

## Relation between Novel Markers with Sperm Quality in Obese Rats

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### Abstract

**Aims:** Obesity and inflammation stemmed from obesity basically affects fertility and male reproductive system by its negative effect on erectile dysfunction and semen variables. Novel factors could be used to determine the fertility and infertility. This study was conducted to evaluate the relation between novel markers with sperm quality in obese rats.

**Materials & Methods:** Animals were grouped into 2 groups including normal group received normal rat pellet diet for 12 weeks of intervention, while other group received a high fat diet. The serum concentrations of IL-1 $\beta$ , IL-6, IL-3, TNF- $\alpha$ , sialic acid, CRP, haptoglobin and fibrinogen were measured. Sperm motility was assessed.

**Findings:** Results showed that progressive motility and non-progressive motility and immotile were respectively lower and higher in obese rats in comparison to control groups ( $P < 0.05$ ). It was observed positive correlation between inflammatory factors and immobility and negative correlation between progressive motility with inflammation ( $P < 0.05$ ).

**Conclusion:** Pro-inflammatory factors had significant correlation with sperm quality and could be used as markers for infertility.

**Keywords:** Fibrinogen; Inflammatory Factors; Obese Rats; Sialic Acid

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## Introduction

Obesity has been known as a common metabolic disorder that occurs due to imbalance between energy consumption and expenditure. It has been known as one of major problems for healthiness [1]. It has been known to have effects on lipid metabolic processes such as lipogenesis and lipolysis [2]. Obesity basically affects fertility and male reproductive system by its negative effect on erectile dysfunction and semen variables [3]. It has been known to be higher infertility in obese men [4]. Obesity has been known to have stimulator effects on reproductive in male individuals that could be attributed to increased reactive oxygen species (ROS) [5].

Infertile men have been shown high levels of ROS on semen that could be attributed to increased level of pro-inflammatory cytokines and leucocyte invasion in their semen [6]. On the other hand, inflammation is known as a natural host response against microbial attack or tissue damage that restores tissue vasculature and functions [7, 8]. It has been shown that inflammation increased spermatogenic arrest and prevented processes of sperm maturation [4]. Inflammation specifically influences spermatocyte and spermatids but no spermatogonia [9]. Stress oxidative influences lipid parameters and obese individuals experience hyperlipidemia. Lipids have been known significant role in the functional activity of sperm cells [10, 11]. Sperm viability, maturity, capacitation and fertilization are influenced by lipid parameters [12]. Phospholipids and cholesterol are known as important variables of human plasma membranes and they are needed for membrane permeability, fluidity and capacitation [13]. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is one pro-inflammatory cytokine in border line of inflammation process. Interleukin-1 $\beta$  (IL-1 $\beta$ ) as other pro-inflammatory cytokine stimulates neutrophils into region of infection [14]. Nuclear factor-kappa B (NF- $\kappa$ B) increases production of TNF- $\alpha$  and IL-1 $\beta$  [14]. C-reactive protein (CRP) is one inflammatory marker that is associated with increased systemic inflammatory response syndrome [15]. Sialic acid has been reported as inflammatory cytokine that their levels are increased during inflammation [16]. Haptoglobin (Hp) is other factor that scavenges hemoglobin released into circulation by hemolysis or normal red blood cell (RBC) turnover [17]. So far, no study has been conducted to evaluate the relation between the mentioned markers and fertility in obese animals. This study was thus conducted to assess the relation between novel markers with sperm quality in obese rats.

## Material and Methods

### Animals

A total number of 36 male Wistar rats (6 weeks of age, 210 $\pm$ 10 g) were acclimatized for one week before trial and maintained on the basis of the animal welfare laws. All the animals were kept in an optimal temperature (25 $\pm$ 1 °C), and humidity (55 $\pm$ 5%) and illumination period (12 h L and 12 h D) were kept in the experimental period. Standard pellet was prepared from Javaneh Khorasan Company-Iran and cholesterol, cholic acid, palm oil were purchased for preparation of the high fat diets. Animals received *ad libitum* water and feed. Animals were grouped into 2 groups (n=18) and each group was divided into 3 sub-groups. Groups were included: The normal group received normal rat pellet diet for 12 weeks of intervention, while other group received a high fat diet. Food consumption and body weight were evaluated in days 1, 42 and 84. In the end of the trial, animals were sacrificed after an overnight fast, and their blood samples were stored for evaluation of sera.

### The serum concentration of pro-inflammatory cytokines

The serum concentrations of IL-1 $\beta$  (Abcam, No. Ab197742), IL-6 (Abcam, No. Ab100713), IL-3 (Abcam, No. Ab113345), TNF- $\alpha$  (Abcam, No. Ab6671), sialic acid (Abcam, No. Ab83375), CRP (Cayman Chemical, No. 10011236), haptoglobin (Abcam, No. Ab108856) and fibrinogen (AssayMax) were measured as recommended by producer Companies. The serum concentrations of triglycerides and cholesterol were assessed by commercial kits (Pars Azmoon, Tehran-Iran).

### Sperm parameters

In the end of trial, following euthanasia, the right vas deferens was gathered. Sperm were collected by one syringe and needle by using internal rinsing with 1.0 mL of modified HTF medium (Human Tubal Fluid, Irvine Scientific) in 34°C. A Makler counting chamber heated up to 34°C was included with a small aliquot of sperm solution. Assessment of sperm motility was conducted by the one person in all the trial and calculated by visual estimation (100 spermatozoa per animal, in duplicate) under a phase-contrast microscope (Leica DMLS) in 200X magnification. Spermatozoa were grouped as immotile, motile lack of progression and motile containing progressive movement. Sperm were also removed from the left vas deferens using internal rinsing by 1.0 mL of saline formula by a syringe and needle. To evaluate the sperm morphology, smears were made in histological slides that were left to dry for 90 min and observed in a phase-contrast microscope (400 $\times$  magnification) [18], and 200 spermatozoa were assessed per rat. Morphological abnormalities were classified into two general categories as reported by others [19].

### Statistical Analysis

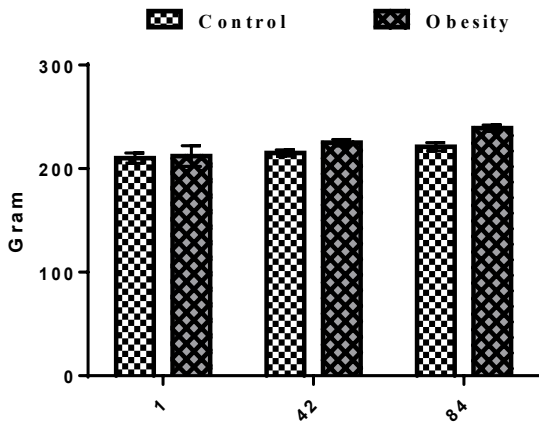
Results were expressed as mean  $\pm$  SD. All the analyses were conducted by T-test and correlation

was conducted by Pearson correlation. Figures were illustrated Graph Pad Prism.

**Findings**

**Body weight**

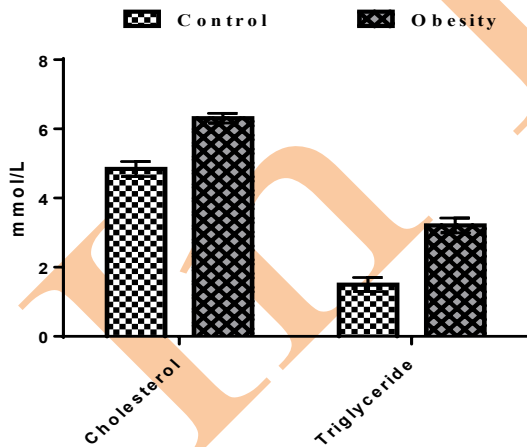
Results in Figure 1 showed that body weight was not influenced by experimental treatments in day 1 ( $P>0.05$ ), but body weight was significantly higher in rats fed with high fat diets in comparison to control group in days 42 and 84 ( $P<0.05$ ).



**Figure 1)** Effects of obesity on body weight in the different days in the rats

**Lipid profile**

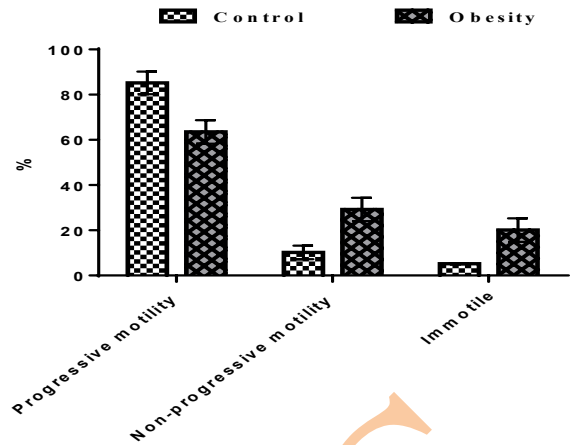
The serum concentrations of cholesterol and triglycerides were significantly higher in obese rats in comparison to control group (Figure 2) ( $P<0.05$ ).



**Figure 2)** Effects of obesity on the serum concentrations of cholesterol and triglycerides in the rats

**Sperm quality**

Our findings for sperm quality are shown in Figure 3. Results showed that progressive motility was significantly higher in control group in comparison to obese rats ( $P<0.05$ ). The non-progressive motility and immotile were significantly higher in obese rats in comparison to control groups ( $P<0.05$ ).



**Figure 3)** Effects of obesity on sperm quality in the rats

**Inflammatory factors**

Effects of obesity on inflammatory factors are shown in Table 1 .

As results show, inflammatory factors were significantly higher in obese rats in comparison to control rats ( $P<0.05$ ). There was negative correlation between progressive motility and also positive correlation between non-progressive motility and immotile with inflammatory factors ( $P<0.05$ ) (Table 2).

**Table 1)** Effects of experimental treatments on inflammatory markers (mg/dl)

Parameters	Control	Obesity	P
IL-1β	4.22±0.22	8.10±0.32	***
IL-6	3.25±0.17	5.41±0.11	***
IL-3	7.53±0.13	10.25±0.21	***
TNF-α	6.17±0.13	9.10±0.12	***
Sialic acid	1.15±0.13	3.53±0.32	***
CRP	2.31±0.11	4.12±0.11	***
Haptoglobin	0.95±0.15	2.20±0.11	***
Fibrinogen	531.21±32.25	785.21±12.21	***

\*\*\* show significant differences in per row.

**Table 2)** Correlation between inflammatory markers and sperm quality in obese rats

Parameters	Progressive	Non-progressive	Immotile
IL-1β	*** (-)	*** (+)	*** (+)
IL-6	*** (-)	*** (+)	*** (+)
IL-3	*** (-)	*** (+)	*** (+)
TNF-α	*** (-)	*** (+)	*** (+)
Sialic acid	*** (-)	*** (+)	*** (+)
CRP	*** (-)	*** (+)	*** (+)
Haptoglobin	*** (-)	*** (+)	*** (+)
Fibrinogen	*** (-)	*** (+)	*** (+)

**Discussion**

The diets used for obesity were efficient to promote obesity, as highlighted by enhanced body weight. Increased body weight could be attributed to hyperlipidic diet. It has been accepted that consumption of high-fat diets raises oxidation. Increased oxidation enhances lipid deposition, i.e. lipid profile, which increases body weight [20, 21]. Our findings showed increased lipid profile in obese

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rats which confirm obesity. Increased oxidation during obesity could be attributed to oxidation that increases lipolysis and in turn increased lipid profile. Unfortunately, we did not evaluate oxidation parameters but our findings for lipid parameters confirm obesity.

With regards to sperm quality, it was negatively influenced by experimental treatments. Sperm motility has been known as one of the most common variables used in the assessment of sperm quality [22-24]. Sperm motility is obtained during sperm transition by the epididymal duct [25-29]. Previous studies have shown decreased motile sperm without changes in testosterone levels [30]. Morphological changes in obese animals show a high probability that obesity adversely influences spermatogenesis. It has been accepted that spermatogenesis is influenced in males with extreme obesity [31]. Our findings confirmed adverse effects of obesity on sperm quality.

Increased inflammatory markers were observed in obese rats. Adipose tissue produces TNF- $\alpha$  and interleukin that play an essential role in energy regulation.

The ROS formed during inflammation is well thought-out as a factor, and amplified mitochondrial ROS production may have significant role in pathogenesis of obesity [32]. Metabolic inflammation is a major factor of obesity [33], and inflammatory signaling which could considerably influence lipid metabolism in the liver [34]. TNF- $\alpha$  initiates inflammation process, then IL-1 $\beta$  as other pro-inflammatory cytokine calls neutrophils into region of infection [14]. CRP increases inflammatory responses [15]. Sialic acid and Hp increases during inflammation [16, 17]. We observed relations between inflammatory markers and sperm quality. Inflammation has been reported as a natural host response against microbial attack or tissue damage that restores tissue vasculature and functions [7,8]. Inflammation increased spermatogenic arrest and inhibited processes of sperm maturation [4]. Inflammation specifically influences spermatocyte and spermatids but no spermatogonia [9]. So far, studies have not been investigated relation between these factors with sperm quality. We could not find any study showing relation between sperm quality and pro-inflammatory factors.

#### Conclusion

In conclusion, there was positive relation between obesity and lipid profile. Results also showed increased serum concentrations of IL-1 $\beta$ , IL-6, IL-3, TNF- $\alpha$ , sialic acid, CRP, haptoglobin and fibrinogen in obese rats. Increased factors was correlated with decreased sperm quality. These novel factors could be used for diagnosis of sperm quality in infertile patients.

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