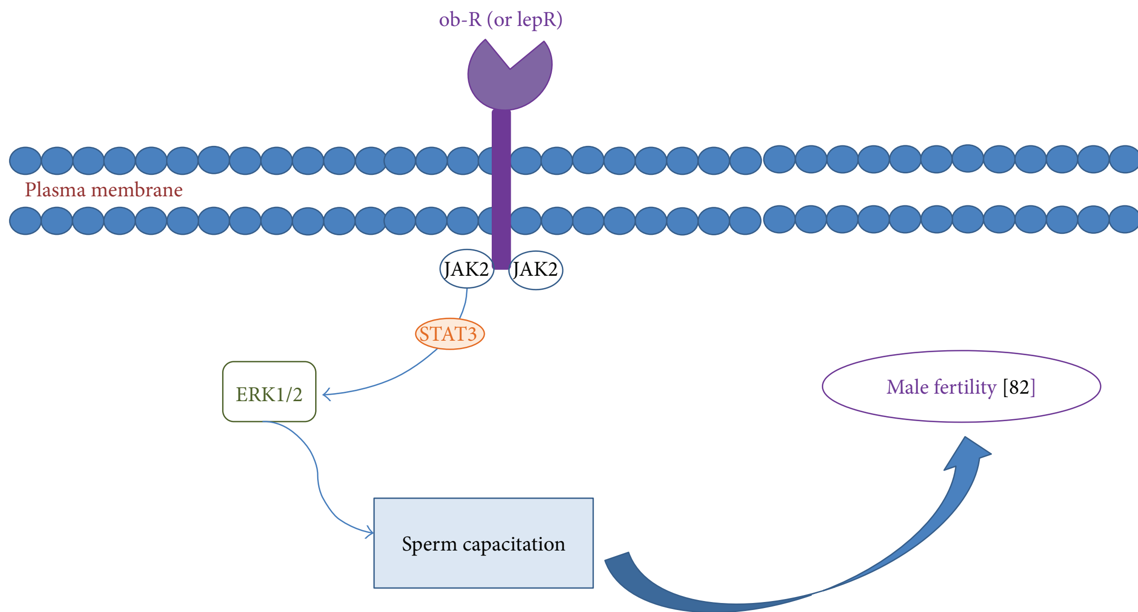

FIGURE 1: Leptin receptor and its interactions with JAK2 and STAT3 system to sperm capacitation [82].

levels: mRNA expression, protein expression, and immunolocalization. In humans, the presence of leptin receptor has been reported in seminiferous tubules [24]; however, only Jope et al. [25] have reported that seminal plasma and sperm contain this receptor [26]. The presence of leptin receptors on the tail of spermatozoa suggests an effect on motility [25] as described in Section 1.1.2 of this review.

Leptin receptor has also been reported to be present in the sperm of certain species, but there are also reports claiming its absence in other species. Hatami-Baroogh et al., using several commercial and noncommercial antibodies and various techniques, were unable to detect leptin receptors at protein levels in human spermatozoa of fertile ($n = 22$) and infertile ($n = 50$) individuals [27]. Ishikawa reported that in humans, the leptin receptor is present in testicular tissue and confined only to Leydig cells and is not expressed by Sertoli cells, germ cells, or spermatozoa [28]. The difference in the leptin receptor location has been related to species differences.

1.1.2. Effects on Semen Parameters. Although different studies showed contrasting results, it is possible to consider a physiological role of leptin on sperm motility. In fact, studies in which the seminal plasma studied had high concentrations of leptin showed that this adipokine was inversely correlated with sperm motility. Glander et al. [24] showed a negative correlation between seminal leptin and progressive ($r = -0.53$, $p = 0.0004$) and straight ($r = -0.3$, $p = 0.029$) motility for 64 male partners of couples consulting for infertility. This team demonstrated an average leptin concentration in seminal plasma of 2.4 ng/mL, and once the separation into two groups "normozoospermic" and "pathozoospermic", the mean concentrations of seminal leptin were 1.5 ng/mL and 3.19 ng/mL, respectively. Two other studies have shown a negative correlation between leptin concentrations in seminal plasma and progressive motility. The first study [26] was performed on 79 men with asthenospermia ([leptin] = 4.72 ng/mL) and 77 control men ([leptin] = 3.75 ng/mL). The second study [20] involved 42 infertile patients with varicocele ([leptin] = 3.01 ng/mL) compared to 10 control men ([leptin] = 1.79 ng/mL). It is important to note that a higher concentration of seminal leptin is often associated with spermatic pathologies, suggesting that high concentrations of leptin in seminal plasma would have deleterious effects.

Finally, other studies have concluded that there is no correlation between seminal leptin and sperm motility [29, 30]. In these studies, patients had relatively low leptin concentrations (0.93 ng/mL and 0.95 ng/mL). Despite high concentrations of seminal leptin (5 ng/mL in the nonobese group versus 12.5 ng/mL in the obese group), a South African team found no correlation between seminal leptin and sperm motility [31]. The obese group nevertheless had significantly lower sperm motility than the nonobese group (42.2% versus 54.4% for total motility), and this group had a higher seminal leptin concentration. Thus, analysis of published studies to date suggests that increased seminal leptin concentration would be associated with decreased motility. It can therefore be hypothesized that, at high concentrations, leptin in seminal plasma is associated with a decrease in sperm motility [2]. At lower or "physiological" concentrations, leptin may either have a physiological effect, beneficial to motility, or have no effect.

The same type of result is found when we explore the relationship between seminal leptin and sperm concentration in the ejaculate. Thus, for low concentrations of leptin (0.83–0.91 ng/mL), there is a positive correlation between seminal plasma leptin and sperm concentration ($r = 0.24$, $p < 0.05$) [2]. On the other hand, studies carried out on patients with high seminal concentrations of leptin show a negative correlation between this adipokine and not only the concentration ($r = -0.187$, $p < 0.05$), but also the spermatozoa count ($p = 0.0001$) [20, 32, 33].

Table 1: Consequences on male fertility phenotypes of animal models with missing adipokine or adipokine receptor.

Type	Phenotype	References
ob/*ob* mice	Testicular atrophy Decrease nuclear volume of Sertoli cells, spermatogonia, and spermatocytes	[40]
ob/*ob* mice	Infertile Reduction of testis weight, multinucleated spermatids, few spermatozoa, and anormal Leydig cells	[41]
db/*db* mice	Infertile	[43]
db/*db* mice	Infertile Impairment of spermatogenesis and sperm motility	[14]
fa/*fa* rat (Zucker rat)	Alteration in sperm production and sperm DNA damage	[45]
AdipoR2	Seminiferous tubular atrophy with aspermia and reduced testicular weight	[54]
AdipoR2	Decrease of testis weight	[58]

Concerning spermatic vitality, two studies did not find any correlation with the seminal levels of leptin [29, 31]. A Chinese team [33], comparing 74 varicocele patients, 70 leukocytospermia patients, and 40 control patients, describes a negative correlation in the case of associated pathology but without supporting their observation by statistical analysis. They showed that patients with varicocele (VC [leptin] = 3.2 ng/mL) and leukocytospermia (LC [leptin] = 2.72 ng/mL) had high concentrations of seminal leptin as well as increased ROS (reactive oxygen species) and apoptosis compared to the control group. They also noted that there was a correlation, for the VC and LC groups, between leptin, apoptosis, and ROS. ROS are markers of oxidative stress, and an increase in these ROS induces a deleterious effect on sperm function [34, 35]. It appears, therefore, that at high concentrations, leptin may be a proapoptotic factor.

The correlation between ejaculate volume and seminal leptin has been poorly studied so far since only two studies presenting contradictory results are at our disposal. On the one hand, Thomas et al. [2] found a negative correlation ($r = -0.34$, $p < 0.01$), whereas Leisegang et al. [31] showed a lack of correlation between levels of seminal leptin and the volume of the ejaculate. It is therefore difficult to decide whether or not there is a link between seminal leptin and ejaculate volume. On the other hand, seminal leptin does not seem to have any effect on sperm morphology since three studies agree on the lack of correlation between these two parameters [2, 29, 31].

To sum up, the analysis of these different studies suggests that there would be an "ideal" seminal concentration of leptin, a concentration at which this adipokine would have a physiological effect, whereas at high concentration, its effects could be deleterious on spermatic parameters. Indeed, at high concentrations, leptin is rather associated with an alteration of certain spermatic parameters, which could have an impact on fertility.

Altered leptin dynamics may contribute to male infertility via at least two mechanisms, both of which may produce hypogonadism. These include leptin resistance or leptin insufficiency at the hypothalamus and leptin modulation of testicular physiology.

1.1.3. Direct Effect on Motility. Leptin may have a physiological role in the male reproductive tract. Thus, an *in vivo* study and an *in vitro* study showed a positive correlation between seminal leptin and motility. The *in vivo* study was performed on 96 men without pathologies associated to spermatogenesis and showed a positive correlation between seminal leptin and progressive ($r = 0.27$, $p < 0.01$) and total ($r = 0.23$, $p < 0.05$) motility [2]. The mean leptin concentrations in seminal plasma were 0.91 ng/mL in normal weight men and 0.83 ng/mL in overweight or obese groups. The *in vitro* work directed by Lampiao and du Plessis [36] aimed to study the effect of leptin on sperm motility: it was shown that after 1, 2, and 3 hours of incubation, leptin significantly increased the total and progressive motility ($p < 0.05$). This study was performed on spermatozoa from normozoospermic donors.

On buffalos, Khaki's team conducted two types of protocols. In the first one, they added 10 ng/mL of leptin on spermatozoa in semen, which was shown to preserve motility and vitality during frozen sequence compared to the control group. For the second protocol, they added 200 ng/mL which had a deleterious effect on semen parameters [37]. This deleterious effect at high levels of leptin consolidates the dual effect of leptin according to the concentration. In male mice, diet-induced obesity induces not only significant impairments of sperm function parameters, but also disruption of the blood-testis barrier integrity [38]. Even if it has been shown that leptin can cross the blood-testis barrier [39], we can hypothesize that obesity could also facilitate the passage of leptin and other adipokines through this barrier.

1.1.4. Transgenic Animal Model (cf Table 1). A recent study showed testicular atrophy in an *ob*/*ob* mice model, with testis weight 13% less than the control group ($p < 0.0001$) despite higher body weight [40]. Likewise, this model displayed a decrease in the nuclear volume of Sertoli cells, spermatogonia, and spermatocytes. The same transgenic mice model already demonstrated these effects in 2006 [41]. Furthermore, leptin treatment of adult *ob*/*ob* males corrects their sterility, an effect that is mediated at least partly by a normalization in testicular weight, spermatogenesis, and Leydig cells morphology [42].

It was shown that LepR gene null mice generate infertile phenotype [43] and present a decrease of gonadal functions [44].

Male *ob/ob* mice are morbidly obese and infertile. Similar phenotypes are observed in Lep-R-deficient mice and the Zucker fatty (fa/fa) rat. Pubertal obese Zucker rats present altered spermatogenesis, as observed in the histological level, which persists up to the adult phase; in the quantitative analysis, sperm production in the fatty animals was reduced as well, but only in the pubertal rats. On the other hand, the increased sperm DNA fragmentation found in the adult rats points out genetic damage generated in the fatty rat gamete, which can be a lead for understanding the obese Zucker rat's infertility [45].

1.1.5. Polymorphisms in Human. Different polymorphisms of leptin do exist and are characterized in many studies. Leptin rate is more increased in AA genotype than in AG genotype [46]. LEP-2548G/A genotype is different between fertile and infertile patients ($p = 0.012$). AA genotype is increased in the infertile group, and AG genotype decreased in this group, which induces that AG genotype has a protective/safety effect on fertility by reducing the risk of male infertility by 3-fold [47]. Sperm count is increased in the infertile group with AG and GG genotypes than AA ($p = 0.0009$ and $p = 0.026$). Leptin's receptor polymorphisms exist too and influence spermatic motility so that progressive motility is increased in RR genotype than QQ and QR ones [47].

All this data supports a local role for leptin in sperm parameters with the consequent potential impact on fertility capacity.

1.2. Adiponectin

1.2.1. Topography in Male Genital Tract and Mechanism of Action. Adiponectin is a protein of 224 amino acids mainly produced by white adipose tissue but also found in other tissues such as bone and muscle [16]. Unlike the majority of adipokines, plasma adiponectin concentration is negatively correlated with BMI and visceral adiposity [48], but it also regulates gonadotropic axis and gonad function [49]. Adiponectin is found in the circulation in various molecular forms: the so-called LMW (low molecular weight) form corresponding to an assembly of 3 adiponectin monomers in trimers, the MMW (medium molecular weight) form corresponding to hexamers (assembly of 2 trimers), and the form called HMW (high molecular weight) which corresponds to an assembly of 3 hexamers [50]. The HMW form of adiponectin is the predominant circulating form (>80%) and would be the most active. Most studies published to date have been conducted with total adiponectin, but some have been performed with the dosage form HMW.

Adiponectin mRNA is present in testis and has been found in Leydig cells [51] and spermatocytes [52]. AdipoR1 and AdipoR2 (adiponectin receptor) are present in testis [53, 54] more particularly in seminiferous tubules and specifically in interstitial tissue of rats [48]. Indeed, these receptors are abundant in Sertoli cells, Leydig cells, and germ cells in rats. Kawwass et al. also reported a presence of adiponectin receptors on spermatozoa themselves [54]. Adiponectin and adiponectin receptors have been immunolocalized on bull's spermatozoa in acrosomal, postacrosomal, equatorial, and tail regions [55].

Adiponectin protein is abundant in the tail region of bull sperm, while AdipoR1 is localized mainly at the equatorial and acrosome region and AdipoR2 is expressed primarily on the sperm head region and on the equatorial line. Adiponectin and its receptors are expressed during pre- and postcapacitation of spermatozoa, suggesting that adiponectin might have a role in sperm capacitation [55]. Thus, local actions of adiponectin in testis are involved in the production of sperm capable of fertilization [56].

1.2.2. Effects on Semen Parameters. To our knowledge, only one team until now [2] has studied the relationship between seminal adiponectin concentrations (total adiponectin form) and sperm parameters in humans. This study suggested that adiponectin would rather have a positive effect on sperm function. Mean seminal adiponectin concentrations of 16.8 ng/mL and 14.2 ng/mL (a thousandfold lower than adiponectinemia) were measured in normal weight subjects and in overweight or obese patients, respectively.

Adiponectin levels in seminal plasma have been shown to be positively correlated with sperm concentration, sperm count, and percentage of typical sperm forms.

An animal study showed an improvement of fertility in bulls positively correlated with the seminal concentration of adiponectin ($r = 0.80$, $p < 0.0001$) and its AdipoR1 receptors on spermatozoa ($r = 0.90$; $p < 0.0001$) and AdipoR2 ($r = 0.65$, $p < 0.0001$) [51]. After capacitation, the levels of adiponectin and its receptors are lowered, suggesting a direct role on sperm motility. Interestingly, a novel association of adiponectin system with sperm motility was shown in rams [57] (Figure 2).

1.2.3. Transgenic Animal Model (Table 1). An adiponectin receptor gene knockdown study performed in mice highlighted the potential importance of the adiponectin pathway in the male genital tract. Indeed, this work has shown that the loss of AdipoR2 was responsible for seminiferous tubular atrophy associated with aspermia and reduced testicular weight [54] (Figure 2). Moreover, a decrease of testis weight was evidenced by Bjursell et al. with the same model [58].

It seems that adiponectin may play a beneficial role in male reproductive function, but this pathway has yet to be studied and confirmed. There are no *in vitro* studies carried out on seminal adiponectin.

1.2.4. Polymorphisms in Human. Polymorphism has only been described for females, to the best of our knowledge with a link to insulin resistance in polycystic ovary syndrome patients [59]. However, no polymorphism has been described with a link to male infertility.

1.3. Resistin

1.3.1. Topography in Male Genital Tract and Mechanism of Action. Resistin is a 12.5 kDa adipokine belonging to a family of cysteine-rich proteins [16]. It is present in testis, in seminiferous tubules, and specifically in Leydig and Sertoli cells [60] of animals, but this has not been demonstrated in

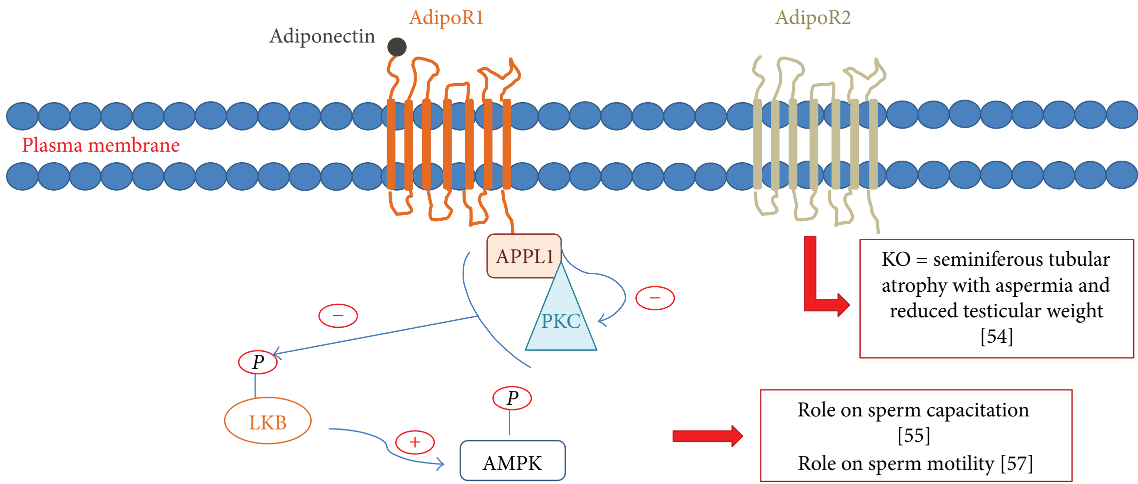

Figure 2: Adiponectin receptors and its possible interactions to fertility.

humans. TLR-4, a binding site for resistin, has been found in human sperm [16].

1.3.2. Effects on Semen Parameters. To our knowledge, only three studies have measured resistin in seminal plasma. The team of Moretti et al. showed that there was a negative correlation between the concentrations of seminal resistin and spermatic motility and vitality [61]. Two other teams [2, 62] studied the relationships between resistin concentrations in seminal plasma and sperm parameters but did not show significant correlation. However, given the low number of studies available, it is difficult to conclude on the role of resistin, which seems to have a rather negative effect on spermatozoa and thus fertility.

However, it has been shown that this adipokine is associated with markers of inflammation in seminal plasma. Indeed, the concentrations of seminal resistin correlate positively with those of proinflammatory mediators such as elastase, interleukin-6 (IL-6) [62], and tumor necrosis factor-α (TNF-α) [61]. During inflammation, the concentrations of cytokines and ROS increase, and this may have a deleterious effect on the male reproductive function [63, 64]. Indeed, it has been shown that an increase in ROS could induce a decrease in spermatic concentration, motility, and sperm count [34]. In the study published by Moretti et al., the seminal concentrations of resistin were significantly higher in cases of leukocytospermia or if the patients were smokers [61]. This increase in resistin concentrations was also associated with a significant increase in TNF-α and IL-6, as well as a sharp decrease in spermatic motility and the number of normal morphology spermatozoa for patients with leukocytospermia. All these results suggest that resistin could be considered as a marker of inflammation, and in pathological situations such as leukocytospermia, the presence of this adipokine would be related to an alteration of sperm parameters.

1.3.3. Polymorphisms in Human. As for adiponectin, we found that resistin polymorphism has only been described for females and more particularly in cases of polycystic ovary syndrome [65].

1.4. Chemerin

1.4.1. Topography in Male Genital Tract and Mechanism of Action. Chemerin, a recently discovered adipokine, is synthesized mainly by the liver, kidney, and adipose tissue [66]. Few studies have been carried out on this adipokine and in particular on its role in the reproductive function. In human as in rodents, chemerin receptors (CMKLR1, GRP1, and CCRL2) are present in testis. Chemerin, CMKLR1, and GPR1 are localized specifically on Leydig cells and poorly on germ cells [16].

1.4.2. Effects on Semen Parameters. To our knowledge, only one study was carried out in humans for this adipokine [2]. Chemerin was detected in the seminal plasma of 96 men with no spermatogenesis abnormalities, and it was shown that this adipokine correlated negatively with spermatic motility and positively with sperm concentration. Thomas' team [2] showed increased chemerin concentrations in the sperm of control subjects compared to a group of vasectomized patients ($p < 0.001$). This data suggests that there would be a local secretion of chemerin in the male genital tract, particularly at the testicular level.

1.4.3. In Vitro Experiment. Surprisingly, it was demonstrated by experiments conducted *in vitro* on rats that chemerin had an inhibitory effect on steroidogenesis [16] (Figure 3). The roles played by this adipokine in human semen needs to be further investigated.

1.5. Visfatin. Visfatin, also known as NAMPT, is a recently discovered adipokine produced primarily by perivascular adipose tissue. It has been found in Leydig cells, spermatocytes, and spermatozoa [16]. Visfatin levels are a hundred times higher in seminal plasma than in blood suggesting a significant local production in the male genital tract [2, 16].

No other studies are available to further understand the effects of this adipokine on male fertility.

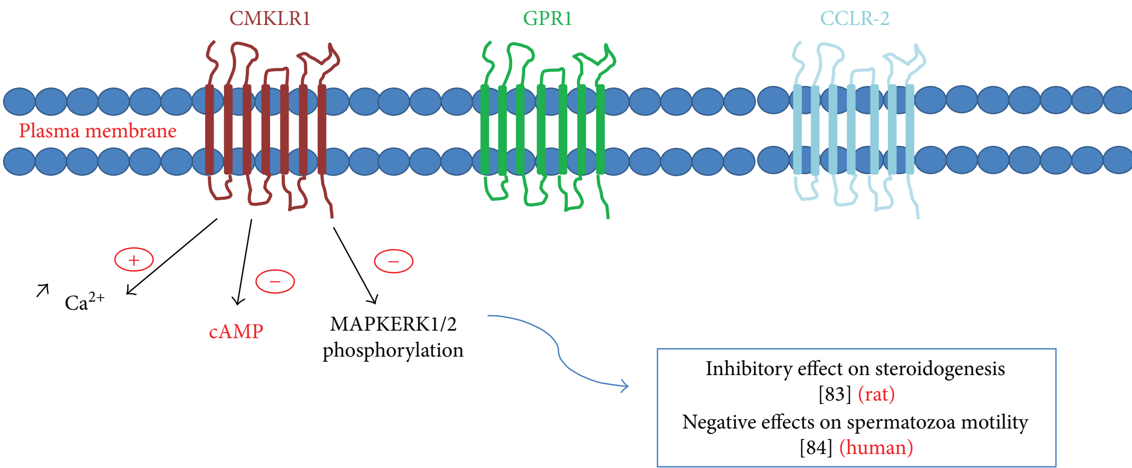

Figure 3: The three transmembrane receptors (CMKLR1, GPR1, and CCLR-2) and its known interactions to male fertility [83, 84].

1.6. Vaspin. Vaspin, another recently discovered adipokine, is expressed in epididymal, retroperitoneal, and mesenteric adipose tissue and is related to the metabolic state [67]. Thomas et al. showed that seminal plasma vaspin was negatively correlated with ejaculate volume ($r = -0.36$, $p < 0.001$) and positively correlated with sperm DNA fragmentation ($r = 0.22$, $p < 0.05$) [2].

1.7. Progranulin. Progranulin is increased in cases of obesity or metabolic syndrome and could contribute to the inflammatory mechanisms found in certain pathologies via a recruitment of macrophages [68]. This adipokine was studied in the seminal plasma only by Thomas et al. [2]. Progranulin is positively correlated with motility ($r = 0.32$, $p < 0.001$), sperm count ($r = 0.23$, $p < 0.05$), and sperm morphology ($r = 0.25$, $p < 0.01$). In vasectomized patients, seminal progranulin levels were significantly decreased ($p < 0.05$), indicating probable local secretion.

1.8. Cytokines

1.8.1. Topography in Male Genital Tract and Mechanism of Action. In the wide family of cytokines, some have been described in semen and related to male fertility. The presence of tumor necrosis factor- (TNF-) α and interferon- (IFN-) γ will be further investigated here. In dogs, TNF is present in testis (more particularly in germ cells, but not in Sertoli cells nor Leydig cells), epididymis, and spermatozoa [69]. Proinflammatory cytokine like TNF-α can directly impair the seminiferous epithelium by damaging the expression and assembly of the junctional proteins leading to an impairment of the blood-testis barrier [70]. Moreover, proinflammatory cytokines disrupt the seminiferous and epididymal epitheliums by creating high levels of ROS [70].

1.8.2. Effects on Semen Parameters. Different authors report that cytokine levels are increased in the seminal plasma of infertile male [71–73]. It is the case for TNF-α and IFN-γ that rise in semen from males with an inflammation linked to infertility [74].

On the one hand, cytokines seem to have a bad effect on sperm motility [75, 76]. This was confirmed by Paradisi that showed a negative correlation between TFN-γ and sperm concentration, motility, and morphology [73]. In 2013, it was also confirmed that TNF-α levels are increased in the seminal plasma of oligozoospermic (42%, $p < 0.01$) and asthenospermic patients (58%, $p < 0.001$) compared to control patients. On the other hand, one study did not find any effect of either TNF-α or IFN-γ on sperm motility [77].

1.8.3. In Vitro Experiments. One study observed *in vitro* effects of TNF and IFN on spermatozoa for 3 hours and showed a decrease of 18% of sperm motility between 60 and 180 minutes and a decrease of 16% of sperm vitality at 180 minutes [78].

1.8.4. Polymorphisms in Human. A polymorphism in the TNF-α_308 gene was associated with a significant decrease of sperm count, sperm motility, normal sperm morphology, and acrosin activity [79]. In the same study, the occurrence of A allele was significantly increased in infertile patients than fertile controls (21.6% versus 9.7%; OR: 0.388, $p = 0.005$). An AA genotype of TNF-α corresponds more to a lowering concentration, motility, and normal morphology sperm profile. Moreover, TNFR-1 36G allele is more found in oligozoospermia associated to a decrease of sperm concentration [80].

2. Discussion and Conclusion

Taken together, leptin is the most studied adipokine in male fertility; fewer data are available for the other adipokines. For example, until now, it is unclear if adiponectin, resistin, visfatin, vaspin, progranulin, and chemerin are able to cross the blood-testis barrier. Leptin is present in germ cells, but there is no consensus for the presence of its receptor on sperm. It could depend on spermatozoa origin because Jope et al. found it on ejaculated spermatozoa [25], whereas Ishikawa researched it on spermatozoa obtained directly from the testis and could not found any LepR on these sperms [28]. Otherwise, the other article, which concluded to a lack of LepR on spermatozoa, found nonetheless by RT-PCR LepR on 1 of 10 controls and 3 of 23 infertile patients [27]. Thus, these

two articles have to be discussed cautiously and checked on ejaculated sperm. Our point of view is that LepR would appear on mature spermatozoa. We promote the dual role of leptin according to its concentration in seminal plasma. We hypothesize a beneficial role of leptin at physiological levels as we can expect it in men with normal BMI. On the contrary, a negative effect of leptin on spermatozoa is suggested for high concentration, corresponding to those determined in overweight or obese men. The mechanism of action of leptin on spermatozoa could be direct because human receptor of leptin has been found on spermatozoa itself. However, it also could be the consequence of higher circulating levels of leptin in obese or overweight men leading to a decrease of testosterone production by Leydig cells, which therefore interferes with the normal cycle of spermatogenesis [15]. Moreover, leptin can also modulate the nutritional support of spermatogenesis by human Sertoli cells [15]. Indeed, exposure of human Sertoli cells to leptin dramatically decreases the production of acetate, which is a central metabolite for spermatogenesis [9].

In animals and humans, adiponectin is less concentrated in seminal plasma than in serum by 180-fold in bulls [81] and 66-fold in humans [2]. Different isoforms of adiponectin circulate in blood, with a large predominance of HMW adiponectin. One hypothesis of this huge difference of concentration between these two fluids is a possible crossing of the blood-testis barrier only by smaller molecules. Even if Heinz's team got proportionally more HMW adiponectin in semen than other forms, which reduce this hypothesis, it is also reported that Ca^{2+} is 3-fold more concentrated in semen than blood and can promote HMW adiponectin forming from small isoforms that come from blood leakage. Adiponectin's effects on spermatozoa seem to be beneficial, which is in agreement with a better fertility in lean men.

Concerning the other adipokines described in the present review, only one study reported their concentration in human seminal plasma in normal weight, overweight, and obese patients. Even if these data need to be confirmed, it is clear that adipokines might be a link between obesity and male infertility. It would be worthwhile to determine seminal adipokines and adipokines expression in testis cells in some pathologies of male genital tract.

The increase of resistin and doubtlessly many other adipocytokines involved in inflammation in seminal plasma is correlated with a decrease of sperm vitality and motility. We need more in vitro experiments to assess the ideal physiologic concentrations of each adipokine and their synergic effects on spermatogenesis and sperm fertilization capacity. We cannot find enough information on combinatory actions of adipokines on male fertility and semen parameters, whereas all these adipokines are present in seminal plasma and many should be increased concomitantly and could interact together. Also, since some circulating adipokines like adiponectin can be modulated by nutrition, it will be very interesting to investigate if dietary supplements could affect seminal adipokines or adipokine testis expression and consequently improve male fertility. More experiments to assess the best levels of each adipokine and synergic effects of these hormones on spermatogenesis and sperm fertilization capacity are necessary.

In conclusion, some adipokines have been found in human and animal semen. Studies performed *in vitro* and *in vivo* by using transgenic animal models confirmed the adipokine's effects observed on semen parameters. Thus, adipokine profiles in seminal plasma could be a biomarker of male fertility. It could be interesting to measure these markers in the semen of infertile men to evaluate their seminal metabolic profile.

Conflicts of Interest

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

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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 6481658, 8 pages
https://doi.org/10.1155/2018/6481658

Research Article

A Randomised, Controlled Study of Different Glycaemic Targets during Gestational Diabetes Treatment: Effect on the Level of Adipokines in Cord Blood and ANGPTL4 Expression in Human Umbilical Vein Endothelial Cells

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Received 4 January 2018; Revised 27 February 2018; Accepted 14 March 2018; Published 14 May 2018

Academic Editor: Agnieszka Rak

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Our aim was to study the expression of adipokine-encoding genes (leptin, adiponectin, and angiopoietin-like protein 4 (ANGPTL4)) in human umbilical vein endothelial cells (HUVECs) and adipokine concentration in cord blood from women with gestational diabetes mellitus (GDM) depending on glycaemic targets. GDM patients were randomised to 2 groups per target glycaemic levels: GDM1 (tight glycaemic targets, fasting blood glucose < 5.1 mmol/L and <7.0 mmol/L postprandial, $N = 20$) and GDM2 (less tight glycaemic targets, <5.3 mmol/L and < 7.8 mmol/L, respectively, $N = 21$). The control group included 25 women with normal glucose tolerance. ANGPTL4 expression was decreased in the HUVECs from GDM patients versus the control group (23.11 ± 5.71, 21.47 ± 5.64, and 98.33 ± 20.92, for GDM1, GDM2, and controls; $p < 0.001$) with no difference between GDM1 and GDM2. The level of adiponectin gene expression was low and did not differ among the groups. Leptin gene expression was undetectable in HUVECs. In cord blood, leptin/adiponectin ratio (LAR) was increased in GDM2 compared to controls and GDM1 ($p = 0.038$) and did not differ between GDM1 and controls. Tight glycaemic targets were associated with normalisation of increased LAR in the cord blood. ANGPTL4 expression was downregulated in HUVECs of newborns from GDM mothers and was not affected by the intensity of glycaemic control.

1. Introduction

The intrauterine hyperglycaemia in women with gestational diabetes mellitus (GDM) is supposed to be an important factor that predisposes offspring to obesity and type 2 diabetes mellitus (T2D) [1, 2]. However, the mechanisms connecting intrauterine exposure to hyperglycaemia with subsequent development of metabolic diseases are not clear enough.

There is some suggestion that the exposure to diabetes in utero increases the risk of offspring obesity via alterations in the "adipoinsular axis," the endocrine loop, linking the brain and endocrine pancreas with insulin- and leptin-sensitive tissues in the control of eating behaviour and energy balance [1, 3].

Adipokines play an important role in the energy metabolism regulation [4]. Leptin (LEP) and adiponectin (ADIPOQ) are well-recognised obesity- and diabetes-related candidate genes through which the adipose tissue influences the regulation of several important physiological functions, including appetite, satiety, energy expenditure, insulin sensitivity, fat

distribution, and endothelial function [4]. Adiponectin and leptin are also factors associated with fetal growth [5] and shown as predictors of early-life weight gain [6, 7].

Another promising adipokine is angiopoietin-like protein 4 (ANGPTL4), a multifunctional signal protein expressed in many tissues. ANGPTL4 is involved in the regulation of multiple physiological processes, including energy metabolism [8], plasma glucose level and tolerance regulation [9], fat storage, and lipid metabolism [10]. The association of ANGPTL4 expression with obesity was confirmed in a study of monozygotic twins [11]. Robciuc et al. revealed that ANGPTL4 expression in the adipose tissue and circulation was inversely correlated to body weight, suggesting a role for ANGPTL4 in acquired obesity [11].

The change in the expression of the abovementioned genes in the fetal tissues may serve as a marker of subsequent metabolic diseases of the offspring. The association between the presence of hyperglycaemia in the mother and altered cord blood levels of leptin, adiponectin, and ANGPTL4 has been identified in previous studies [5, 12, 13]. An increased placental LEP expression level has been also described in women with GDM [14].

However, it is not obvious that maternal hyperglycaemia causes such alterations. Perhaps, on the contrary, the altered gene expression functions in GDM pathogenesis (e.g., due to activation of hormone-encoding genes evoking insulin resistance or reduction of insulin secretion). It is also possible that both phenomena (maternal hyperglycaemia and changes in the expression of adipokines in the fetus and/or the mother) result from other pathological processes.

Randomised controlled trials (RCT) comparing changes of newborn gene expression level in groups of women with different target glucose levels during the treatment of GDM are supposed to help clarifying the cause-and-effect relations.

The human umbilical vein endothelial cells (HUVECs) represent a good cellular model for studying the effect of maternal hyperglycaemia on the fetal cardiovascular system and can serve as a marker of the predisposition of the fetus to metabolic diseases [15].

In this study, we investigated the alterations in ANGPT4, ADIPOQ, LEP, and leptin receptor gene (LEPR) expression levels in HUVECs and concentrations of these adipokines in the cord blood from newborns of women with GDM with different glycaemic targets compared to healthy women.

2. Materials and Methods

This study was carried out at the Almazov National Medical Research Centre (ANMRC) as part of the ongoing RCT "Genetic and epigenetic mechanisms of developing gestational diabetes mellitus and its effects on the fetus" (GEM GDM) which started in July 2015. This study was approved by the local ethical committee (Protocol 119); informed written consent was obtained from all subjects.

2.1. Design and Study Population. Forty-one women with GDM and 25 controls were randomly selected to assess the levels of expression of genes in HUVECs. The women with GDM were randomised to 2 groups according to target glycaemic levels: group 1 (target fasting blood glucose < 5.1 mmol/L and <7.0 mmol/L 1-hour postprandial) (GDM1, $N = 21$) and group 2 (target fasting blood glucose < 5.3 mmol/L and <7.8 mmol/L 1-hour postprandial) (GDM2, $N = 20$).

GDM was diagnosed according to the Russian National Consensus [16] and the recommendations of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) based on the results of 2-hour oral glucose tolerance test (OGTT) performed at 24th–28th week of gestation [17]. Pregnant women without diabetes were included as controls.

None of the patients had previous history of diabetes mellitus or any known medical condition affecting glucose metabolism.

They were all followed until delivery at ANMRC. Anthropometric variables (height and blood pressure) were measured using standardised procedures. Prepregnancy body mass index (BMI) was calculated based on the prepregnancy weight recalled by participants. Women with GDM were consulted by the endocrinologist and provided the results of their self-measurements of blood glucose every 2-3 weeks. In case of exceeding the target blood glucose levels (in 2 or more measurements per week in group 1 and in more than 1/3 of measurements per week in group 2), insulin therapy was started. The participants were asked to keep electronic nutrition and glycaemic control diaries with the help of a specially developed mobile application and send data to the doctor. The mobile application is described elsewhere [18]. According to the personal diaries, automatic calculations of the integral indicators characterising the self-control of glycaemia (fasting, postprandial, and average glycaemia) were accomplished. Electronic diary data were available for almost all women with GDM (N GDM1 = 19, N GDM2 = 20) and 8 women from the control group.

2.2. Blood Sample Processing and Analysis. Cord blood samples were collected immediately after delivery. Blood glucose measurements were made on fresh plasma samples. The cord blood serum samples were stored at −80°C for further analysis of C-peptide, leptin, adiponectin, and ANGPTL4. Plasma glucose (PG) concentration was determined by the glucose oxidase method. Serum C-peptide level was measured by the chemiluminescent microparticle immunoassay (Architect C-peptide assay, Abbott Laboratories, IL, USA). Serum adiponectin (BioVendor Laboratory Medicine Inc., Modrice, Czech Republic) and leptin (Diagnostics Biochem Canada Inc., Canada) levels were measured using an enzyme-linked immunosorbent assay (ELISA) as recommended by the manufacturer. Serum level of ANGPTL4 was determined by DuoSet ELISA Development kits (DY3485) from R&D Systems (USA). The limit of detection for ANGPTL4 is 1.25 ng/mL. The detection range is 1.25 ng/mL–80 ng/mL. The following factors prepared at 800 ng/mL were assayed and exhibited no cross-reactivity or interference: recombinant human angiopoietin-1, angiopoietin-2, angiopoietin-4, and angiopoietin-like 3 and recombinant mouse angiopoietin-3 and angiopoietin-like 3.

The limit of detection for Leptin is 0.5 ng/mL. The detection range is 0.5–100 ng/mL.

The following substances were tested at 1000 ng/mL and exhibited no cross-reactivity: mouse leptin, TNF-α, IL-2, IL-3, IL-4, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF, and EGF.

The limit of detection for adiponectin is 26 ng/mL. The detection range is 26 ng/mL–100 ug/mL. No cross-reactivity has been observed for human leptin and leptin receptor.

Intra-assay coefficients of variation (CVs) for leptin assay were between 3.7% and 5.5%, and interassay CVs were 5.8–6.8%. For adiponectin assay, the intra- and interassay CVs were 3.9–5.9% and 6.3–7.0%, respectively.

2.3. Isolation and Identification of the HUVECs. The HUVECs were isolated using a standard collagenase digestion method [19] as we do routinely in our laboratory [20]. Immediately after isolation, the cells were cultured and expanded in endothelial cell medium (ECM cat number 1001, ScienCell, San Diego, CA) containing 5% fetal bovine serum, 1% penicillin/streptomycin, and endothelial cell growth supplement in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. For this study, 80% of confluent HUVEC monolayers (passages 2-3) were used.

The purity of primary HUVEC cultures was evaluated by flow cytometry analysis performed on Guava EasyCyte8. Briefly, detached cells were resuspended in 200 μL of PBS containing 1% bovine serum albumin (Sigma-Aldrich, Saint Luis, MO, USA) and incubated for 15 min at 20°C with the following antibodies (Ab): FITC-conjugated anti-CD31, PE-A-conjugated anti-CD144, PE-Cy7-A-conjugated anti-CD146 (BioLegend, San Diego, CA, USA), PE-A-conjugated anti-CD105 (Bioscience Pharmingen, San Jose, CA, USA), and APC-A-conjugated anti-CD45 (DAKO, Santa Clara, CA, USA). Data files were collected and analysed using the FACSDiva software program (version 6.1.3; BD Bioscience, San Jose, CA, USA).

2.4. Evaluation of Apoptosis and Immunocytochemical Assay. The viability of HUVEC was assessed by flow cytometry with the determination of the number of viable cells, as well as those in early and late apoptosis and necrosis evaluated by Annexin-V/PI (BioLegend, San Diego, CA, USA) double staining.

The expression of von Willebrand factor and CD146 (BioLegend, San Diego, CA, USA) in HUVECs was detected by immunocytochemical staining. Cell nucleuses were stained with $4'$,6-diamidino-2-phenylindole (DAPI).

2.5. RT-qPCR. Total RNA was extracted from HUVEC using ExtractRNA reagent (BC032, Evrogen, Moscow, Russia) according to the manufacturer's protocol. One microgram of total RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT) kit (SK021, Evrogen, Moscow, Russia). After cDNA synthesis, quantitative real-time PCR was performed in 25 μL reaction mixture containing: 5x qPCRmix-HS LowROX (PK154L, Evrogen, Moscow, Russia) diluted to a final concentration of 1x, 20x primers diluted to 1x, 50 ng cDNA, and deionised distilled water. Reaction mixtures were incubated for an initial denaturation at 95°C for 10 min, which was followed by 40 PCR cycles, each consisting of exposure to 95°C for 15 sec and 60°C for 1 min. Gene expression was evaluated by real-time PCR using Applied Biosystems TaqMan Gene Expression Assays (ADIPOQ: Hs00605917_m1; LEP: Hs00174877_m1; LEPR: Hs00174497_m1; and ANGPTL4: Hs01101127_m1). All data are expressed as ratio to the reference gene GAPDH (forward AATGAAGGGGTCATTG ATGG, reverse AAGGTGAAGGTCGGAGTCAA) (Alkor-Bio, Saint-Petersburg, Russia).

Relative expression was evaluated according to the $2^{-\Delta\Delta Ct}$ method [21]. In order to confirm the correctness of the method of detection of LEP and ADIPOQ expression, we used RNA samples derived from our previous adipose differentiation experiments [22]. RNA samples from adipose tissue multipotent mesenchymal stromal cells (MSC) and from differentiated in vitro adipose tissue were used as negative and positive controls, respectively.

2.6. Data Analysis. Statistical analysis was performed using SPSS 22.0 (SPSS Inc., USA). Mean and standard deviation are reported for continuous variables, and numbers and percentages are reported for categorical variables. Differences among the groups were analysed by Mann–Whitney test (for comparison between two groups), Kruskal-Wallis test (for comparison of more than two groups) or chi-square test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the Participants. Baseline characteristics of the participants are described in Table 1. The women from all three groups did not differ in terms of age and pre-pregnancy BMI. The GDM1 group had higher diastolic BP compared to controls ($p = 0.003$). The GDM1 and GDM2 groups had higher levels of fasting PG ($p = 0.004$ and $p = 0.003$, resp.) and higher levels of PG 1 h and 2 h in OGTT ($p < 0.001$ in comparison with controls).

Mean levels of fasting, 1-hour postprandial and average blood glucose measured by the participants during the study are described in Table 2. The GDM1 group achieved significantly lower average and 1-hour postprandial glucose levels compared to GDM2. Mean postprandial BG was lower in GDM1 even compared to the control group, though the difference did not reach statistical significance ($p = 0.088$). Gestational weight gain did not differ between GDM1 and GDM2 groups and was lower in both groups compared to controls (Table 2). The percentage of women treated with insulin was 40% and 29% in the GDM1 and GDM2 groups, respectively, and did not significantly differ ($p = 0.495$).

3.2. Pregnancy Outcomes. Pregnancy outcomes and biochemical markers in cord blood are shown in Table 3.

There was no statistically significant difference among the groups in terms of pregnancy outcomes (percent of large for gestational age (LGA) and small for gestational age (SGA) newborns, delivery by caesarean section) and the level of C-peptide, adiponectin, and ANGPTL4 in cord blood serum

Table 1: Characteristics of the participants at study entry.

	GDM 1 ($N = 20$)	GDM2 ($N = 21$)	Control ($N = 25$)	p	p control-GDM1	p control-GDM2	p GDM1-GDM2
Maternal age, years	30.9 ± 5.4	32.3 ± 5.0	30.8 ± 4.2	0.566			
Prepregnancy BMI, kg/m^2	25.4 ± 7.2	26.1 ± 6.5	23.4 ± 4.2	0.287			
BP syst, mmHg	120 ± 13	118 ± 12	112 ± 14	0.114			
BP diast, mmHg	76 ± 8	73 ± 10	69 ± 8	0.016	0.003	0.155	0.195
Fasting PG, mmol/L	5.1 ± 0.8	5.0 ± 0.6	4.5 ± 0.4	0.007	0.004	0.003	0.396
OGTT 1 h PG, mmol/L	10.2 ± 1.4	9.9 ± 1.6	6.9 ± 1.9	<0.001	<0.001	<0.001	0.454
OGTT 2 h PG, mmol/L	8.0 ± 1.6	8.8 ± 1.6	5.9 ± 1.5	<0.001	<0.001	<0.001	0.231
Fasting leptin, ng/mL	22.2 ± 20.7	29.5 ± 26.2	26.6 ± 17.0	0.561			
Fasting adiponectin, ng/mL	7.2 ± 3.3	9.1 ± 3.3	8.9 ± 2.5	0.077			

Note: BMI: body mass index; BP: blood pressure; PG: plasma glucose; OGTT: oral glucose tolerance test.

Table 2: Blood glucose data from electronic diaries and gestational weight gain.

	GDM 1 ($N = 20$)	GDM2 ($N = 21$)	Control ($N = 25$)	p	p control-GDM1	p control-GDM2	p GDM1-GDM2
Gestational weight gain, kg	9.9 ± 4.9	9.5 ± 5.9	15.2 ± 7.8	0.006	0.023	0.023	0.970
BG average, mmol/L*	5.6 ± 0.3	5.9 ± 0.4	6.0 ± 0.5	0.004	0.110	0.893	0.005
Fasting BG, mmol/L*	4.7 ± 0.4	4.8 ± 0.3	4.7 ± 0.3	0.499	0.835	0.421	0.735
1 h postprandial BG, mmol/L*	5.9 ± 0.3	6.4 ± 0.5	6.5 ± 0.7	0.002	0.088	0.818	0.002
Number of BG measurements	140 ± 78	147 ± 60	42 ± 21	0.001	<0.001	<0.001	0.946
% (N) treated with insulin	40% (8)	29% (6)	N/A	0.495			

Note: *derived from electronic diaries filled in by participants (N GDM1 = 19, N GDM2 = 20, N control = 8) during the study period. BG: blood glucose; N/A: nonapplicable.

Table 3: Pregnancy outcomes, biochemical markers in the cord blood, and ANGPTL4 gene expression in HUVECs.

	GDM 1 ($N = 20$)	GDM2 ($N = 21$)	Control ($N = 25$)	p
Gestational age at delivery, weeks	39.2 ± 1.5	39.3 ± 1.0	39.7 ± 1.0	0.261
Caesarean section, % (N)	30% (6)	19% (4)	20% (5)	0.723
Birth weight, g	3572 ± 488	3584 ± 577	3513 ± 555	0.856
Height, cm	52.1 ± 2.5	52.4 ± 2.3	52.1 ± 2.5	0.990
LGA, % (N)	20% (4)	23% (5)	12% (3)	0.235
SGA, % (N)	5% (1)	9.5% (2)	4% (1)	0.819
Apgar score 1 min	7.5 ± 0.7	7.7 ± 1.1	7.7 ± 0.6	0.204
Apgar score 5 min	8.6 ± 0.5	8.7 ± 0.9	8.8 ± 0.4	0.208
Glucose, mmol/L	4.7 ± 1.2	5.3 ± 1.3	4.5 ± 1.2	0.203
C-peptide, ng/mL	0.8 ± 0.5	1.0 ± 0.6	0.9 ± 0.4	0.379
Leptin, ng/mL	8.8 ± 6.6[a]	18.3 ± 16.1	10.6 ± 10.4	0.042
Adiponectin, ng/mL	15.9 ± 11.5	16.3 ± 14.4	18.3 ± 14.3	0.843
LAR	0.97 ± 1.31	1.70 ± 1.66[b]	0.72 ± 0.46	0.038
ANGPTL4 in cord serum, ng/mL	19.9 ± 15.0	14.1 ± 4.5	13.9 ± 5.2	0.248
ANGPTL4 relative expression in HUVECs	23.1 ± 25.6[c]	21.5 ± 25.8[c]	98.3 ± 104.6	0.001

Notes: LAR: leptin/adiponectin ratio; LGA: large for gestational age; SGA: small for gestational age. LGA was defined by a birth weight exceeding the 90th percentile on standard charts. SGA was defined by a birth weight below the 10th percentile on standard charts. [a]$p < 0.05$ versus GDM2; [b]$p < 0.05$ versus the control group; [c]$p < 0.01$ versus the control group.

and glucose in cord blood plasma. The level of leptin in cord blood serum was higher in the GDM2 group than in the GDM1 ($p = 0.036$) and the control group, but the difference from the control group did not reach statistical significance ($p = 0.066$). After adjustment by insulin therapy, age, and prepregnancy BMI, the difference in the level of leptin

between GDM1 and GDM2 remained significant ($p = 0.01$). The leptin/adiponectin ratio (LAR) in cord blood serum was higher in the GDM2 group compared to controls ($p = 0.011$) with no difference between the GDM1 and control group ($p = 0.404$).

3.3. HUVEC Characterisation. HUVECs were obtained from the umbilical vein, expanded in vitro, and characterised for expression of endothelial markers by flow cytometry and immunohistochemistry. All samples demonstrated characteristic endothelial morphology and immunophenotype CD45−/CD144+/CD31+/CD146+/CD105+ and stained positively for endothelial markers, von Willebrand factor, and CD146 (as demonstrated in our previous work [20]). There was no difference in the parameters of viability and replicative aging of HUVEC cultures from different patient groups (data not presented).

3.4. Gene Expression in HUVECs. ANGPTL4 expression was downregulated in the HUVECs derived from GDM patients compared to control group (23.11 ± 5.71, 21.47 ± 5.64, and 98.33 ± 0.92, respectively, for GDM1, GDM2, and control groups; $p < 0.001$ for comparison among the 3 groups) while no difference between GDM1 and GDM2 groups was observed (Figure 1(a)).

We did not detect the expression of LEP in HUVEC but found out that they expressed LEPR, and the expression of LEPR demonstrated a decline in the GDM1/GDM2 groups compared to the control group, though the differences were not statistically significant (Figure 1(c)). The expression of LEPR did not correlate with the level of LEP in cord plasma (Figure 1(d)) which indicates that there is no reciprocal regulation between LEP and its receptor in HUVECs. The expression of ADIPOQ was detected in HUVEC samples, but the level of its expression was as low as in negative control samples and lower by four orders of magnitude in HUVEC compared to adipocytes (positive control) (Figure 1(b)).

4. Discussion

Our RCT has shown that GDM treated according to the most widely accepted current guidelines was associated with the increased LAR in cord blood and that LAR did not differ from the control group if GDM was treated aiming at tighter glycaemic targets. The GDM1 group (with tighter glycaemic targets) had lower levels of leptin compared to GDM2. We also found a decrease in the level of expression of ANGPTL4 in HUVEC of newborns from women with GDM in comparison with the control group. However, there was no difference in the level of expression of ANGPTL4 between the two groups of GDM with different glycaemic targets.

The most appropriate target levels of glycaemia for the management of GDM are not universally defined [23]. Most organisations [24–26] suggest the targets for glycaemic control for women with GDM based on recommendations from the Fifth International Workshop-Conference on Gestational Diabetes Mellitus [27].

These targets were used by the Maternal-Fetal Medicine Units Network (MFMU) trial showing benefit for the treatment of GDM [28]. We used these targets for group 2.

However, there are no reliable data from controlled trials of lower versus higher target levels of glycaemia to identify ideal glycaemic targets for prevention of fetal risks [29].

The glycaemic targets used in our study for group 1 were tighter in accordance with current Russian guidelines [16]. Our data suggest that achieving tighter glycaemic targets during GDM treatment reduces LAR in the cord blood. However, this data should be interpreted with caution considering the small sample size. The full information about pregnancy outcomes is needed to guide clinical practice regarding target glycaemic levels during pregnancy. Our current study with a small sample size was not designed for this purpose. However, our findings may serve as a confirmation of the cause-and-effect relationship between maternal hyperglycaemia and alteration of LAR in newborns. Our results are in line with the data by Pirc et al. that reported treatment of mild GDM reduces cord blood leptin [12]. The authors hypothesise that hyperleptinaemia of the babies born to women with untreated mild GDM may persist for some time. It could reduce appetite and food intake and might contribute to the phenomenon of catch-down growth seen in macrosomic infants following birth [12, 30].

Follow-up studies are needed to understand the impact of tight glycaemic targets during pregnancy on obesity development in the offspring of women diagnosed with GDM according to IADPSG criteria. It is especially important taking into consideration the evidence that low early-life leptin concentrations may promote faster weight gain in infancy [31–33].

There is controversy about the association of adiponectin levels in cord blood with GDM. Pirc et al. reported decreased levels of adiponectin in the cord blood of newborns from mothers with GDM [12], whereas several other studies, like ours, find no effect of maternal DM on cord blood adiponectin concentration [5, 34, 35]. The reasons for these differences are unclear but may reflect different assay methodologies, different study populations, and different criteria used to diagnose GDM.

The level of expression of LEP in HUVECs turned out to be below the detection threshold. The expression of ADIPOQ was detected in HUVEC samples, but the level of its expression was as low as in negative control samples, which confirms that umbilical vein endothelium is not a place of adiponectin production. We are not aware of any other study addressing the expression of these genes in HUVEC. However, our results are in line with some of the previous studies which have shown that ADIPOQ is not expressed in the placenta [14, 36]. Thus, the levels of cord adiponectin may be attributed to other fetal tissues, while LEP has been shown to be expressed in the placenta [14]. It should be noted that there are also conflicting results of the studies which indicated the expression of ADIPOQ in human placenta [36, 37].

In contrast to LAR changes associated with tight glycaemic targets of treatment of GDM, the level of expression of ANGPTL4 was lower in both the GDM groups regardless of glycaemic targets compared to the control group. Possibly,

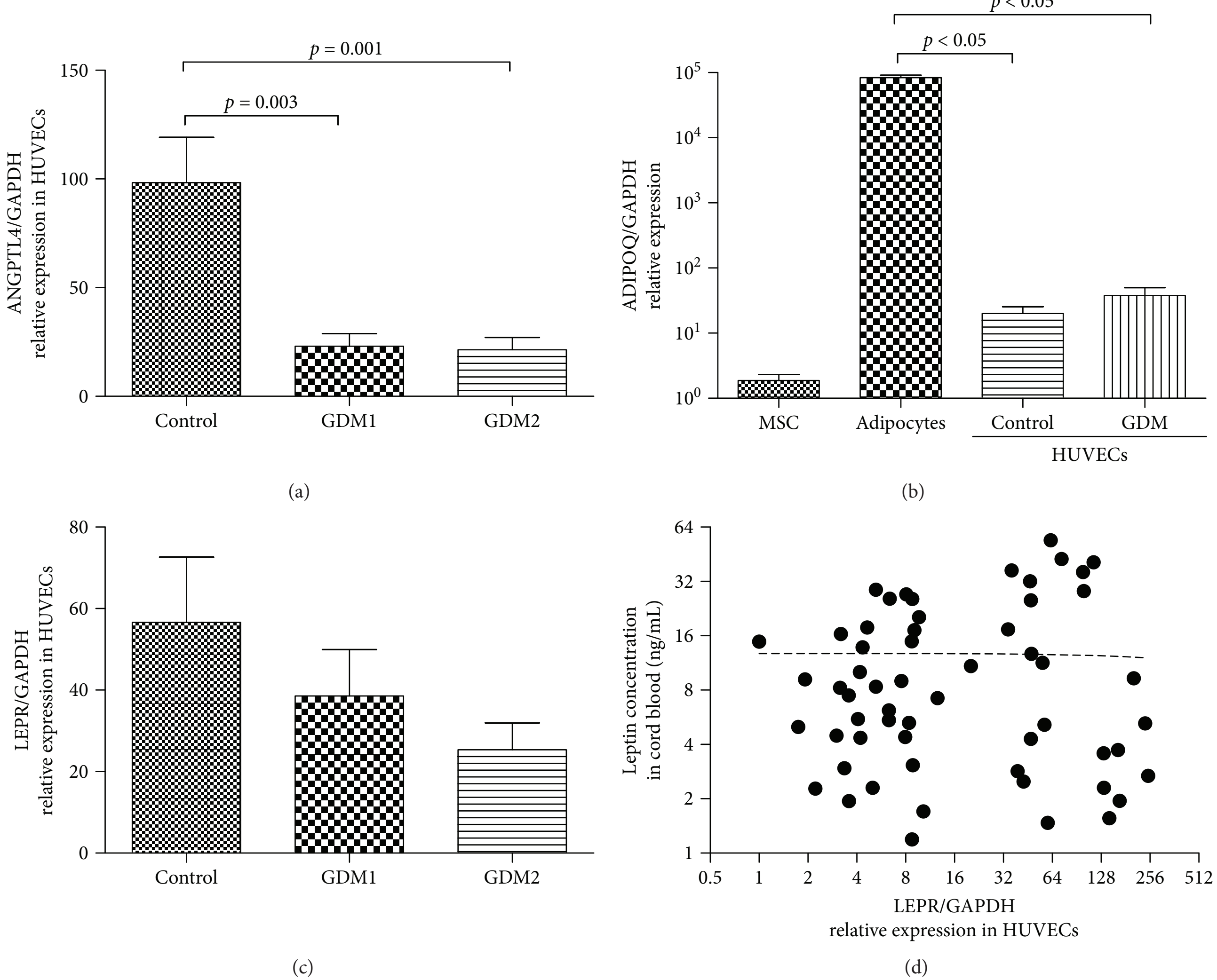

Figure 1: Gene expression analysis. (a) The level of relative ANGPTL4 mRNA expression in HUVECs from healthy (control) and GDM patients. (b) The level of relative ADIPOQ mRNA expression in multipotent mesenchymal stromal cells (MSC) (as negative control), adipocytes (as positive control), and HUVECs. (c) The level of relative LEPR mRNA expression in HUVECs from healthy (control) and GDM patients. (d) Correlation between the relative LEPR mRNA expression in HUVECs and the level of leptin in the cord blood.

it is due to the fact that the difference in target glycaemic levels is not significant enough to affect the expression level of ANGPTL4, at least on such a small sample. Another plausible explanation is that the reduced level of activity of ANGPTL4 is transmitted at the genetic level to newborns from their mothers. Maybe, the reduced level of activity of ANGPTL4 contributes to the development of GDM in the mothers, that is, it is the cause, not the consequence of hyperglycaemia.

This hypothesis is supported by the data of Xu et al. on the lower level of ANGPTL4 in patients with type 2 diabetes whose pathogenesis is close to GDM [9]. However, this hypothesis is contradicted by the data of Ortega-Senovilla et al., indicating that maternal serum ANGPTL4 concentrations showed no difference between the control and GDM women [13].

Moreover, opposite to our data, Ortega-Senovilla et al. showed that serum ANGPTL4 concentrations in cord serum were higher in those from GDM than those from control pregnancies [13]. We found no difference in the level of ANGPTL4 in the cord serum. There seems to be no correlation between the levels of ANGPTL4 protein in the cord serum and ANGPTL4 gene expression in one of the fetal tissues (HUVEC). It is plausible that the main source of cord serum ANGPTL4 is some other fetal tissue (e.g., the liver). The functional consequences of the downregulation of ANGPTL4 mRNA levels in HUVECs in GDM remain to be identified.

In addition, other factors besides intrauterine hyperglycaemia may affect the activity of a number of genes, including ANGPTL4, in the fetus. Known factors that affect the weight of the newborn are the body mass index (BMI) of the mother and maternal gestational weight gain. Obviously, these parameters are influenced by the mother's lifestyle (the quantitative and qualitative composition of the diet and the level of physical activity).

It is known that ANGPTL4 can be regulated by diet [38, 39]. The diet interventions leading to NEFA increase in the blood (high-fat diet, a very low-energy diet, and fasting) were shown to upregulate the plasma level of ANGPTL4 [38].

This upregulation has been confirmed likewise in vitro as the expression of ANGPTL4 is upregulated in response to exposure to fatty acids in cell studies [40].

Our study established a significantly lower pregnancy weight gain in GDM patients compared to controls which is obviously due to diet adherence by patients.

Further studies are needed to clarify the cause-and-effect relationship between GDM and the level of expression of ANGPTL4 gene in HUVEC.

The level of C-peptide in the cord blood is commonly used as a marker of fetal hyperinsulinemia [41]. The data presented by HAPO study has shown associations between increasing levels of fasting, 1-hour, and 2-hour plasma glucose obtained on oral glucose-tolerance testing and cord blood serum C-peptide level above the 90th percentile [41]. We did not reveal any difference in the level of C-peptide among the groups. It could be a result of the treatment that was efficient to reduce fetal hyperinsulinemia in both GDM groups or it may be due to a small sample size.

The strength of our study is the design of the RCT of different glycaemic targets for women with GDM which allows at testing cause-and-effect relationships. The weakness of the study, besides its relatively small sample size, is the lack of information about maternal levels of the studied gene expression.

5. Conclusion

Our study established positive association of cord leptin levels and LAR with target levels of glycaemia during pregnancy in women with GDM. Further investigation into long-term consequences of cord leptin concentrations is required.

We also found a decrease in the expression of ANGPTL4 in HUVECs of neonates from mothers with GDM. However, we could not prove the causal relationship between intrauterine hyperglycaemia and the expression of the ANGPTL4 gene, given the absence of differences between the level of expression of ANGPTL4 in groups with different glycaemic targets. This relationship remains to be clarified.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the institutional committee of ANMRC (Protocol 119).

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The study was funded by the Russian Science Foundation (Project no. 17-75-30052).

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Hindawi
International Journal of Endocrinology
Volume 2018, Article ID 7965071, 12 pages
https://doi.org/10.1155/2018/7965071

Review Article

Adiponectin: A New Regulator of Female Reproductive System

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Received 20 December 2017; Revised 11 March 2018; Accepted 22 March 2018; Published 29 April 2018

Academic Editor: Joëlle Dupont

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Adiponectin is the hormone that belongs to the group of adipokines, chemical agents mainly derived from the white adipose tissue. The hormone plays pleiotropic roles in the organism, but the most important function of adiponectin is the control of energy metabolism. The presence of adiponectin and its receptors in the structures responsible for the regulation of female reproductive functions, such as hypothalamic-pituitary-gonadal (HPG) axis, indicates that adiponectin may be involved in the female fertility regulation. The growing body of evidence suggests also that adiponectin action is dependent on the actual and hormonal status of the animal. Present study presents the current knowledge about the presence and role of adiponectin system (adiponectin and its receptors: AdipoR1 and AdipoR2) in the ovaries, oviduct, and uterus, as well as in the hypothalamus and pituitary, the higher branches of HPG axis, involved in the female fertility regulation.

1. Introduction

Until the late 80s of the XX century, adipose tissue was considered only as an organ responsible for an energy storage. Since 1987, when Siiteri [1] reported that adipose tissue actively metabolizes steroid hormones, the tissue has begun to be considered as an endocrine organ and active factor in the energy metabolism regulation. For now, adipose tissue was found to be the source of a number of bioactive peptides called adipokines, which may act at both autocrine/paracrine and endocrine levels.

Adiponectin belongs to the adipokine family and initially was considered as a hormone produced exclusively by the white adipose tissue (WAT) [2–5]. A number of further studies proved that adiponectin is produced and secreted not only in the WAT but also in other tissues, like skeletal muscles, cardiomyocytes, hypothalamus, pituitary, ovaries, uterus, or placenta [6–12]. The expression of adiponectin and its receptors has been identified in the reproductive organs of many animals, including rats, mice, humans, and pigs [11–16], which indicates a potential involvement of this hormone in the reproductive system functions.

The aim of this review is to compile and systematize the current knowledge about the adiponectin system (adiponectin and adiponectin receptors) role in the structures responsible for the regulation of reproductive functions (the hypothalamus-pituitary-ovarian axis and uterus) during the reproductive cycle and early gestation.

1.1. The Hormone. Adiponectin is a 244-amino-acid protein with molecular weight of 30 kDa. The hormone contains four domains: the amino-terminal signal sequence, a nonconserved variable region, a collagenous domain, and a carboxy-terminal globular domain [3]. Adiponectin circulates in the serum in three main homomultimer fractions: trimer (low molecular weight, LMW), hexamer (medium molecular weight, MMW), and multimer, containing 12 to 18 adiponectin molecules (high molecular weight, HMW) [4]. Fruebis et al. [17] identified the fourth fraction of the adipokine, globular adiponectin, which is formed by the proteolytic cleavage of

full-length hormone. In the serum, adiponectin occurs at approximately 0.01% of total plasma proteins, at the μg/ml concentrations [18]. Concentration of this adipokine in the plasma estimates from 3 to 30 μg/ml in humans, 2 to 7 μg/ml in rats, and 3 to 4 μg/ml in pigs [18–20]. Despite the fact that adiponectin is produced mainly by the WAT, its serum concentration is reversely correlated with the body mass index and the overall mass of WAT [18]. Adiponectin plasma concentration was found to be sex dependent. Sexual dimorphism of the hormone serum concentration was confirmed in human and mice [21, 22]. Physiological concentrations of the adipokine were higher in women (12.5 ± 0.3 μg/ml) than in men (8.7 ± 0.3 μg/ml) and prepubertal individuals. For more, in pubescent boys, adiponectin concentrations were significantly lower (5.6 ± 0.5 μg/ml) than in girls of the same age (7.1 ± 0.5 μg/ml), which suggests that adiponectin serum concentration may be dependent on the androgen concentration [22–24].

Adiponectin exhibits pleiotropic properties. The hormone is known for its involvement in the control of metabolism and insulin sensitisation. In the liver, adiponectin promotes glucose transport, inhibits gluconeogenesis, activates oxidation of fatty acids, and enhances insulin sensitivity promoting phosphorylation of the insulin receptor [25, 26]. In WAT, adiponectin promotes basal glucose uptake, insulin-stimulated glucose uptake, and regulates fat lipid metabolism inhibiting lipolysis [25, 27]. Adiponectin is also known for its anti-inflammatory properties. The adipokine may attenuate inflammation processes in different types of tissues, like endothelial cells, muscle, epithelial cells, and macrophages [28–30]. Its antiatherogenic and anticancerogenic properties have also been proven [31–34].

Expression of adiponectin gene and protein was confirmed in the reproductive system. Adiponectin expression was noted, among others, in the human endometrium as well as uterus, trophoblasts, and conceptuses of mice and pigs [12, 14, 16]. For more, in pig the concentration of adiponectin in plasma, as well as, the expression of the hormone and its receptors in the ovary and uterus were found to be dependent on the phase of the oestrous cycle or the stage of gestation. In the porcine ovaries, adiponectin expression on both gene and protein expression levels was found to be enhanced during the luteal phase of the oestrous cycle, when compared to the follicular phase of the cycle. In the porcine uterus, the highest expression of the adiponectin gene was observed on days 14 to 16 and 2 to 3 of the oestrous cycle, in the endometrium and myometrium, respectively, whereas during the early gestation period, on days 15 to 16 of gestation in both the endometrium and myometrium. During the oestrous cycle, the hormone concentration in the blood plasma was constant during the luteal phase (from days 2 to 3 to days 14 to 16) and decreased during the follicular phase (days 17 to 19 of the cycle). During the early gestation period, the highest concentration of adiponectin in the porcine plasma was observed on days 15 to 16 of gestation, whereas the lowest on days 30 to 32 of pregnancy. Taken together, data presented above indicates that adiponectin actions may be dependent on the actual hormonal status of animals [15, 16, 20, 35, 36]. The above also suggests that adiponectin may be involved in the regulation of the reproductive functions.

1.2. Adiponectin Receptors. In the organism, adiponectin actions are mediated *via* two distinct receptors: adiponectin receptor type 1 (AdipoR1) and adiponectin receptor type 2 (AdipoR2). Mouse AdipoR1 and AdipoR2 share 66.7% homology in its amino acid sequence. Both receptors are integral membrane proteins consisting of seven transmembrane domains, which make them similar to the G-protein-coupled receptors family. However, the N-terminus of the proteins is located internally and the C-terminus externally, which is opposite to the topology of G-protein-coupled receptors [37]. Human AdipoR1 protein consists of 375-amino-acids and has a molecular weight of 42.4 kDa. Human, mouse, and porcine AdipoR1 gene are located on the chromosome 1p36.13-q41, 1 E4, and 10p11, respectively. The receptor has a greater affinity to the trimers and globular domain of adiponectin and is mostly expressed in the skeletal muscles [37–39]. AdipoR1 acts *via* AMP kinase and mitogen-activated protein kinase [40]. Human AdipoR2 protein consists of 386-amino-acids with a molecular weight of 48.3 kDa. Its gene is located on the chromosome 12p13.31, 6 F1, and 5q25 for human, mouse, and pig, respectively. AdipoR2 has a higher affinity for the multimeric forms of adiponectin and is highly expressed in the liver [37–39]. The receptor acts primarily through the peroxisome proliferator-activated receptor α (PPARα) pathway [39]. Hug et al. [41] reported the existence of third adiponectin receptor, T-cadherin. The protein is expressed mainly in the vascular endothelial cells and smooth muscles. T-cadherin binds MMW and HMW adiponectin; however, it has no influence on adiponectin cell signaling since its protein has no intracellular domain. It is hypothesized that T-cadherin acts only as an adiponectin binding protein [38, 41–43].

Similar to adiponectin, AdipoRs' (AdipoR1 and AdipoR2) were found to be expressed in many tissues, including the structures responsible for the reproduction. AdipoRs' expression was confirmed in all structures of the hypothalamic-pituitary-ovarian axis (HPG axis). The presence of adiponectin receptors was observed in human, rodent and porcine hypothalami [44–46], human, rat and porcine pituitaries [9, 47, 48], and rat and porcine ovarian follicles, and corpora lutea (CL) [20, 48]. Moreover, AdipoRs' expression was observed also in the human endometrium and uteri and conceptuses of mice and pigs [12, 14, 16].

2. Adiponectin and H-P-G Axis

2.1. Hypothalamus. Adiponectin receptor expression in the hypothalamus was confirmed in many species, including humans, rodents, and pigs [44–46]. In the hypothalamus, AdipoRs' were expressed in the porcine preoptic area, mediobasal hypothalamus and stalk median eminence, human and rat arcuate and paraventricular nuclei, and human lateral hypothalamus [8, 37, 44–46]. Expression of adiponectin hormone was confirmed in the murine and chicken brains; however, no immunopositive cells for adiponectin were observed in the human hypothalamus or infundibular stalk [8, 49, 50] (Figure 1). Adiponectin may be also supplied by the blood. The hormone has been detected in human, mice, and rat cerebrospinal fluid (CSF) [44, 51–54]. The hormone

The effect of adiponectin
on hypothalamic-pituitary-gonodal axis

Hypothalamus

Expression of adiponectin
(i) In the mouse [51]
(ii) In the chicken [50]

Adiponectin receptor expression
(i) In the porcine preoptic area, mediobasal hypothalamus and stalk median eminence [46]
(ii) In the human arcuate and paraventricular nuclei and lateral hypothalamus [8, 37, 45]
(iii) In the arcuate and paraventricular nuclei of rat [45]

Hypothalamus

The effect of adiponectin
through activation of AMPK pathway
(i) Regulatioon of food intake and energy expenditure [56]
(ii) Inhibition of kisspeptin1 (*KISS1*) gene transcription [58]
(iii) Decrease in the GnRH neuronal activity and inhibition of GnRH secretion [57, 59]
(iv) Hyperpolarization of plasma membrane potentials and reduction of calcium influx in GT 1-7 hypothalamic GnRH-produced neurons [57]

Pituitary

Expression of adiponectin
(i) In the pig [61]
(ii) In the chicken [60]

Adiponectin receptor expression
(i) In the porcine preoptic area, mediobasal hypothalamus and stalk median eminence [46]
(ii) In the human arcuate and paraventricular nuclei and lateral hypothalamus [8, 38, 45]
(iii) In the rat arcuate and paraventricular nuclei [45]

Pituitary

The effect of adiponectin
(i) Reduction of LH secretion and GnRH-induced LH release [9, 63]
(ii) Stimulation of the basal FHS release [61]
(iii) Inhibition of growth hormone (GH) secretion and the release of GH under the influence of ghrelin [9]
(iv) Increase in the expression of PPARA and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC-1*), genes involved in the regulation of energy homeostasis [66]

Ovary

Expression of adiponectin hormone
(i) In the human theca interna cells [13]
(ii) Rat theca interna, granulosa, corpora lutea, and oocytes [49]
(iii) In the bovine theca interna, granulosa, corpora lutea, and oocytes [67, 68]
(iv) In the porcine theca interna, granulosa and corpora lutea [36]

Adiponectin receptor expression
(i) In the human granulosa cells [13]
(ii) In the rat follicular cells and corpora luteaof rats [46]
(iii) In the bovine follicular cells and corpora lutea of cows [36, 68]
(iv) In the porcine granulosa cells of prepubertal pigs [71], corpora lutea, theca and granulosa cells of mature gilts [11, 20]
(v) In the hens ovarian follicles [72]

Ovary

The effect of adiponectin
(i) Stimulation on of the expression of genes associated with the ovarian follicles remodeling [72]
(ii) Stimulation of meiotic oocytes maturation *via* p38MAPK pathway (may differ between the species) [74]
(iii) Regulation of corpus luteum growth and activity [75]
(iv) Modulation of steroidogenic enzymes genes expression and basal and gonadotrophin/insulin/IGF-I-induced release of ovary hormones [36, 49]

FIGURE 1: The evolvement of adiponectin system in the regulation of hypothalamic-pituitary-gonadal axis (H-P-G axis) in different animal species. The left side of the figure presents the expression of adiponectin and adiponectin receptors in the particular tissues of the H-P-G axis. The right side of the figure presents the effect of the hormone on the target tissues.

concentrations in the CSF are many times lower (0.1%), when compared to blood plasma. In the CSF, adiponectin occurs only in the LMW and MMW forms, with the dominant contribution of the LMW form, which suggest an inability of high-molecular complexes to cross the blood-brain barrier [52].

The pleiotropic effect of adiponectin in the mouse hypothalamus has been observed. The hormone, *via* AdipoR1, enhanced AMPK activity in the arcuate hypothalamus, which resulted in the stimulation of food intake and decreased energy expenditure. Moreover, in the adiponectin-deficient mice, the AMPK phosphorylation was decreased, which caused an increase in the energy expenditure and decreased food intake [55]. The second function of adiponectin in the hypothalamus is the hormone involvement in the regulation of gonadoliberin (GnRH) secretion. Adiponectin, *via* activation of the AMPK, inhibited GnRH secretion and caused a hyperpolarization of plasma membrane potentials and reduction of calcium influx in GT1-7 mouse hypothalamic GnRH-produced neurons [56]. For more, adiponectin, also through AMPK pathway, inhibits the gene transcription of kisspeptin 1 *(KISS1)*, the upstream signal for GnRH release [57]. Klenke et al. [58] reported that mouse GnRH neurons express AdipoR2 and adiponectin may decrease the GnRH neuronal activity rapidly *via* the AMPK pathway. The above findings indicate the potential role of adiponectin as a metabolic regulator of the reproductive functions *via* its influence on GnRH release (Figure 1).

2.2. Pituitary. The expression of adiponectin mRNA in the pituitary gland was described in a number of species [9, 59–61] (Figure 1). In humans, adiponectin expression was localized mainly in growth hormone (GH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone- (TSH-) producing cells [8]. The expression of adiponectin receptors in the pituitary was also confirmed in many species including humans, rats, and pigs [8, 9, 47, 48]. Psilopanagioti et al. [8] localized AdipoRs' expression in the human gonadotrophs, somatotrophs, and thyrotrophs, but not in corticotrophs or lactotrophs. The expression of AdipoRs' in the pituitary suggests that the hormone may regulate central endocrine axes and participate in the control of metabolic homeostasis.

Rodriguez-Pacheco et al. [9] proposed that locally produced adiponectin may affect pituitary hormone secretion. The presence of this adipokine in the rat and mouse pituitary cell cultures resulted in the reduction of LH secretion and LH release induced by GnRH [9, 62]. For more, in rats, adiponectin has been observed to inhibit growth hormone (GH) secretion and the release of GH under the influence of ghrelin [9]. Adiponectin had also a stimulatory effect on ACTH secretion in the primary culture of pituitary cells *via* an AMPK-dependent mechanism in the rat's pituitary corticotroph cells [63]. In the *in vitro* studies on the porcine primary pituitary cells, adiponectin stimulated the basal FSH release. Administration of the hormone affected GnRH- and insulin-induced LH and FSH secretion dependently on the phase of the oestrous cycle [60]. As mentioned in the previous subsection, adiponectin had an inhibitory effect on the GnRH release [56]. However, GnRH suppressed adiponectin expression in the rat primary pituitary cell culture as well as in mouse LβT2 gonadotroph cell line. The inhibitory action of GnRH was mediated *via* the calcium and protein kinase A intracellular pathways. Interestingly, GnRH did not affect the expression of both AdipoRs' [61]. As we observed in our unpublished studies (Szeszko et al., unpublished data), adiponectin affects the global gene expression in the porcine primary pituitary cell culture. Results of the studies show that adiponectin influences the group of genes responsible for MAPK cascade, which plays a key role in the transduction of extracellular signals to cellular responses and regulates the expression of several gene encoding gonadotroph functions. The activation of MAPK ERK1/2 signaling pathway is necessary for, among others, steroidogenesis and steroidogenic gene expression in granulosa cells [64]. For more, adiponectin increased the expression of PPARA and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC-1*) genes involved in the regulation of energy homeostasis, especially fatty acid oxidation and carbohydrate metabolism (Figure 1). Wang et al. [65] hypothesize that adiponectin may act as a mediator of nutrition and reproduction in sheep. Fasting of prepubertal eves increased serum adiponectin concentrations and enhanced *AdipoR1* and *AdipoR2* gene expression, which showed the negative correlation with the LH β-subunit and FSH β-subunit gene expression.

The presence of adiponectin system in the pituitary, especially in the gonadotroph cells, and its influence on the LH and FSH release, *via* the modulation of the AMPK and MAPK signaling pathways, indicates the important role of this hormone in the regulation of the reproductive functions at the higher branches of HPG axis, in response to the actual metabolic status of female.

2.3. Ovaries. To assent adiponectin as a key factor involved in the reproductive system regulation, it is important to investigate its direct role in gonads. The expression of adiponectin on both gene and protein level was noted in the women theca interna cells [13], theca interna, granulosa, corpora lutea, and oocytes of rats [66] and cows [67, 68], in theca interna, granulosa, and corpora lutea of pigs [36] (Figure 1). The concentration of this adipokine was measured in human (2.209 $\pm$ 0.85 μg/ml) and cattle (19.4 $\pm$ 1.4 μg/ml) follicular fluid [69, 70]. In turn, the AdipoRs' expression was reported in the human granulosa cells [13], follicular cells, and corpora lutea of rats [46] and cows [36, 68], in granulosa cells of prepubertal pigs [71], corpora lutea, theca and granulosa cells of mature gilts [11, 20], and in the ovarian follicles of hens (mRNA only) [72].

It has been postulated that adiponectin may take part in the initiation of preovulatory changes in the ovary and modulate the ovarian steroidogenesis process. The presence of adiponectin in the primary granulosa cell culture from prepubertal gilts stimulated the expression of genes associated with the remodeling of the ovarian follicles, including cyclooxygenase-2, prostaglandin E synthase, and vascular endothelial growth factor genes [72]. In pigs, adiponectin

modulates steroidogenic enzymes and steroidogenic acute regulatory protein (*StAR*) gene expression, increasing *StAR* transcript abundance and reducing the cytochrome P450 aromatase (*CYP19A3*). It has been observed that the adipokine affects basal and gonadotrophin-/insulin-induced release of progesterone (P_4), oestradiol (E_2), and testosterone by the porcine luteal and follicular cells, respectively [36]. In the rat primary granulosa cells, adiponectin had no effect on the basal steroid secretion but caused an increase in P_4 and E_2 production, when combined with the insulin-like growth factor-I (IGF-I) [66]. In the *in vitro* studies on bovine ovaries, Lagaly et al. [71] observed that adiponectin decreased insulin-induced P_4 and androstenedione (A_4) production, inhibited IGF-I, as well as induced *LH receptor*, P450 side-chain cleavage enzyme *(CYP11A1)*, and cytochrome P450c17 *(CYP17A1)* gene expression in theca cells, and decreased *LH receptor* gene expression in granulosa cells. For more, adiponectin may also take part in the oocyte maturation process. AdipoR1 and AdipoR2 were found to be expressed in the porcine oocytes and cumulus cells in both small and large follicles, and adiponectin was found to stimulate meiotic maturation of oocytes derived from large follicles *via* p38MAPK pathway [73]. On the other hand, in the bovine *in vitro* studies, adiponectin did not affect oocyte maturation [68], which implies that adiponectin role in the oocyte maturation may differ between the species. The microarray analysis of Szeszko et al. [74] indicates the modulatory effect of adiponectin on the porcine ovarian cells during the luteal phase of the oestrous cycle. The researchers observed that adiponectin influences the number of genes, including steroidogenic enzymes, genes responsible for prostaglandin synthesis, or genes responsible for vascularization, which suggests the important role of this adipokine in the regulation of CL growth and activity (Figure 1).

Disorders in the adiponectin serum concentrations and its influence on the ovarian steroidogenesis have been linked with the polycystic ovary syndrome (PCOS). PCOS is one of the commonest endocrine disorders in women, and hyperandrogenism is one of its hallmarks. Theca cells are recognized as one of the primary sources of excess androgen biosynthesis in women with PCOS [75]. In the PCOS women, the concentration of adiponectin in the blood plasma was 16 μg/ml which is about 23.5% less than in the plasma of healthy individuals (about 20 μg/ml) [76]. Another characteristic issue for PCOS is the difference in the adiponectin multimer ratio and concentrations, when compared to the control group. Aroda et al. [77] reported lower HMW adiponectin serum levels in women with PCOS, whereas O'Connor et al. [78] observed selectively reduced HMW fraction in PCOS-positive individuals, independently to the body mass index. In the bovine theca cells, adiponectin suppressed A_4 production and gene expression of key enzymes in the androgen synthesis pathway. In turn, the knockout of AdipoRs' genes resulted in an increase in the A_4 secretion by the bovine theca cells. For more, in women polycystic ovaries, a significantly lower proportion of theca cells expressing AdipoR1 and AdipoR2 was observed, when compared to the normal ovaries [79]. Also in the mouse ovary, adiponectin reduced A_4 secretion and oxidative stress protein concentrations, which may be potentially linked with the pathogenesis of PCOS associated to obesity [80].

The presence of adiponectin and its receptors in the ovaries during all periods of the oestrous cycle, taken together with the data presented above, indicates the important role of this adipokine in the regulation of oocyte maturation, CL formation and activity, and a proper course of the oestrous/menstrual cycle *via* its influence on the steroid production process. A clear confirmation of this statement may be a link between adiponectin and its receptor concentrations and the PCOS disorder.

3. Reproductive Tract

Adiponectin system is supposed to influence reproductive functions not only at the central level *via* the H-P-G axis regulation but also locally *via* the hormone actions across the reproductive tract. In mammals, the oviduct, besides its obvious role in the oocyte and embryo transport, is an important source of hundreds of macromolecules derived by oviduct epithelium, such as enzymes, protease inhibitors, growth factors, and a group of oviductins [81–83]. The expression of adiponectin gene and protein in the rat oviduct has been confirmed by Archanco et al. [84]. In the oviductal secretory epithelial cells of cyclic rats, adiponectin expression was changing throughout the oestrous cycle, increasing from proestrous to oestrous. Oses et al. [85] indicated that in the *in vitro* primary cultures of ciliated cells from the rat epithelium adiponectin treatment resulted in an increased ciliary beat frequency (Figure 2). In the uterus, the expression of the adiponectin system was reported, among others, in human endometrial stromal and epithelial cells, mouse epithelial cells of the uterine glands, rabbit myometrium, and endometrial stromal and epithelial cells, as well as in porcine endometrium, myometrium, and trophoblasts [12, 15, 16, 86] (Figure 2). In the porcine uterine luminal fluid, adiponectin concentration varies between the oestrous cycle (4.7 μg/ml) and early pregnancy period (8.7 μg/ml), which taken together with the data presented above suggests the important role of adiponectin in the maintenance and proper course of gestation [35]. Adiponectin system has been suggested to play an important role during early pregnancy period, especially implantation. Adiponectin receptor expression was found to be increased in the primary cultures of human stromal cells during the decidualization process, what may be an evidence for the modulatory influence of adiponectin on the uterine receptivity during pregnancy [87]. Expression of adiponectin, as well as AdipoRs', was found in both rabbit and mouse trophoblasts and embryoblasts, suggesting that during the pre- and peri-implantation period, adiponectin may be involved in the crosstalk between mother and embryo [86]. Another evidence for the regulatory function of adiponectin during early gestation was provided by Dos Santos et al. [88], who indicated the lower expression of adiponectin receptor genes in the uteri of women with iterative implantation failures. For more, in women with endometriosis, in which high rate of implantation failure was observed, adiponectin

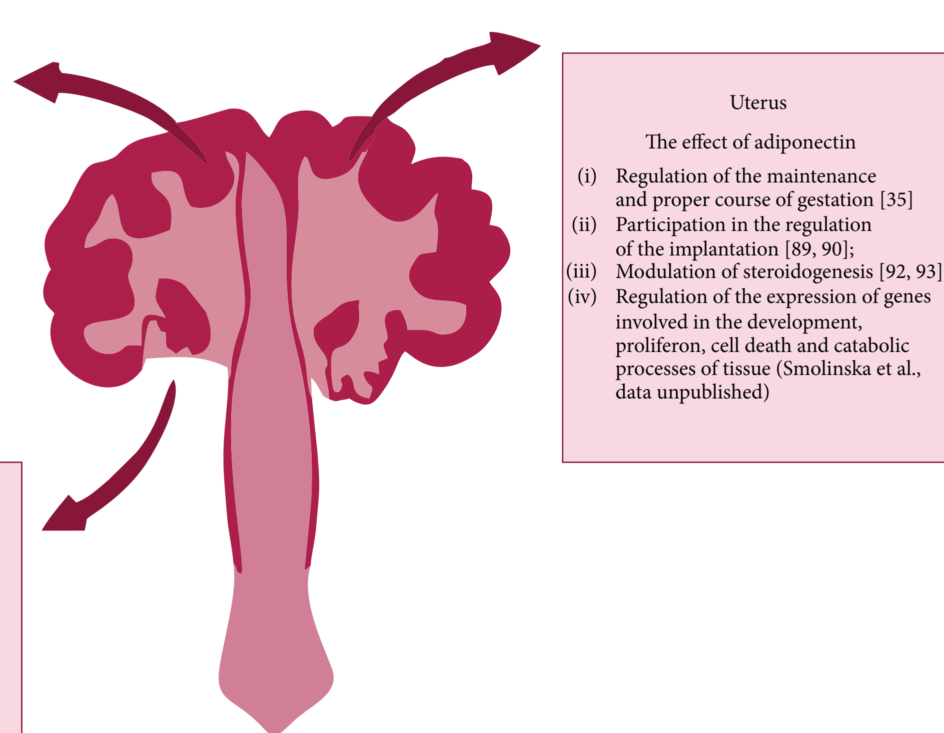

Figure 2: The effect of adiponectin on the female reproductive tract. The figure presents the expression of the adiponectin and adiponectin receptors, as well as the hormone action in the oviduct and uterus.

serum level was lower (13.1 μg/ml), when compared to the healthy individuals (15.9 μg/ml) [89].

As described in the above subsection, it has been proven that adiponectin exerts a modulatory influence on the steroidogenesis process in the ovary. In 2008, Franczak and Kotwica [90] indicated the steroidogenic activity of the porcine endometrium and myometrium and indicated that the uterus is an alternative source of steroid hormones. Smolinska et al. [91] proved that adiponectin may modulate not only ovarian but also endometrial and myometrial steroidogenesis. In the *in vitro* studies on porcine uterine tissues, adiponectin modulated the gene expression of key enzymes involved in the steroid synthesis: *StAR*, *CYP11A1*, and *HSD3B1* (3β-hydroxysteroid dehydrogenase), as well as influenced the secretion of P_4 and A_4 by the tissues. For more, adiponectin has been found to modulate also the prostaglandin synthesis pathway in the porcine uterus. On day 15 of pregnancy, the presence of adiponectin and insulin in the *in vitro* cultures of porcine endometrial cells enhanced the expression of gene encoding enzymes important for the prostaglandin synthesis, such as cyclooxygenase 2, as well as vascular endothelial growth factor and peroxisome proliferator-activated receptor gamma gene expression [92]. Our unpublished data, concerning the influence of adiponectin on the global gene expression in the porcine endometrium during early pregnancy indicates that the hormone is involved in the regulation of a number of processes important for the tissue growth and development. We found that adiponectin provoked an increase in the group of genes responsible for development and proliferation and suppressed the expression of genes connected with the cell death and catabolic processes. For more, the adipokine modulated the expression of genes involved in the steroid and prostaglandin synthesis, or metabolism, which confirmed the previous results and expanded our knowledge about adiponectin's interaction networks in the pregnant endometrium (Smolinska et al., data unpublished) (Figure 2).

Adiponectin and adiponectin receptor genes and protein expression in the porcine uterus, as well as adiponectin concentration in the porcine uterine luminal fluid, change during both the oestrous cycle and early pregnancy period, which suggests that adiponectin system expression in the uterus strongly depends on the animals hormonal status [15, 16, 35]. Those speculations were confirmed by the *in vitro* studies of Dobrzyn et al. [93–95], which indicated the modulatory effect of steroid hormones, P_4, oestrone, and E_2, as well as prostaglandins: E_2 and $F_{2\alpha}$ on the adiponectin system expression in the porcine pregnant uterus. The effect of the steroids and prostaglandins on the adiponectin system expression in the endometrium and myometrium was found to be tissue specific and dependent largely on the period of early gestation. The modulatory effect of steroid

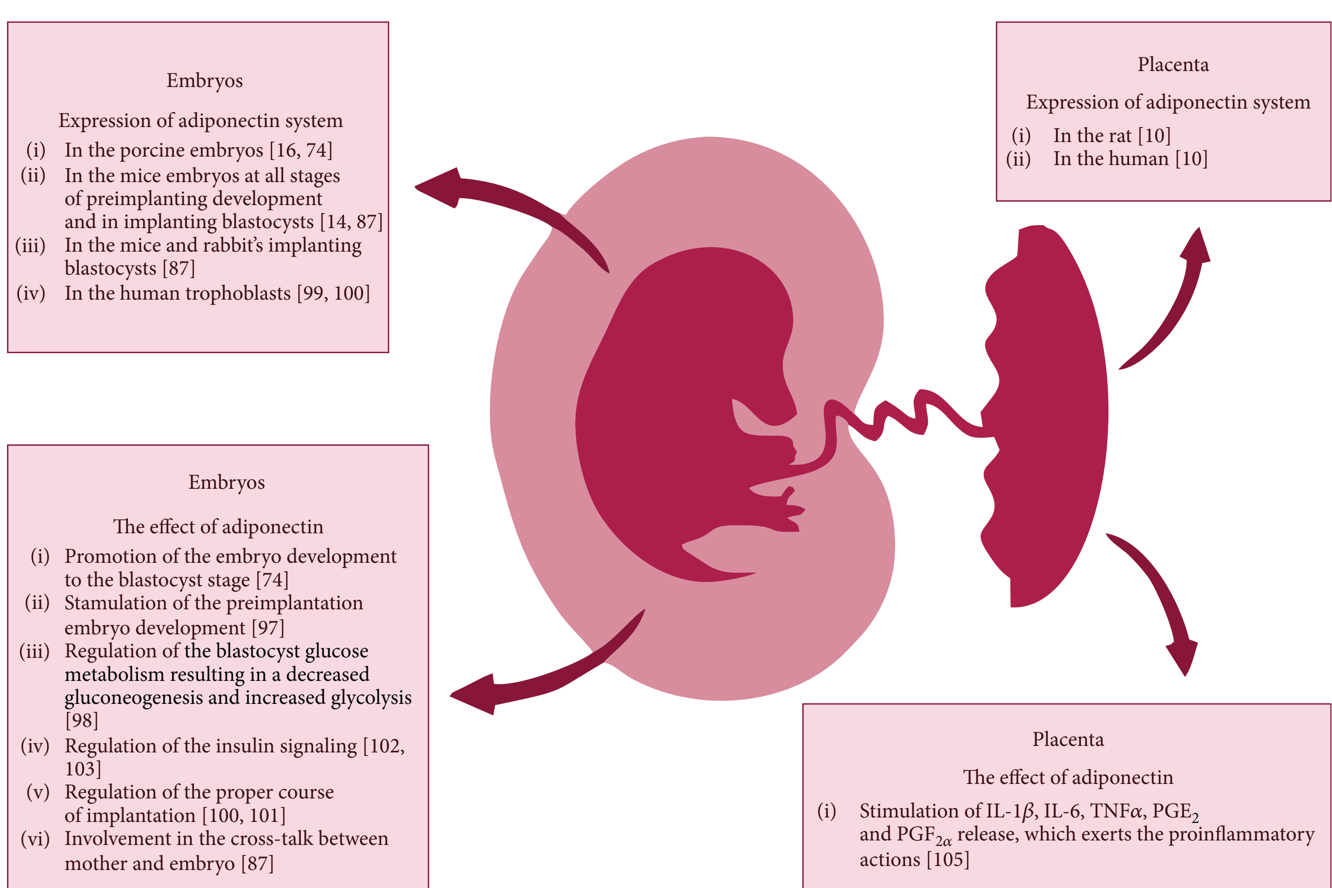

Figure 3: The effect of adiponectin on the embryos and placenta. The left side of the figure presents the expression of adiponectin system, as well as adiponectin effect on the embryos of different mammalian species. The right side of the figure presents the expression of adiponectin and its receptors and the hormone action in the mammalian placenta.

hormones on the adiponectin system was confirmed also in the cultures of human endometrial stromal and epithelial cell lines, in which E_2 and P_4 stimulated the expression of both AdipoRs' genes during the decidualization process [87].

Taken together, the data presented above indicates that adiponectin act as an important hormonal regulator in a number of processes occurring in the reproductive tract during both the oestrous cycle and early pregnancy, *via* its influence on, among others, steroid hormones and prostaglandin synthesis by the tissues.

4. Embryos and Placenta

Adiponectin system is suspected to take part in the maternal-foetus interactions. The elements of adiponectin system were found in early developing embryos of pig [16, 73], mouse embryos at all stages of preimplantation development [14], and rabbit and mouse-implanting blastocysts [86]. It was shown that adiponectin may promote embryo development to blastocyst stage in the porcine *in vitro* cultures [73]. For more, it was proven that adiponectin treatment resulted in doubling of mouse blastocyst formation, when compared to the control group, which indicates the regulatory role of the hormone in cell proliferation during the embryo development. In the mice *in vitro* blastocyst culture, the influence of adiponectin resulted in an increased proportion of embryos with high cell numbers, indicating that adiponectin may affect the development of the preimplantation embryo [96]. It is hypothesized that in early developing embryos, adiponectin, via AdipoRs', may downstream fatty acid oxidation and replete energy stores in the developing embryo before the implantation [14]. What is more, in rabbit blastocysts, adiponectin was found to enhance PRKA alpha 1/2 (PRKAA1/2) phosphorylation and decrease the expression of key regulator of gluconeogenesis, the phosphoenolpyruvate carboxykinase 2 (PCK2). It was indicated that *via* the phosphorylation of PRKAA1/2, adiponectin influences the glucose metabolism of blastocyst, which results in a decrease of gluconeogenesis and an increase in glycolysis [97].

Adiponectin system expression was confirmed also in the porcine and human trophoblasts [16, 98, 99]. However, human trophoblast cells expressed only AdipoRs' [98, 99] (Figure 3).

Adiponectin was found to act stimulatory on the trophoblast cell migration and invasion in the *in vitro* culture of human HTR-8/SVneo cell lines, which suggests that adiponectin may be a positive regulator of the early invasion process [99]. On the other hand, the adipokine was also found to exert an antiproliferative effect on human trophoblastic JEG-3 and BeWo choriocarcinoma cell lines, which points out that the hormone may act also as a regulator of trophoblastic cell proliferation and, in consequence, on the proper course of implantation [100]. Adiponectin was also found to attenuate insulin signaling in the primary human trophoblast cells, which, in result, inhibited insulin-stimulated amino acid transport. Those findings may have important implications in the pregnancy disorders linked with altered maternal adiponectin levels [101, 102] (Figure 3).

The gene expression of adiponectin and its receptors was described in the rat and human placenta [10]. Similar to the trophoblast, also in the placenta, adiponectin is supposed to play a role in adapting energy metabolism at the maternal-fetal interface. In the human placenta, adiponectin system was found to be regulated by the cytokines (including TNFα, IFN-γ, and IL-6) and leptin [103]. Adiponectin was also found to enhance the release of IL-1β, IL-6, TNFα, PGE_2, and $PGF_{2\alpha}$, exerting proinflammatory actions in human placenta [104] (Figure 3). The above findings suggest the existence of autoregulatory loop between proinflammatory cytokines and adiponectin in the placenta, which may be important for the proper growth and functioning of this organ.

5. Conclusion

Adiponectin, the adipocyte-derived hormone is an important factor taking part in the regulation of organism energy metabolism. Herein, the authors gathered available data concerning the inherence of adiponectin system in the structures responsible for the female reproductive system functioning and proving the regulatory role of adiponectin in these organs. Data presented above indicate that adiponectin is an important factor regulating the reproductive functions dependently on the actual metabolic status of animal and *vice versa*, regulating female metabolism according to the hormonal status of animal during both menstrual/oestrous cycle and pregnancy.

Conflicts of Interest

The author declares that there is no conflict of interests regarding the publication of this paper.

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