ISSN: 0067-2904

The Role of Resistine in the Fertility of Obese Infertile Males

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Abstract:

Fertility is a major need for all humans and obesity has been shown an important factor that may affect it. Resistin is one of the adipokines that have a relation with both fertility and obesity. The study aimed to measure the resistin levels in serum and seminal fluid of normal fertile (n=10), obese infertile (n=20) and Non obese infertile (n=10) individuals using the enzyme linked immunoassay method (ELIZA), Also conventional semen analysis was performed for each sample. Results revealed a highly significant increase ($p<0.01$) in serum resistine levels in normal fertile males (7.4 ng/ml) compared to both non-obese and obese infertile males (1.13, 3.01 ng/ml) respectively the same results for seminal plasma resistin levels (12.1 ng/ml) compared to (2.1, 5.35 ng/ml) respectively. The results give an indication that resistine may have an influence on the fertility of obese males and this need further investigations in the molecular field of research.

Keywords: Resistine, Fertility of males, Obesity.

دور الرزستين في الخصوبة لدى الذكور الذين يعانون السمنة

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الخلاصة:

الخصوبة هي حاجة رئيسية لكل البشر والسمنة اظهرت ان لها دورا فاعلا في التاثير على الخصوبة. الرزستين هي احد انواع الحركيات التي لها علاقة مع كل من الخصوبة والسمنة. هدفت الدراسة الى قياس مستوى الرزستين في كل من مصل الدم وبلازما السائل المنوي لدى 10 ذكور طبيعيين و 20 ذكر غير خصيب يعاني السمنة و 10 ذكور غير خصيبين لا يعانون السمنة، باستخدام تقنية التحري المناعي الممتز المرتبط بالأنزيم (ELISA). كما تم اجراء الفحص المنوي التقليدي لكل عينة سائل منوي ولكافة المجاميع . اظهرت النتائج زيادة معنوية في مستوى الرزستين في عينات المصل لمجموعة الذكور الطبيعيين(7.4ng/ml) مقارنة مع كل من مجموعة غير الخصيبين الذين يعانون من السمنة (1.13 ng/ml) وغير الخصيبين الذين لا يعانون من السمنة (3.01 ng/ml)، ($p<0.01$) وعدم وجود فرق معنوي بين مجموعتي غير الخصيبين ضمن عينة المصل. اظهرت النتائج ايضا زيادة معنوية في مستوى الرزستين في عينات بلازما السائل المنوي لمجموعة الذكور الطبيعيين (12.1 ng/ml) مقارنة مع كل من مجموعة غير الخصيبين الذين يعانون من السمنة (5.35ng/ml) وغير الخصيبين الذين لا يعانون من السمنة (2.1 ng/ml) ($p<0.01$) مع وجود زيادة معنوية بين مجموعتي غير الخصيبين ضمن عينة السائل المنوي($p<0.01$). هذه النتائج تشير الى ان الرزستين له علاقة بخصوبة الرجال ممن يعانون السمنة مما يحتاج الى دراسات اخرى على المستوى الجزيئي.

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Introduction:

Obesity is an increasing health problem today because it cause different complications such as, atherosclerosis, depression, orthopedic diseases, type 2 diabetes mellitus, and infertility. Recently, a correlation between obesity and male infertility has been widely discussed [1]. Normal weight men have normal sperm counts [2], compared to obese men. Adipokines which are known to be proteins secreted mainly by adipose tissue, could be related to the molecular mechanisms of obesity involved in male infertility [3]. Adipocytes as well as lymphocytes, leucocytes, trophoblasts, and fibroblasts [4, 5] secrete adipokines into the peripheral blood, which has been proved to effect especially carbohydrate metabolism, satiation, and subclinical inflammation [6]. In the last several years, leptin, adiponectin, resistin, has been revealed to be main adipose tissue-related players in central and peripheral actions [7]. Leptin and resistin are presented in the male reproductive tract and may be important in the pathogenesis of impaired spermatogenesis [8]. Serum adipokines could be transferred into or expressed in the male reproductive tract and could have a direct or indirect influence on the maturation and functionality of spermatozoa and [9] that obesity-associated changes in adipokine levels could be directly linked to male infertility. To test these hypotheses, we performed an observational study on the serum and seminal plasma of normal weight-fertile, obese infertile and non-obese infertile donors to investigate the association of resistine with the main functional parameters of spermatozoa.

Materials and Methods:

1) Study Population: This study was carried out at Al-Samaraiy hospital from March 2014 until August 2015. Twenty obese infertile males (who their couples had no history of pregnancy or abortion for more than one year from marriage with regular unprotected sex), were recruited in this study, along with two control groups; the first: Healthy fertile males, n=10 and the second: Non-obese infertile males, n=10.

The ages of all study groups ranged between (25-45) years, with a mean of 34 ± 7.1 years.

2) Samples Collection : two kinds of samples were collected through this study:

a. Serum samples: Blood was drained from each individual in a volume of 5 ml by vein puncture using a disposable syringe, then transferred to plane plastic tubes and left to clot for 30 min. at room temperature, then centrifuged at 3000 r.p.m for 5 min. the serum was separated to clean sterile Eppendorf tubes and frozen immediately at -20 C^o for further investigations. Serum levels of resistin was determined using enzyme linked immunosorbent assay (ELISA) (SPI bio Company, France) in all study groups.

b. Semen plasma: semen samples were taken from each subject by masturbation after 3-5 days of abstinence. Conventional semen analysis was performed for each sample according to the protocol of WHO [10]. After incubation and liquification period (30-60 min.) semen plasma was collected by centrifugation of semen for 15 min. at 3000 r.p.m and the supernatant was then transferred to clean sterile Eppendorf tubes and frozen at -20 C for further investigations.

3) Body Mass Index (BMI): To evaluate the weight of each individual of the study group the BMI was used to determine whether the male recruited in the study was obese or had normal weight by using this formula :

$$\textbf{BMI} = \frac{\textbf{body weight kg}}{\textbf{m}^2} = \textbf{kg/m}^2$$

If the BMI ranged between (23-29) it is considered as a normal weight, If > 30 considered obese.

Results and Discussion:

Obesity is one of the most important factors that contribute to infertility, therefore in this study the contributing males were grouped into three groups depending on their BMI and fertility: Normal fertile, Non-obese infertile and obese infertile, Table-1.

Table 1- Parameters of study groups

Parameters	Mean ± SD		
	Control n=(16)	**Obese infertile n= (16)**	**Non-Obese infertile n= (16)**
Age (yr.)	34 ± 9.0	31± 8.0	32 ± 8.5
BMI (kg/m^2)	24.1 ± 3.21	32.6 ± 8.1*	25.9 ± 3.49

***P<0.01**

Seminal fluid analysis was performed on all specimens of semen taken from all groups, there was a significant increase in the percentage of active sperms 41.3% in fertile group compared to both

infertile groups (22%,15.6%) respectively at ($p<0.01$). There was also a significant increase in the percentage of dead sperms in both obese and non- obese infertile groups (62.2%, 58.0%) respectively among the normal fertile group (21.5%) at ($p< 0.01$). As well as a significant increase in the total sperm count of sperms/ml of fertile group $58.2*10^6$ /ml compared to the two other infertile groups (23.6, 15.8 * 10^6 /ml) at ($p<0.01$). From the previous results it is obvious that sperm status has disability to be fertile in both non-obese and obese infertile males, which in fact confirm their infertility, Table-2.

Table 2- Semen parameters of study groups

Semen parameters	Mean ± SD		
	Fertile	Non-Obese infertile	Obese infertile
Volume (ml)	2.8 ± 0.64	2.04 ± 0.70	2.2 ± 0.81
pH	7.9 ± 0.11	8.01 ± 0.12	8.1 ± 0.21
Motility:			
Active %	41.3 ± 16.1*	22 ± 15.8*	15.6 ± 10.4
Sluggish %	28.2 ± 11.42	20.0 ± 13.4	22.2 ± 13.8
Dead %	29.5 ± 12.12	58.0 ± 25.5*	62.2 ± 26.1*
Total sperm count Million/ml	58.2 ± 25.2*	15.8 ± 8.4*	23.6 ± 10.2
Morphology:			
Normal %	55.19 ± 23.2	65.1 ± 28.1	52.2 ± 20.6
Abnormal %	44.78 ± 21.4	34.9 ± 18.3	47.8 ± 23.1

***p< 0.01**

Resistine levels were measured in two kinds of specimens: serum and seminal plasma. Results showed a significant increase in serum resistine levels in normal fertile males (7.4 ng/ml) compared to both non-obese and obese infertile males (1.13, 3.01 ng/ml) respectively at ($p<0.01$), Figure-1, Table-3. The same results for seminal plasma resistine levels (12.1 ng/ml) compared to (2.1, 5.35 ng/ml) respectively, Figure-2, Table-3.

Table 3-Resistine levels in serum and semen plasma of study groups.

Resistine level ng/ml	Mean ± SD		
	Fertile	Non-Obese infertile	Obese infertile
Serum	7.4 ± 3.5 *	1.13 ± 1.81	3.01 ± 0.81
Seminal plasma	12.1 ± 5.1*	2.1 ± 1.15	5.35 ± 2.3*

***P < 0.01**

Resistin belong to the family of cysteine-rich peptide called resistin-like molecules. In rodents serum resistin levels are increased in obesity, while resistin mRNA levels in adipose tissue are reduced [11], it is also abundantly expressed in adipose tissue and has been linked to reduce insulin tolerance, via increased hepatic glucose production [12] Further more resistine decreases glucose uptake by adipocytes and skeletal muscles indicating that miscle, adipose tissue and liver contribute to impaired glucose sensitivity.

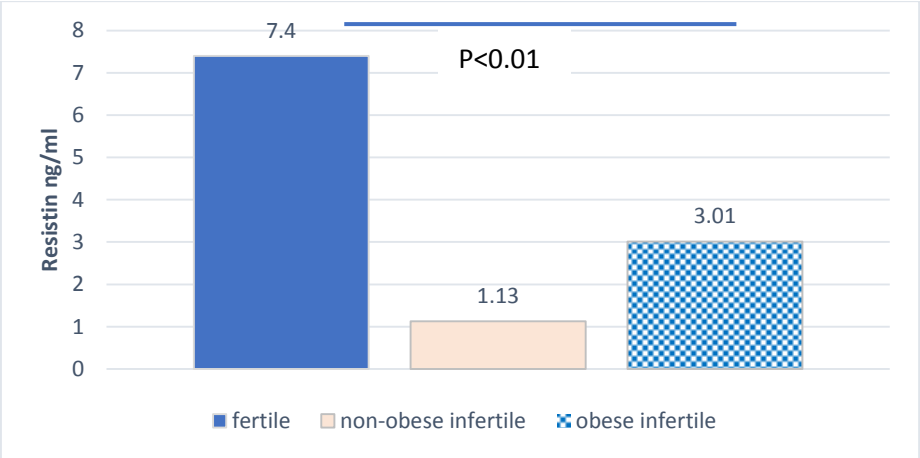

Figure 1- Resistine level in serum of study groups.

In contrast, resistin mRNA expression in human adipocytes is comparably low [13]. Although resistin altered proliferation of cultured human adipocytes [14], it didn't affect glucose uptake, which raises the possibility that human resistin is not directly involved in glucose homestasis in adipocytes. Resistin is however highly expressed in macrophages and monocytes [15], suggesting it may influence insulin resistance via effects of inflammation. Therefore, in rodents the direct role of resistin in obesity and glucose utilization is more apparent than in humans, where it may be related to the functioning of adipose tissue rather than insulin resistance.

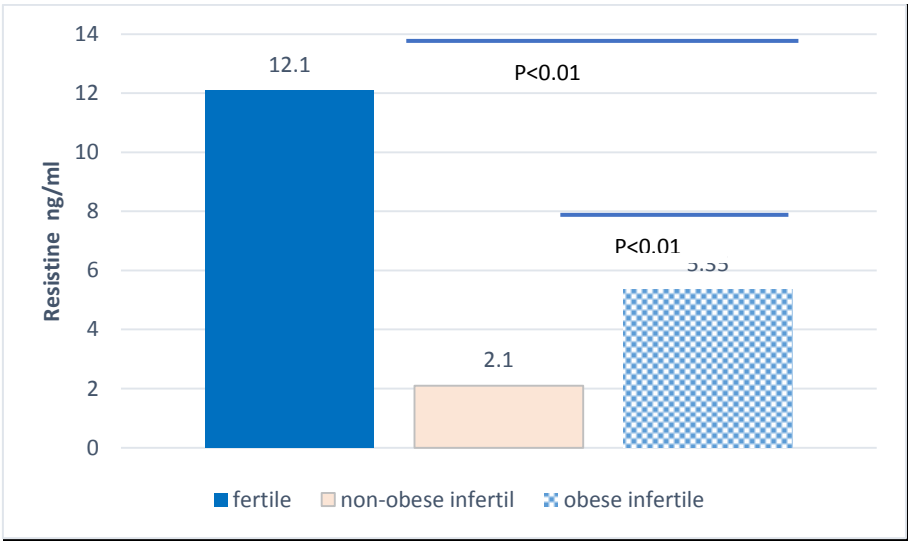

Figure 2- Resistine levels in semen plasma in all study groups

Resistin levels have been linked to obesity and lipid and glucose abnormalities in some studies [16] although others have not found such relation [17]. Obese men are 3 times more likely than normal healthy men of normal weight to have a sperm count fewer than 20 million/ml, an indicator of oligospermia [18]. A lower sperm concentration was observed in not only obese and overweight males but also in males who were significantly underweight. This can serve as an indication that there may be an ideal range of BMI for normal spermatogenesis. Subjects whose BMI was within normal range showed a higher sperm concentration as well as a higher total sperm count and lower percentage of abnormal spermatozoa [19].

Resistin inhibits adipogenesis whereas resistine deficiency increases body weight and fat and improves insulin sensitivity [20], thus resistine has profound effects on energy and glucose metabolism. It was found that loss of resistin in mice increase body weight and fat by decreasing energy expenditure [21].

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