REVIEW

Seminal plasma adipokines: involvement in human reproductive functions

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ABSTRACT. Infertility, which increased worldwide over the past few decades, has recently been linked to obesity prevalence. Adipokines, produced by adipose tissue, could be the link between obesity and infertility. The association between circulating adipokines and female infertility has been extensively studied in the last ten years. However, the male aspect has been less investigated, although some adipokines are present in seminal plasma. We have attempted to analyze published studies that measured seminal plasma adipokines and their relationships with semen parameters. Apart from leptin, other seminal adipokines have rarely been studied. Indeed, leptin seems to have a differential role depending on its concentration in the seminal plasma. Thus, it could have a beneficial effect at lower concentrations but a deleterious effect at higher seminal levels. Although some studies are currently available, the roles of leptin and other adipokines in seminal plasma on sperm parameters and their consequences on male fertility remain to be clarified.

Key words: adipokines, semen, infertility, metabolic disorders

ADIPOKINES: A PUTATIVE LINK BETWEEN METABOLIC DISORDERS AND REPRODUCTIVE DYSFUNCTION?

For a long time, white adipose tissue (WAT) was considered as an organ that preferentially stores energy molecules such as triacylglycerols during energy excess and releases fatty acids in the periods of energy need. Over the last few decades, WAT has been proven to be more than just a tissue that stores fat; rather it is a metabolically dynamic tissue that also serves as not only an endocrine organ capable of synthesizing a number of biologically active compounds that regulate metabolic homeostasis, but also a large panel of other physiological functions [1]. Among these compounds, adipokines are a family of proteins mainly secreted by WAT, but also secreted into the peripheral blood by adipocytes as well as lymphocytes, macrophages and fibroblasts [2, 3].

The prevalence of male obesity in reproductive-age has nearly tripled in the past 30 years and coincides with an increase in male infertility worldwide [4]. Although it is generally thought that reproductive issues concern women, infertility affects men and women equally [5]. There is an increasing awareness that male obesity reduces sperm quality, in particular altering the physical and molecular structure of germ cells in the testes and mature sperm [4]. As obesity is associated with changes in the levels of adipokines in peripheral blood, it is conceivable that this disturbed secretion might be involved in the molecular mechanisms of obesity-related male infertility. Indeed, a recent study has demonstrated the presence of adipokines including adiponectin, chemerin, vaspin,

Abbreviations

WAT	white adipose tissue
IL	interleukin
TNF	tumor necrosis factor
PCOS	polycystic ovary syndrome
BMI	body mass index
IVF	in vitro fertilization
WHO	world health organization
MMP	mitochondrial membrane potential
ROS	reactive oxygen species
PR	progressive motility
NP	non-progressive motility
IM	immotility
VCL	curvilinear velocity
VSL	straight line velocity
VAP	average path velocity
DNA	deoxyribonucleic acid
WC	waist circumference

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leptin, resistin, progranulin, and visfatin in seminal plasma [3]. In addition, several cytokines are produced by adipose tissue (such as Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-α), and many of these contribute to local or systemic inflammation [6, 7]. Altogether, these results have pointed toward the indication that dysregulations of these adipokines could play a role to the mechanistic link between increased fat mass and impaired male fertility. It is well known that there are many risks associated with obesity that target the female reproductive function such as menstrual irregularity, polycystic ovary syndrome (PCOS), endometrial pathology, and infertility [8-11]. It is established that an increase in Body Mass Index (BMI) is associated with a decrease in oocyte and embryo quality in In Vitro Fertilization (IVF) patients and that embryos from women with overweight or obesity display phenotypic and metabolic abnormalities [8, 9, 12, 13]. Many studies have documented the tight interconnection between energy metabolism and female reproductive function and discussed the impact that metabolic status and related hormones such as adipokines could have on female fertility [8]. One study has even considered insulin and adipokines to be the most involved molecular players linking obesity and reproductive impairment [9]. For example, it has been shown that women with obesity have lower circulating levels of adiponectin, an important anti-inflammatory adipokine [14]. *In vitro* and *in vivo* studies have demonstrated that in the ovary, adiponectin stimulates steroidogenesis via granulosa cells [15, 16]. It was also reported that in women with PCOS, the concentration of adiponectin is impaired and associated with abdominal obesity and hyperandrogenism [17, 18]. These findings led us to believe that the decrease of adiponectin in women with obesity could partially explain the disruption of the reproductive function. It appears that adipokines could play an important role on the reproductive function. Their effects on female reproduction have been analyzed in many studies, but their impact on the male function and sperm parameters has yet to be determined. Several studies have looked into the different serum levels of adipokines and their impact on male and female reproductive function. For example, abnormal serum leptin levels were significantly associated with fertility disorders in males [19]. It was also demonstrated that serum leptin has a significant negative correlation with sperm concentration and motility, and a positive correlation with abnormal sperm morphology [20]. Very few studies have analyzed the presence of adipokines specifically in the seminal plasma and their possible effect on sperm parameters and general reproductive function. Thus, the aim for the present work was to review as many studies as possible and to propose several hypotheses on the impact of adipokines in the seminal plasma.

SPERM ABNORMALITIES IN PATIENTS WITH ABNORMAL BMI

Numerous studies have attempted to find a link between infertility and BMI, which is the most commonly used index to identify patients with obesity [21]. It has been shown that overweight and obesity are associated with an increased prevalence of azoospermia or oligozoospermia [22]. In 2004, Jensen *et al.* carried out one of the largest studies (1558 men) on BMI in relation to semen quality and reproductive hormones [23]. This study showed that a significantly lower sperm concentration and also a lower total sperm count were observed not only in men with obesity or overweight but also in men with underweight. This shows that there could be an ideal BMI for an optimal spermatogenesis. Indeed, patients with body mass within the ideal normal range had a higher sperm concentration and a lower percentage of abnormal spermatozoa. The concept of an ideal BMI range is not novel. Indeed, a study in 2010 demonstrated that, in 1.46 million white adults, underweight, overweight, and obesity are associated with an increase in all-cause mortality. All cause-mortality was found to be generally lowest for patients with a BMI of 20.0-24.9 [24]. In 2008, Hammoud *et al.* found among their 526 patients that the prevalence of a low progressively motile sperm count and low sperm concentration was greater with increasing BMI [25]. Another study performed on 794 patients in 2010 found a negative association between BMI and sperm mobility [26]. Furthermore, Qin *et al.* showed that the associations between BMI and semen quality were statistically significant even after an adjustment for reproductive hormones. Thus, reproductive hormones (such as testosterone) cannot fully explain the association between BMI and semen quality [27]. It has been established that WAT plays an essential role on the reproductive function. For example, it was demonstrated on female mice that removal of the ovary fat pad, the nearest adipose tissue to ovary, has adverse effects on fertility. The authors showed that the ovary fat pad-removed mice showed decreased fertility, less ovulated mature eggs, lower secretion levels of estrogen and FSH and higher levels of LH compared to control groups [28]. It was also reported that surgical removal of the epididymal white adipose tissue in hamsters decreases spermatogenesis [29]. This is why we focused our interest on adipokines: we hypothesize that abnormal adipokine profiles could explain the association between BMI and altered semen parameters and could represent important factors that interfere with the male reproductive function. Seminal plasma reportedly contains adiponectin at concentrations approximately 300 fold lower than serum [3] and concentrations correlate significantly between both sample matrices. In contrast, concentrations of progranulin and visfatin are 50 fold higher in the seminal plasma than that in the blood [3]. There was no correlation found for adipokines other than adiponectin between both sample matrices. This suggests a compartment-specific regulation of adipokines in the reproductive tract and peripheral blood. Thus, adipokines could exert a local function in the reproductive tract. However, this has yet to be determined.

DISORDERS OF SPERMATOZOA FUNCTIONS IN RELATION TO SEMINAL ADIPOKINES (TABLE 1)

An assessment of sperm quality based on the World Health Organization's guidelines [30] is usually performed to estimate the fertilization potential of male partners. The spermogram is the first semen diagnosis carried out when a patient is consulting for infertility problems. This test assesses multiple sperm parameters in order to conduct a thorough study of the semen. The sperm parameters

Table 1
Relationship between seminal adipokines and sperm characteristics

Sperm parameter	Motility	Volume	Concentration	Count	Vitality	Morphology	DNA frag.	MMP	ROS
Leptin	3 studies Negative (Glander *et al.* 2002, Guo *et al.* 2014, Ni *et al.* 2015) 1 study + in vitro : Positive (Lampiao & du Plessis 2008, Thomas *et al.* 2013) 3 studies No (Camina *et al.* 2002, Li *et al.* 2009, Leisegang *et al.* 2014)	Negative (Thomas *et al.* 2013) No (Leisegang *et al.* 2014)	Positive (Thomas *et al.* 2013) Negative (Ni *et al.* 2015) 2 No (Camina *et al.* 2002, Leisegang *et al.* 2014)	Negative (von Sobbe *et al.* 2003) 2 No (Thomas *et al.* 2013, Leisegang *et al.* 2014)	2 studies No (Camina *et al.* 2002, Leisegang *et al.* 2014) Negative (Wang *et al.* 2015)	3 studies No (Camina *et al.* 2002, Thomas *et al.* 2013, Leisegang *et al.* 2014)	2 studies No (Thomas *et al,* 2013, Leisegang *et al.* 2014)	No (Leisegang *et al.* 2014)	Positive (Wang *et al.* 2015)
Resistin	Negative (Moretti *et al.* 2014)	No (Moretti *et al.* 2014)	No (Moretti *et al.* 2014)	NS	Negative (Moretti *et al.* 2014)	No (Moretti *et al.* 2014)	NS	NS	NS
Adiponectin	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	NS	NS
Chemerin	Negative (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	NS	NS
Vaspin	No (Thomas *et al.* 2013)	Negative (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	NS	NS
Progranulin	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	Positive (Thomas *et al.* 2013)	No (Thomas *et al.* 2013)	NS	NS

No: no correlation, Positive: positive correlation, Negative: negative correlation, NS: not study.

reported in this paper are as follows: motility, volume, concentration, sperm count, sperm vitality and morphology. It is also possible to conduct further analysis of the spermatozoa, in order to carry out more in depth research, by examining DNA fragmentation, mitochondrial membrane potential (MMP) and the presence of reactive oxygen species (ROS).

MOTILITY

According to the World Health Organization's [30] 5th edition of "normal semen analysis", motility of each spermatozoon is graded as: progressive motility (PR), which includes spermatozoa moving actively either linearly or in a large circle, non-progressive motility (NP), which includes all other patterns of motility with an absence of progression, and immotility (IM) [30]. The systems that grade sperm movement must distinguish these different types of motility; the technique often used in studies is computer-aided sperm analysis [31]. As reported by the WHO, the lower reference limit for total motility (PR + NP) is 40% and 32% for progressive motility. CASA systems are capable of recording "sperm speed" variables such as curvilinear velocity (VCL), straight-line velocity (VSL), and average path velocity (VAP).

Numerous articles have reported significant correlations between certain adipokines and sperm motility, in particular between leptin and sperm motility, nevertheless many discrepancies were found. Indeed, Glander *et al.* found that semen leptin correlated negatively with progressive ($r=-0.46$; $p = 0.0005$) and straight-line velocity (VSL) ($r = -0.3$; $p = 0.029$) for 64 male partners of couples consulting for infertility problems [32]. Two other studies established a negative correlation between seminal plasma leptin and progressive motility ($r = -0.431$; $p<0.001$ [33] and $r = -0.234$; $p<0.01$ [34]). The first study by Guo *et al.* was carried out on 79 asthenozoospermic men and 77 normozoospermic men [33], the study by Ni *et al.* included 42 infertile patients with varicocele [34]. It was shown that the seminal leptin level of asthenozoospermic men was significantly higher than in the control group which suggests a potential link between leptin and sperm motility. On the other hand, Thomas *et al.* found a positive correlation between seminal leptin and progressive ($r = 0.27$; $p<0.01$) and total motility ($r=0.23$; $p<0.05$) for ninety-six male volunteers without spermatogenesis-associated diseases [3]. Completing this finding, a study was carried out in 2008 to investigate the *in vitro* effect of leptin on human sperm motility, acrosome reaction and nitric oxide production [35]. The results showed that leptin significantly increased total and progressive motility after a 1h, 2h or 3h incubation ($p<0.05$). It was also demonstrated that leptin increased acrosome reaction ($p<0.05$). Many studies performed either on 40 healthy men and 5 vasectomized [31], 24 human subjects with normal and subnormal semen parameters [36], or 23 males with obesity and 19 without obesity [37] were not able to establish any links between leptin and sperm motility. Li *et al.* also claim that leptin has no effect on sperm capacitation or acrosome reaction [36]. Overall, many studies have analyzed the effect of leptin on motility and the results are contradictory. However, it is possible to explain these differences by analyzing the seminal plasma leptin concentrations and this clarifies the different effects of leptin on motility. Indeed, for the studies that showed that leptin correlates negatively with sperm motility, it is noticeable that the patients with sperm abnormalities exhibited the highest mean concentrations of seminal leptin ([32]: 2.4 ng/mL total semen samples, 1.5 ng/mL normospermia group, 3.19 ng/mL pathozoospermia group; [33]: 3.75 ng/mL for normozoospermic men; 4.72 ng/mL for asthenozoospermic men; [34]: 1.79 ng/mL control group, 3.01 ng/mL varicocele preoperative group, 2.63 ng/mL varicocele 3 months post-operative group and 2.35 ng/mL varicocele 6 months post-operative group). On the contrary, the study that established a positive correlation between seminal plasma leptin and sperm motility, showed lower levels of semen leptin for the 96 male volunteers [3]. Indeed, the mean concentration of seminal leptin was 0.91 ng/mL for men with normal weight ($n = 41$) and 0.83 ng/mL for men with overweight or obesity ($n = 55$). In addition, it must be emphasized that these patients had no spermatogenesis-associated diseases. Moreover, the *in vitro* experiment was carried out on washed human spermatozoa from normozoospermic donors [35], which is in accordance with the results of Thomas' study. Finally, three studies found that seminal leptin did not correlate with spermatozoa motility. For two of these studies, the patients involved also presented low mean concentrations of seminal leptin (respectively 0.93 ng/mL and 0.95 ng/mL) [31, 36]. Leisegang *et al.* found high seminal leptin concentrations for patients with obesity and without obesity [37]. This is in contrast to the hypothesis that we have put forward; it is possible, however, that the number of subjects in this study could be too limited ($n = 42$) to detect a significant difference. Although sometimes contradictory, these findings suggest that when leptin is present in the seminal plasma at higher concentrations (>1.5 ng/mL, often in cases of spermatogenesis associated diseases), this adipokine could have an adverse effect on sperm motility. In contrast, at lower and possibly "physiological" concentrations, leptin could be beneficial for spermatozoa motility [3].

A smaller amount of studies have found significant correlations between other adipokines and sperm motility. For example, Thomas *et al.* found a positive correlation between seminal plasma progranulin and progressive ($r = 0.32$; $p<0.001$) and total motility ($r = 0.25$; $p<0.01$) and a negative correlation between semen chemerin and progressive ($r = -0.25$; $p<0.01$) and total motility ($r = -0.22$; $p<0.05$) [3]. Others discovered a significant negative correlation between resistin concentration in the seminal plasma and sperm progressive motility ($r = -0.2$; $p<0.001$) for 110 non-azoospermic men with a normal karyotype [7]. No correlation between seminal plasma concentrations of adiponectin and vaspin and progressive and total sperm motility were described [3]. Further studies concerning the other adipokines' effect on sperm motility are, however, still lacking.

VOLUME

The volume of the ejaculate is contributed mainly by the seminal vesicles and prostate gland, with a small amount from the bulbourethral glands and epididymis [30]. An accurate measurement of the volume is important for the

evaluation of semen, with a lower reference limit for semen volume of 1.5 mL.

One study has shown that there is a negative correlation between seminal plasma concentrations of vaspin ($r = -0.36$, $p<0.01$), leptin ($r = -0.34$, $p<0.01$) and semen volume [3]. On the contrary, Leisegang found no such correlation between seminal leptin and semen volume [37]. A study found no correlation between seminal plasma levels of resistin and semen volume [7], and another work established no correlation between semen concentrations of adiponectin, chemerin, progranulin and ejaculate volume [3]. Overall, it seems that contradictory results exist on the relationship between seminal adipokines (particularly for leptin) and semen volume. More studies are necessary in order to have a better understanding of the role of adipokines on semen volume.

CONCENTRATION

According to the World Health Organization, the concentration of spermatozoa in the semen is related to fertilization and pregnancy rates and is influenced by the volume of secretions from the seminal vesicles and prostate [30]. The lower reference limit for sperm concentration is 15×10^6 spermatozoa per mL. Semen containing a concentration of sperm lower than the reference limit is referred to as oligozoospermia; the term azoospermia refers to semen containing no spermatozoa in the ejaculate before and after centrifugation, and the ejaculate must be tested at least twice with a 3-month interval between examinations. Concerning the relationship between semen leptin and spermatozoa concentration, results are yet somewhat contradictory. Thomas *et al.* discovered a positive correlation between semen leptin and sperm concentration ($r = 0.24$, $p<0.05$) for ninety-six male patients without spermatogenesis-associated diseases [3]. By contrast, Ni *et al.* established a negative but low correlation between seminal plasma leptin and the concentration of spermatozoa in the semen ($r = -0.187$, $p<0.05$) of 42 infertile patients with varicocele [34]. Two other studies both found that there was no correlation between semen leptin and sperm concentration [31, 37]. Fewer results are available for other adipokines. Thomas *et al.* discovered a positive correlation between semen adiponectin ($r = 0.25$, $p<0.01$), chemerin ($r = 0.27$, $p<0.01$), progranulin ($r = 0.23$, $p<0.05$) and sperm concentration in almost one hundred spermatogenesis disease-free individuals [3]. Moretti *et al.* studied the seminal concentrations of resistin and found no correlation between semen resistin and sperm concentration [7]. Finally, Thomas *et al.* found no correlation between seminal vaspin and sperm concentration [3]. Altogether, these correlations studies between semen adipokine concentrations and sperm motility are scarce, and with discordant results for leptin. Functional studies are mandatory to identify a potential role of these factors on spermatozoa motility.

SPERM COUNT

The sperm count parameter, which is the amount of spermatozoa contained in an ejaculate, is calculated according to semen volume and sperm concentration.

In the study including patients without spermatogenesis-associated diseases, Thomas *et al.* found a positive correlation between seminal plasma concentrations of adiponectin ($r = 0.22$, $p<0.05$), progranulin ($r = 0.23$, $p<0.05$) and sperm count [3]. In contrast, they showed that there is no correlation between sperm count and semen levels of chemerin, vaspin and also leptin [3] which was confirmed by another study (Leisegang *et al.* 2014). Von Sobbe *et al.* studied the semen of 65 men with different andrological diseases and found that seminal leptin and sperm count correlated negatively ($p = 0.0001$) [38]. Indeed, semen leptin levels of patients with azoospermia due to hypergonadotropic hypogonadism were significantly elevated, while seminal leptin levels of patients with obstructive azoospermia and low-grade oligospermia were comparable to those of normospermic men. For the sperm count parameter, one out of the three studies found a significant inverse correlation with seminal leptin [38]. Interestingly, this study separated the patients according to different andrological diseases, and it was shown that for the groups with a "normal", or slightly altered (low grade oligozoospermia), or obstructive azoospermia, there is a lower mean seminal leptin concentration (0.21-0.54 ng/mL) compared to the groups with high grade oligozoospermia and non-obstructive azoospermia (1.09-1.19 ng/mL). The two other studies did not divide the patients into groups according to semen quality and no correlation was found [3, 37]. Overall, it seems that a higher concentration of seminal leptin is correlated to a lower sperm count.

VITALITY

Sperm vitality is determined by assessing the membrane integrity of the cells and the most commonly used method is the eosin-nigrosin test. This is a staining technique, indeed, nigrosin is used to increase the contrast between the background and the sperm heads (which makes them easier to visualize) and eosin stains only the dead sperm. Spermatozoa with red or dark pink heads are considered dead (damaged membrane), whereas spermatozoa with white heads are considered alive (intact membrane). This test can also provide a check on the motility evaluation, since the percentage of dead cells shouldn't exceed the percentage of immotile spermatozoa. The lower reference limit for vitality (membrane-intact spermatozoa) is 58%. The term necrozoospermia refers to semen containing a low percentage of live spermatozoa and a high percentage of immotile spermatozoa in the ejaculate [30]. Two studies used the eosin-nigrosin technique to determine sperm vitality [31, 37]. They concluded that seminal plasma concentrations of leptin have no correlation with sperm vitality. It must be noted however, that the number of subjects could be too limited (respectively $n = 45$ and $n = 42$).

Other techniques were used to determine cell vitality. Moretti *et al.* studied spermatozoa apoptosis and necrosis [7]. Apoptosis is a genetically regulated form of cell death, yet it has a role in biological processes, including embryogenesis, ageing, and many diseases [39]. Despite the physiological role of apoptosis, there is also a pathological side. Indeed, lots of diseases are associated with altered cell survival. Apoptosis has several characteristic

traits such as: decreased cell volume, presence of apoptotic bodies, discrete changes of organelle morphology, chromatin condensation, no rupture of the plasma membrane and no inflammatory response. Apoptosis is triggered by a physiological stimulus or an accidental stimulus with "low intensity" and targets one of few cells [40]. Necrosis, in contrast, is another form of cell death that concerns a larger group of cells and is triggered by an accidental stimulus with high intensity. Necrotic cells have an increased volume, a release of intracellular contents, flocculation of the chromatin, loss of integrity of organelles, rupture of the plasma membrane and an inflammatory response. Moretti *et al.* showed on one hundred and ten non-azoospermic men with a normal karyotype that there is a positive correlation between seminal plasma concentrations of resistin and spermatozoa apoptosis ($r = 0.21$, $p<0.05$) and necrosis ($r = 0.63$, $p<0.001$) [7]: this shows that there is a negative correlation between semen resistin and sperm vitality. Wang *et al.* also showed that, within groups of patients with varicocele or leukocytospermia, there is a positive correlation between seminal leptin and spermatozoa apoptosis [41]. Unfortunately, there was no statistical analysis test mentioned to complete this finding. Despite the lack of statistic tests, the number of subjects for this study was large: 74 varicocele patients and 70 leucocytospermia patients compared to 40 normospermic men. These findings generally suggest that seminal plasma resistin and leptin correlate negatively with sperm vitality.

MORPHOLOGY

The study of spermatozoa morphology is important since there is a typical appearance of potentially fertilizing, thus morphologically normal, spermatozoa. Indeed, by the application of certain criteria of sperm morphology, relationships between the percentage of normal forms and various fertility endpoints have been established [30]. The observation of sperm morphology consists of several steps: preparing a smear of semen on a slide, fixing and staining the slide, and examining the slide with bright field optics. The total number of morphologically normal spermatozoa in the ejaculate is obtained by multiplying the total number of spermatozoa in the ejaculate by the percentage of normal forms: the lower reference limit for normal forms is 4% (Kruger classification).

One study established that seminal plasma concentrations of adiponectin ($r = 0.28$, $p<0.01$) and progranulin ($r = 0.25$, $p<0.01$) positively correlated with total normomorphic spermatozoa [3]. Two studies found no correlation between semen levels of leptin and the total number of normomorphic spermatozoa [31, 37], and another discovered no correlation between semen resistin and sperm morphology [7]. Finally, Thomas *et al.* found no correlation between semen levels of chemerin, vaspin, leptin and spermatozoa morphology [3]. These results suggest that semen leptin has no effect on spermatozoa morphology. Numerous studies have established correlations between adipokines and spermogram parameters. In addition, further functional analysis of spermatozoa, such as DNA fragmentation and mitochondrial membrane potential (MMP), is considered as an important additional marker of male fertility. Oxidative stress is a result of increased levels of ROS above physiological levels, and it is known that an excessive presence of ROS has damaging effects on sperm parameters, particularly motility [42]. In order to analyze further the integrity of the spermatozoa, several articles studied the correlations between adipokines and DNA fragmentation, mitochondrial membrane potential, reactive oxygen species and the integrity of the acrosome.

DNA FRAGMENTATION

It is important to evaluate the potential effect of adipokines on DNA integrity, since impaired fertilization [43], altered embryo development [44] and lower implantation rates [45] have been reported in cases of increased sperm DNA fragmentation.

Thomas *et al.* determined the DNA fragmentation index with acridine orange and found that semen vaspin correlated positively ($r = 0.22$, $p<0.05$) with DNA fragmentation [3]. The same study found that there was no correlation between seminal plasma concentrations of adiponectin, chemerin, leptin, progranulin, and DNA fragmentation. Another study confirmed that semen leptin does not correlate with sperm DNA alteration [37]. Overall, it seems that seminal plasma concentrations of leptin have no effect on DNA fragmentation.

MITOCHONDRIAL MEMBRANE POTENTIAL (MMP)

Spermatozoal MMP was assessed in a study using a fluorescence microscope for analysis after staining sperm with a mitochondrial marker [37]. Sperm exhibiting a green fluorescence within their mid pieces were regarded as having disturbed MMP, those showing red fluorescence were regarded as having intact MMP. A percentage of spermatozoa with damaged mitochondria (MMP) higher than 36% is considered abnormal [37]. Damage to the sperm mitochondria function could negatively affect oxidative phosphorylation, reduce ATP synthesis and thus, energy available for motility [46]. The study concluded that there was no correlation between semen leptin and MMP but that both MMP and DNA fragmentation correlate with BMI and waist circumference (WC).

REACTIVE OXYGEN SPECIES (ROS)

High ROS production may cause peroxidative damage and loss of sperm function, as well as DNA damage, in both the nuclear and mitochondrial genomes. Reactive oxygen species are metabolites of oxygen (superoxide anion, hydrogen peroxide, hydroxyl and hydroperoxyl radicals, nitric oxide): when present in excess, they can initiate pathological changes by inducing oxidative damage to cellular lipids, proteins and DNA [30]. Increased levels of ROS have been correlated to decreased motility parameters (i.e. total motility, progressive motility and rapid motility), which were concluded to have adverse effects on fertility [42].

In 2015, Wang *et al.* studied the effect of seminal plasma leptin on sperm apoptosis [41] and discovered a positive correlation between semen leptin and ROS. The subjects consisted of 74 varicocele patients (VC), 70

leucocytospermia patients (LC), and 40 normospermic men who had successfully fertilized eggs for *in vitro* fertilization (IVF). In both the VC ($p<0.01$) and LC ($p<0.05$) groups, there was a significant positive correlation between seminal leptin levels and ROS. These findings suggest that, at high concentrations (LC group 2.72 ng/mL, VC group 3.2 ng/mL, control group 1.69 ng/mL), leptin is positively correlated with reactive oxygen species.

ACROSOME STATUS

The acrosome is a Golgi-derived exocytotic organelle located at the tip of the sperm head [47]. Spermatozoon must have properly formed acrosomes to be fully functional in the process of binding and penetrating the zona pellucida, the extracellular matrix surrounding the egg [48]. Men carrying mutations affecting the formation of the sperm acrosome are infertile or display subfertility [49, 50]. Thus, it is important to evaluate if the acrosome is intact or if it is damaged, in which case this may lead to fertility problems.

Thomas *et al.* showed that there is no correlation between seminal concentrations of adiponectin, chemerin, vaspin, progranulin, leptin, and the levels of intact acrosomes [3]. It seems that, for the 96 volunteers without spermatogenesis-associated diseases, the seminal concentrations of adipokines have no effect on acrosome integrity.

DISCUSSION

It is clear that adipokines play a crucial role on several physiological functions, and previous findings have illustrated the various effects of these peptides on male reproduction. Nevertheless, studies are still lacking, as only a few have analysed the specific correlations between adipokines and sperm parameters. It has been found that leptin plays an important role in reproduction; however, its role in male reproduction is less studied than in female reproduction. Leptin is a hormone secreted mostly by adipocytes in proportion to the fat stores of the body. Many studies have established the presence of leptin in the seminal plasma [3, 31, 32] and it was shown that human ejaculated spermatozoa secrete leptin [51]. More recently, it was discovered that a leptin receptor isoform is localized at the tail of the spermatozoa [52]. This receptor could be a target of leptin action in the male genital tract, which suggests that leptin in the seminal plasma may exert a local function on spermatozoa, possibly even on motility since the tail of the sperm, the flagellum, confers motility upon the spermatozoa. Indeed, the global analysis of many studies has led us to believe that seminal leptin could have several roles on sperm motility. When leptin is present in the seminal plasma at high concentrations, it seems that this adipokine is correlated negatively with sperm motility [32-34]. However, for lower, physiological concentrations, leptin either has no effect [31, 36] or could even have a positive effect on spermatozoa movement [3]. The same effect is observed for sperm concentration, it seems that for low semen concentrations, leptin is positively correlated with sperm concentration. However, the studies that include patients with relatively high seminal concentrations of leptin establish negative correlations between leptin and sperm concentration, sperm count and sperm vitality [34, 38, 41]. It was also observed that for high concentrations of seminal leptin, there is a positive correlation with levels of ROS.

Several studies were carried out on animal models. It was shown *via* a study on 10 ejaculates each from 10 buffalo bulls that the seminal leptin negatively correlated with sperm concentration [53]. Another study explored the relationships between varicocele rated spermatogenesis dysfunction and the expression of leptin in rats [54]. It was shown that, compared to control groups, the rats with varicocele had a significantly higher amount of seminal leptin (4.26 compared to 1.74 ng/mL), decreased sperm motility and a significantly lower sperm concentration, as it was observed in humans [34]. Spermatozoon function is affected by increased levels of ROS and nitric oxide levels, which is mediated by the immunomodulators tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) [42, 55]. The results of the study showing that human seminal plasma leptin levels are significantly correlated with spermatozoa apoptosis for varicocele and leucocytospermia patients [41] could suggest that seminal leptin, when in excess presence, might be an apoptosis promoting factor. Indeed, the two pathological groups showed increased levels of ROS, seminal leptin and spermatozoa apoptosis. Within the varicocele group (VC) seminal plasma leptin levels and seminal plasma ROS correlated positively. For the leucocytospermia group (LC), seminal plasma leptin, ROS, and TNF-α levels all had a positive correlation. It is thought that for the VC group, leptin could promote apoptosis via a pathway that is not immune-mediated since there was no correlation with TNF-α levels. In contrast, this study hypothesized that leptin induces apoptosis *via* TNF-α for the LC group. Overall, these findings suggest that there could be an "ideal" range for seminal leptin concentrations, where this adipokine has no adverse effect on sperm parameters, and perhaps even a positive effect. However, it is noticeable that increased semen leptin levels are correlated with an alteration of several sperm parameters.

In 2007, it was shown that leptin is overexpressed in the testes of patients with Sertoli cell-only syndrome thus might be linked to a reproductive dysfunction [56]. Indeed, a dysfunction of spermatogenesis is associated with an increase in leptin and leptin receptor expression in the testis. Put together with the previous results, it seems that an over expression of seminal leptin could be associated with a decline of sperm parameters which could eventually lead to fertility problems. In humans, the adipokine resistin is mainly expressed in mononuclear leukocytes, macrophages [57] and endothelial cells [58] under the stimulation of inflammation cytokines [59]. The presence of resistin in the seminal plasma has been described by several studies [3, 6, 7], but there is a lack of knowledge on the implications of this adipokine in seminal plasma and sperm parameters. One study showed that seminal plasma resistin levels are negatively correlated with sperm motility and vitality [7]. On the other hand, two others studies [3, 6] found no such correlation between resistin and sperm parameters. It is indeed difficult to conclude on the role of resistin in the semen but it has been shown that this adipokine is associated with inflammation biomarkers in human seminal plasma. Resistin levels in the seminal plasma correlate positively with the concentrations of

proinflammatory mediators such as elastase, IL-6 [6] and TNF-α [7]. It is well known that, in other cell types, resistin is involved in inflammation [59, 60], and it can target several human cells and enhance inflammatory and autoimmune processes [61]. The present studies have established that resistin, IL-6 and TNF-α are correlated negatively with sperm motility and sperm vitality. It is known that, during inflammation, the levels of cytokines and ROS increase, which can impair male fertility [62, 63]. Indeed, abnormal increases in ROS levels are associated with male infertility and can cause a decrease in sperm concentration, motility and morphology [64]. It is also noticeable that semen resistin concentrations increase in cases of smoking and leucocytospermia, which are associated with an increase of TNF-α and IL-6 levels as well as a decrease of sperm motility and normal morphology for leucocytospermia patients. Overall, these findings seem to suggest that resistin is an inflammation biomarker and, in pathological cases such as leucocytospermia, can be associated with an impairment of sperm parameters.

Adiponectin is a protein mainly produced by WAT. On the contrary to most adipokines, the production and secretion of adiponectin is inversely correlated to the severity of obesity. The presence of adiponectin in human seminal plasma has been demonstrated [3], as well as in certain animal models such as bulls [65]. Kasimanickam *et al.* demonstrated by immunolocalization the presence of adiponectin and its receptors in sperm [65]. They found that mean adiponectin and corresponding receptors (AdipoR1, AdipoR2) mRNA abundance was greater for average and high fertility bulls than for low fertility bulls. It was also shown that adiponectin protein was abundant in the tail region of sperm and that the protein concentrations of adiponectin and its receptors differed after capacitation. The study suggested that adiponectin has crucial roles in the organization of sperm structural and functional traits and might affect fertility. It was also shown a novel association of adiponectin with sperm motility in rams [66]. Specific studies looking into the correlations between adiponectin and functional characteristics of spermatozoa are still lacking, only Thomas *et al.* studied this aspect [3]. Overall, it seems that adiponectin has a positive correlation on sperm parameters particularly with sperm concentration, count and normal morphology. This suggests that adiponectin could have a beneficial effect on spermatogenesis, but more studies are necessary to confirm this effect.

Chemerin is a novel adipokine that regulates adipocyte development and metabolic function. In humans, plasma chemerin concentrations are correlated with body fat, glucose, lipid metabolism and inflammation [10]. It has been demonstrated that on *in vitro* rat models, chemerin has an inhibitory effect on steroidogenesis [17]. One human model study [3] showed that chemerin is negatively correlated with sperm motility and positively correlated with sperm concentration. Again, studies analysing the specific correlations between chemerin and sperm parameters are lacking, as well as studies concerning vaspin and progranulin.

Similarly, only one study managed to detect seminal concentrations of vaspin and progranulin and find certain correlations with sperm parameters [3]. Vaspin is a serine protease inhibitor highly expressed in visceral adipose tissue [67]. Circulating levels of vaspin were reported to be associated with impaired insulin sensitivity and obesity [68]. Thomas *et al.* showed that this adipokine correlated negatively with semen volume and positively with DNA fragmentation. This could suggest an inhibitory effect of vaspin on sperm parameters, but more data is necessary. Progranulin is an adipocytokine secreted particularly by adipose tissue and macrophages [67]. It has been found that patients with obesity and metabolic syndrome display increased circulating levels of progranulin, and it has been hypothesized that progranulin contributes to adipose tissue inflammation by macrophage recruitment [69]. Thomas *et al.* established a positive correlation between semen progranulin levels and sperm motility, sperm concentration, sperm count and normal sperm morphology [3]. Thus, it seems that this adipokine could exert a beneficial effect on sperm quality.

It is clear that leptin is the most studied adipokine; further experiments on resistin, adiponectin and chemerin should be done. Adipokines may be considered as regulators of the male reproductive function, but most studies performed on plasma and seminal fluid are only correlative. Experimental studies are necessary to establish the molecular mechanisms of these adipokines and how they influence sperm function. Studies should include experiments with different ranges of adipokines, in order to interpret the effect of abnormal concentrations on sperm parameters. In addition, it will be a challenge to establish whether locally produced adipokines, and/or circulating adipokines entering into the testes could contribute to modulate sperm functions. Altogether, these results and those of future studies could reveal an interesting pharmacological target which could positively impact fertility.

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