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RESEARCH PAPER

Impact of Diabetes and obesity on Human Fertility and Semen Quality

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A B S T R A C T:

The present study was done to assist the effect of obesity and diabetes on semen quality and fertility of adult males in Erbil city. For this purpose, one hundred twenty adult males were used in this study. The subjects were divided into four groups. The control group included 30 healthy males, the obese group included 30 males with BMI ≥ 30. The diabetes group included 30 males with diabetes mellitus type 2 (T2 DM)and obese and the diabetes group included 30 males with both BMI ≥ 30 and T2 DM. Semen analysis and sex hormones were determined to evaluate semen quality and fertility. The incidence of abnormal viscosity in patient groups is significantly higher than that of the control group. Semen volume, sperm concentration, normal morphology, total motility, and grade activity in the control group is significantly higher than that of the other 3 groups. Lower sex hormones LH, FSH, and testosterone were recorded in a group of obese with diabetes group. A lower concentration of the serum malondialdehyde (MDA) is recorded in the control group. Despite the decreasing of the semen volume, sperm concentration, normal morphology, and motility in the patient's groups, all these values are not threshold and within the normal ranges which were recommended by World Health Organization (WHO). In our results concluded that obesity and diabetes have a minor effect and not detrimental to the fertility of males.

KEY WORDS: Semen quality; Fertility; Sex hormones; Obesity; Diabetes; Spermatozoa.
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1.INTRODUCTION:

Infertility remains a global public health problem and a major clinical issue concern which affects 15% of reproductive-age couples (Mascarenhas *et al.*, 2012, Jiang *et al.*, 2015). Low quality of semen is well known as a large disorder causing male infertility (Radwan *et al.*, 2016, Levine *et al.*, 2017).

An estimated 70 million people are Worldwide, couples suffer subfertility or infertility, and around 40% of the reasons for the cases are a male (Légaré *et al.*, 2014, Salas-Huetos *et al.*, 2017).

A rigorous and comprehensive meta-analysis recent of studies carried out between 1973 and 2011, that was reported a decrease of the sperm counts by more than 50%. The same goes for several other studies that have also recorded a continuous decline in semen quality (Li *et al.*, 2016, Sengupta *et al.*, 2017).

Now the epidemic of obesity is spreading across the world and according to the latest World Health Organization estimates that 650 million people worldwide were obese and more than 1.9 billion were overweight in 2016 (Moussa *et al.*, 2016). Obesity has been related to an increased risk for many medical conditions include cardiovascular disorders, osteoarthritis, diabetes, liver, kidney disease, depression, and infertility (Bieniek *et al.*, 2016, Dubeux *et al.*, 2016). Recent studies have looked at the relationships between abnormal BMI and semen quality but contradictory findings remain (Sermondade *et al.*, 2013, Eisenberg *et al.*, 2014, Tsao *et al.*, 2015). Chavarro *et al.* (2010) for example, proposed that the BMI of approximately 35 kg/m^2 was associated with lower ejaculate volume and sperm count among 483 men attending the clinic for infertility. Wang *et al.* (2017a) also observed an increase in BMI appeared to be linked to sperm count among 2384 subfertile men in northern China. In contrast, 31 studies have a pooled meta-

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analysis, proved there were no significant associations between the BMI and volume of semen, and sperm concentration (MacDonald *et al.*, 2010).

Diabetes mellitus (DM), a chronic non-communicable illness, was deemed one of the most important health risks, impacting 9% (422 million) of the world's population as of 2014. Diabetes mellitus is considered to cause multiple medical complications; impotence-based male infertility, retrograde ejaculation, and hypogonadism are not commonly accepted as one of these. In recent years, definitive findings from multiple research have disputed the views that DM has adverse effects on the male reproductive system (Omolaoye and Du Plessis, 2018).

Of the 21 research studies in a total of 1218 cases and 1171 controls, the results indicated that semen volume, sperm concentration, total sperm motility, progressive sperm motility, and normal sperm morphology were significantly lower in DM patients than in nondiabetic controls (Zhu *et al.*, 2017). Reportedly, DM affects male reproductive function through various pathways and mechanisms. Several studies have studied and identified the adverse effects of reactive oxygen species and the subsequent production of oxidative stress that occurs due to DM (Omolaoye and Du Plessis, 2018).

Due to no data about the effect of obesity and diabetes on male fertility in Erbil city. The present study was done and aimed to evaluate the influence of obesity and diabetes type 2 on semen quality and fertility of adult males by performing of semen analysis, and measurement of sex hormones and oxidative stress in the serum of patients groups.

2.MATERIALS AND METHODS

2.1Subjects

The study included 120 male persons and divided into four groups:

1- Control group: Included 30 healthy males.

2- Obese group: included 30 males with BMI ≥ 30.

3- Diabetes group: included 30 males with T2 DM.

4- Obese and diabetes group. Included 30 males with both BMI≥30 and T2 DM.

The study was carried out between April 2019 and February 2020 in the college of Science, Biology department, Salahaddin University-Erbil. The samples were taken and collected in Leila Qasim Diabetes hospital. Before starting the evaluation of semen analysis, other information was taken from the male persons. This information was recorded in a prepared data form. The ages of all individuals in all groups ranged between 35-45 years. The mean ages of the control group were (37.26 ± 4.56); obese group (40.34 ± 3.56), diabetes group (42.75 ± 3.87), and obese and diabetic group (43.24 ± 2.45) with no significant differences between them.

2.2 Semen and serum collection

Semen samples were collected after 3 days of abstinence via masturbation and sexual container, in wide mouth disposable plastic containers. The semen samples were incubated at 37 ºC for 30 minutes to liquefy (Pal *et al.*, 2006). In the liquefied semen samples, the following routine parameters were evaluated according to the methods described in the WHO (WHO, 2009, 2010). The parameters were including; the volume, pH, viscosity, and, appearance of the semen, sperm (count, motility, and morphology). Also, the blood samples were obtained from the individuals and centrifuged at 3000 (rpm) for 15 minutes to obtain the serum. The serum was stored in -20 ºC for further examinations, such as determination of sex hormones and malondialdehyde.

2.3 Semen analysis

Upon liquefaction, the sample's viscosity can be measured by aspirating gently in a wide-boring plastic disposable pipette (about 1.5 mm in diameter), enabling the semen to drop by gravity and to follow the length of every thread. The standard sample leaves a small discrete decrease in the pipette. Where viscosity is anomalous, a thread greater than 2 cm long can shape the drop. A typical sample of liquefied semen has a homogeneous, grey-opalescent look. If the sperm concentration is very small, it can appear less opaque; color alternatively, red-brown can be different when red blood cells are present

(haemospermia), Or yellow in jaundice, or taking other vitamins or medications. The volume of the ejaculate was measured with a graduated cylinder tube (WHO, 2010). The semen sample pH was estimated using a 6.1-10 pH paper range. Whatever form of pH paper is used for this analysis, its accuracy should be tested before being used in routine semen analysis according to established requirements (Comhaire and Vermeulen, 1995).

The sperm concentration was estimated by multiply the mean of sperm number in ten fields with 10^6. Total sperm count = Sperm concentration × volume. Sperm motility was assessed by examining a drop of ejaculate, covered with a cover glass, under a microscope 400x equipped with heat plate 37 ^oC. A minimum of around 200 spermatozoa should be counted; both motile and immotile sperms are counted in at least 5 separate fields (WHO, 2009). Motility % = number of motile spermatozoa/total number of spermatozoa (motile and immotile) ×100. Progressive motility was measured by counting the spermatozoa with straight-line forward movement only in percent of motile spermatozoa. In each sample, sperm motility is graded to 0, 1, 2, 3, or 4, depending on whether it shows:

0 = no movement

1 = movement but not forward progression

2 = movement with slow forward progression

3 = movement in an almost straight line with good speed

4 = movement in a straight line with high speed. (Seaman *et al.*, 1994). The sperm motility index was calculated by multiplying the grading activity with the percentage of motility (Makler *et al.*, 1979).

The normal morphology of spermatozoa was determined by using the hematoxylin and eosin staining procedure (Jequier and Crich, 1986).

2.4 Sex hormones

Serum FSH, LH, and Testosterone hormones were measured by using (Cobas e 411 Roche/Hitachi) in the advanced laboratory of Malaekat Al-Rahma in Erbil city.

2.5 Malondialdehyde

The Serum MDA level was determined by a modified procedure described by (Guidet and Shah, 1989). In short; apply the following to 150 µl of serum: 1 ml of trichloroacetic acid 17.5%, 1 ml of 0.6% thiobarbituric acid, combined well with vortex, incubated for 15 minutes in a boiling water bath, and then allowed to cool. Then add 1 ml of 70% trichloroacetic acid TCA, then let the mixture stand at room temperature for 20 minutes, centrifuged for 15 minutes at 2000 rotation per minute, and remove the supernatant for spectrophotometric scanning (Muslih *et al.*, 2002).

The conc. of MDA = absorbance at 532 nm × D/L × $E_{\circ}$

L: light bath (1 cm)

$E_{\circ}$: extinction coefficient 1.56×105 $M\text{-}1.Cm^{-1}$

D: dilution factor = 1 ml volume. used in Ref./0.15=6.7

2.6 Statistical analysis

Data were observed as means ± standard errors of means. Fishers' Chi-square test is used to compare the semen viscosity and appearance between control, obese, and diabetes groups. Analysis of variance (ANOVA) and Duncan post-hoc test was used for the comparison of semen volume, sperm (concentration, motility, and morphology), sex hormones, and malondialdehyde between different groups. The level of statistical significance established was 0.05. The data were analyzed using the Statistical Package for Social Science (SPSS), version 17.

3.RESULTS

3.1Semen analysis

The results presented in Table 1 illustrate the comparison of the semen viscosity and appearance between control, obese, and diabetes groups. A significant difference $p\leq0.05$ of semen viscosity has appeared between the groups. Males with both obesity and diabetes have a high incidence of the abnormal semen viscosity 33.33% compared with the other groups, while the control group has lower abnormal viscosity 3.33%. No significant differences in semen appearance were observed between control and other patients group. The volume of the semen in the control group (4.22 ± 0.14ml) is significantly $p\leq0.05$ higher than that of the obese (3.19 ± 0.42 ml), diabetes (2.70 ± 0.45 ml), and males with obesity and diabetes (2.34 0.26 ± ml), Table 2.

As shown in Table 3, a significantly higher concentration of the sperm and total sperm count was observed in the control group (88.86×10^6/ml and 374.98×10^6/ejaculate respectively), while lower sperm concentration count and total sperm count is recorded in the males with both obesity and diabetes (46.85×10^6/ml and 109.63

$\times 10^6$/ejaculate respectively). Regards the sperm morphology, significantly lower concentration of the normal morphology was found in diabetes (50.45± 3.22%) and males with both obesity and diabetes (45.67 ± 3.77%) as compared with control (70.67 ± 2.87%) and

males with obesity only (68.32± 4.32%), Table 4.

The results presented in Table 5, compare the motility between the groups and which are considered the most important factors in semen analysis and fertility of males. The results found significant differences ($p \leq 0.001$) in the total sperm count, sperm grade activity, and sperm motility index between the groups. Significantly higher total sperm count, sperm grade, and sperm motility index was observed in the control group (56.34 ± 1.45%, 3.90 ± 0.14, and 219.72 ± 10.42) was compared with a lower value in the obese (48.43 ± 2.52 %, 3.34 ± 0.05 and 161.75 ± 12.67), diabetes (40.22 ± 1.7676%, 2.50 ± 0.07, and 100.55 ± 8.55), and males with both obesity and diabetes (38.21 ± 2.33%, 2.22 ± 0.09, and 84.82 ± 9.26).

3.2Sex hormones

As shown in Table 6, the concentration of the serum sex hormones LH and FSH, are significantly ($p \leq 0.05$) higher in control (6.56 ± 0.27µIU/ml and 7.26 ± µIU/ml) and diabetes groups (5.65 µIU/ml and 6.78 µIU/ml) as compared with obese (4.21 µIU/ml and 5.34 µIU/ml) and males with both obesity and diabetes (3.36 ± 0.24µIU/ml and 4.35 ± 0.37µIU/ml). While the concentration of the testosterone is significantly higher ($p \leq 0.001$) in control (6.77 ± 0.43 ng/ml) as compared with the other 3 groups (4.21± 0.08 ng/ml in obese, 4.00 ± 0.64 in diabetes and 2.65 ± 0.09 ng/ml in males with both obesity and diabetes).

3.3Malondialdehyde

Significantly higher concentration ($p \leq 0.001$) of the serum MDA is recorded in males with both obesity and diabetes (6.45 ± 0.88 µmol/L), while the lower concentration of it is found in the control group (2.14 ± 0.05µmol/L), Table 7.

Table 1. Viscosity and appearance of semen of control, obese and diabetic men.

Groups	Viscosity		Appearance	
	Normal viscosity	Abnormal high viscosity	Normal appearance	Abnormal appearance
Control	29 (96.66%)	1 (3.33%)	28 (93.33%)	2 (6.66%)
Obese	23 (76.66%)	7 (23.33%)	28 (93.33%)	2 (6.66%)
Diabetes	23 (76.66%)	7 (23.33%)	28 (93.33%)	2 (6.66%)
Obese and Diabetes	20 (66.66%)	10(33.33%)	27 (90.00%)	3 (10%)
Calculated x^2	**6.46**		**3.27**	
Tabulated x^2	**4.32**		**3.34**	
p-value	**0.05**		**0.87**	

Table 2. The pH and volume of semen (Mean ± SEM) of control, obese and diabetic men.

Groups	pH	Semen volume (ml)
Control	7.64 ± 0.13 [a]	4.22 ± 0.14 [a]
Obese	7.82 ± 0.34 [a]	3.19 ± 0.42 [b]
Diabetes	7.72 ± 0.21 [a]	2.70 ± 0.45 [b]
Obese and Diabetes	7.78 ± 0.12 [a]	2.34 ± 0.26 [b]
p-value	0.20	0.05

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

Table 3. Sperm concentration and total sperm count (Mean ± SEM) of control, obese, and diabetes men.

Groups	Sperm concentration ($\times 10^6$/ml)	Total sperm count ($\times 10^6$/ejaculate)
Control	88.86 ± 5.89 [a]	374.98 ± 20.35 [a]
Obese	56.66 ± 3.76 [b]	180.74 ± 12.26 [b]
Diabetes	51.57 ± 5.46 [b]	139.23 ± 10.87 [c]
Obese and Diabetes	46.85 ± 8.77 [b]	109.63 ± 10.34 [d]
p-value	**0.001**	**0.001**

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

Table 4. Sperm morphology (Mean ± SEM) of control, obese, and diabetes men.

Groups	Normal morphology (%)		Abnormal morphology (%)
Groups	**Total sperm motility (%)**	**Grade activity**	**Sperm motility index**
Control	56.34 ± 1.45 [a]	3.90 ± 0.14 [a]	219.72 ± 10.42 [a]
Obese	48.43 ± 2.52 [b]	3.34 ± 0.05 [b]	161.75 ± 12.67 [b]
Diabetes	40.22 ± 1.76 [c]	2.50 ± 0.07 [c]	100.55 ± 8.55 [c]
Obese and Diabetes	38.21 ± 2.33 [c]	2.22 ± 0.09 [c]	84.82 ± 9.26 [c]
p-value	**0.001**	**0.001**	**0.001**

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

Table 5. Sperm motility (Mean ± SEM) of control, obese, and diabetes men.

Groups	Total sperm motility (%)	Grade activity	Sperm motility index
Control	56.34 ± 1.45 [a]	3.90 ± 0.14 [a]	219.72 ± 10.42 [a]
Obese	48.43 ± 2.52 [b]	3.34 ± 0.05 [b]	161.75 ± 12.67 [b]
Diabetes	40.22 ± 1.76 [c]	2.50 ± 0.07 [c]	100.55 ± 8.55 [c]
Obese and Diabetes	38.21 ± 2.33 [c]	2.22 ± 0.09 [c]	84.82 ± 9.26 [c]
p-value	**0.001**	**0.001**	**0.001**

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

Table 6. Sex hormones (Mean ± SEM) of control, obese, and diabetes men.

Sex hormones	LH (µIU/ml)	FSH (µIU/ml)	Testosterone (ng/ml)
Control	6.56 ± 0.27 [a]	7.26 ± 0.88 [a]	6.77 ± 0.43 [a]
Obese	4.21 ± 0.08 [b]	5.34 ± 0.64 [b]	4.21 ± 0.25 [b]
Diabetes	5.65 ± 0.74 [a]	6.78 ± 0.78 [a]	4.00 ± 0.64 [b]
Obese and Diabetes	3.36 ± 0.24 [b]	4.35 ± 0.37 [b]	2.65 ± 0.09 [c]
p-value	**0.05**	**0.05**	**0.001**

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

Table 7. Malondialdehyde (Mean ± SEM) of control, obese, and diabetes men.

Groups	MDA (µmol/L)
Control	2.14 ± 0.05 **c**
Obese	4.32 ± 0.24 **b**
Diabetes	4.56 ± 0.76 **b**
Obese and Diabetes	6.45 ± 0.88 **a**
p-value	0.001

P- value ≤ 0.05 considered significant
Post Hoc Duncan-test, no differences between groups with the same letter
SEM = standard error of the mean

4.DISCUSSION

Overweight and obesity are well known to affect female fertility, their effects on male fertility and semen parameters are less clear (Alshahrani *et al.*, 2016). There are inconsistent data on the effect of obesity on seminal fluid and men's fertility (Alahmar *et al.*, 2018). Some studies showed a correlation between obesity and low sperm concentration, motility, morphology, and integrity of the DNA (Bieniek *et al.*, 2016, Sermondade *et al.*, 2013, Wang *et al.*, 2017b). However, other studies have not recorded any link between obesity and poor quality of semen

(Hadjkacem Loukil *et al.*, 2015, Dubeux *et al.*, 2016). The decrease in the semen volume and sperm concentration of obese males in our results are consistent with the results of (Hammoud *et al.*, 2008, Chavarro *et al.*, 2010, Wang *et al.*, 2017b). While it differs from (MacDonald *et al.*, 2010, M. Al-Ali *et al.*, 2014, Alshahrani *et al.*, 2016), who observed no significant differences in semen volume and sperm concentration between obese and normal weight. A negative correlation between BMI and sperm concentration was found in both fertile and infertile men (Anderson *et al.*, 2015).

As with sperm concentration, data are inconsistent on the impact of obesity on sperm morphology and motility. The study of (Macdonald *et al.*, 2013) in New Zealand revealed no effect of BMI on sperm motility, but sperm morology was decreased with increasing BMI. Current results agreed with the findings of (Lazaros *et al.*, 2012, Belloc *et al.*, 2014), that observed a negative correlation between BMI and sperm motility. While the results of other studies differ from the present results, which revealed no association between obesity and sperm motility (Eisenberg *et al.*, 2014, Alshahrani *et al.*, 2016). Jensen *et al.* (2004) recorded no link between obesity a motility percentage. The results of the study of (Hammoud *et al.*, 2008) revealed a lower sperm grade activity in overweight and obese men. In population-based researches, obesity is the single most significant factor that results in a deficit of testosterone (Tajar *et al.*, 2010). The pathophysiology behind the relationship between abnormal BMI and consistency of semen is uncertain and possibly complex. Overweight and obesity have been shown to affect the GnRH – FSH / LH pulse, which can disrupt the role of Leydig or Sertoli cells and interfere with sex hormone release and mature sperm production (Hammoud *et al.*, 2008).

In current results, the concentration of the sex hormones, LH, FSH, and testosterone decrease in obese men, these results are in agreement with the findings of other investigations. Overweight and moderate obesity is primarily correlated with decreases in total testosterone, while free testosterone rates stay within the reference range, especially for younger men (Fui *et al.*, 2014). Decreases in overall testosterone levels attributed to obesity-associated hyperinsulinemia are primarily a result of decreases in sex hormone-binding globulin (SHBG). Nonetheless, although problematic, the calculation of free testosterone levels may provide a more precise androgen status evaluation than the (usually preferred) total testosterone calculation in circumstances where SHBG levels are below the reference range (Bhasin *et al.*, 2010). Testosterone deficiency may cause increased adipogenesis and visceral obesity as evidenced by the rapid weight gain observed in men after androgen deprivation therapy or surgical castration (Tsai *et al.*, 2000, Saylor and Smith, 2009). Weight loss achieved by pharmacologically improving testosterone and gonadotrophin levels with liraglutide (Jensterle *et al.*, 2019) or bariatric surgery, and was able to reverse the hypogonadotropic hypogonadism caused by obesity (Pellitero *et al.*, 2012, Escobar-Morreale *et al.*, 2017).

Serum testosterone (total and free) and SHBG rates are decreased in obese male person (Diaz-Arjonilla *et al.*, 2009). Epidemiological studies have shown that levels of both serum testosterone and, to a lesser extent, free testosterone is reduced in obese men (Allen *et al.*, 2002, Gapstur *et al.*, 2002, Jensen *et al.*, 2004). The decline of overall and free testosterone is correlated not only with intra-abdominal fat but also with total body fat and body fat subcutaneously (Tsai *et al.*, 2004). Obesity is correlated with reduced production of the luteinizing hormone (LH) (Diaz-Arjonilla *et al.*, 2009). The increased levels of estrogen modulate the response of pituitary LH to the hormone-releasing gonadotropin (GnRH) (Castro-Fernandez *et al.*, 2000). However, more obvious obesity is correlated with an unequivocal decrease of free testosterone rates, where rates of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are generally small or excessively high, indicating that the dominant repression happens at the hypothalamic-pituitary rate or level. This may be because adipose tissue expresses aromatase, which transforms testosterone to estradiol (E2), particularly in the inflamed, insulin-resistant state (Fui *et al.*, 2014).

Obesity was confirmed to be triggering systemic oxidative stress (Ozata *et al.*, 2002, Furukawa *et al.*, 2017). The obese infertile males displayed fewer sperm motility as opposed to other classes, fewer sperm function tests values.

The concentration of Malondialdehyde (MDA) in seminal plasma of obese infertile subjects showed higher value compared with those of non-obese infertile men and controls (Najafi *et al.*, 2012). Obesity may cause oxidative stress and decrease testosterone rates, it can modify testicular functions and therefore it can be hypothesized that obesity could be a significant causative factor in the male infertility etiology (Erdemir *et al.*, 2012). Body mass index (BMI) was correlated positively with reactive oxygen species (ROS) and MDA (Han *et al.*, 2018). There was a study done in 2016 showed that the fertile obese men had significantly higher seminal ROS compared to fertile normal-weight men and men with overweight (Taha *et al.*, 2016), these results are in line with our results. A correlation has been identified between oxidative stress and obesity (Karaouzene *et al.*, 2011), and a combination of increased sperm DNA fragmentation in obese men and poor quality of spermatogenesis has also been reported (Smit *et al.*, 2010). Concentrations of seminal MDA are negatively associated with sperm concentration and motility which could offer an easy and effective method for predicting sperm parameters (Hsieh *et al.*, 2006).

Regarding diabetes, our results found a negative impact of diabetes on semen parameters, other investigation approved these results. Studies of infertility prevalence in DM male partners of infertile couples showed decreased sperm motility and increased irregular sperm morphology (Li *et al.*, 2004, Delfino *et al.*, 2007). In a study conducted on 52 diabetic men, semen analysis revealed a significant decline in sperm motility, including the number of rapid progressive cells (Bhattacharya *et al.*, 2014). Semen collected from men with diabetes showed significant sperm parameters decrease compared to men's groups with autoimmune disorders, kidney disease, ulcerative colitis, and cardiac disease (Ranganathan *et al.*, 2002). A few further kinds of research have revealed a significant decline in semen volume, sperm motility, and morphology of diabetic men's semen (Agbaje *et al.*, 2007, Ali and Rakkah, 2007). The findings showed that in DM patients, semen volume, sperm concentration, complete sperm motility, progressive sperm motility, and normal sperm morphology were considerably lower than in nondiabetic controls (Zhu *et al.*, 2017). Semen in patients with T2 DM is of low volume, abnormal motility and morphology compared with non-diabetic subjects (Ibrahim *et al.*, 2018). Ali *et al.* (1993) reported a highly important increase in overall sperm count and sperm concentration in type 1 and type 2 DM patients. Sperm motility and volume of semen were, therefore, lower as for non-diabetics, though sperm morphology and sperm motility rate were not impaired. A high prevalence of irregular sperm motility and morphology in patients with DM was also identified (Amiri *et al.*, 2011, ADA, 2014). Another research carried out in Sudanese males shows a substantial decrease in all parameters of semen (semen volume, sperm count, motility, and morphology) was found in patients with diabetes as opposed to non-diabetics (Abdullah *et al.*, 2014).

There are inconsistent data on the effect of diabetes on sex hormones, A cross-sectional analysis of 355 men with type 2 diabetic aged > 30 in the United Kingdom found that testosterone rates with type 2 diabetes are frequently low, and most of these men have hypogonadism symptoms (Kapoor *et al.*, 2007). Type 2 DM patients will be diagnosed with a moderate but not major decrease of testosterone relative to the regular subjects (Mohammed *et al.*, 2018), these results are consistent with our results. The results of the (Chandel *et al.*, 2008) found that the concentrations of LH and FSH were beyond acceptable limits in type 2 diabetic patients with small free testosterone concentrations. Ali and his colleges found high levels of serum and urinary FSH and LH in diabetics with low levels of total serum free testosterone (Ali *et al.*, 1993).

In current results, a high concentration of MDA was found in diabetes patients. In diabetes patients, MDA is negatively associated with the main sperm parameters (La Vignera *et al.*, 2012). Diabetes mellitus is associated with impaired sperm quality, which involves oxidative stress in pathogenesis, in particular with poor glycemic control (Omu *et al.*, 2014). The semen parameters are reduced and the MDA standard for diabetics is raised whereas type 2 diabetes mellitus harms the capacity for male fertility relative to the non-diabetic (Singh *et al.*, 2014). Excessive development of reactive oxygen species by mitochondria in hyperglycemia is the catalyst that propels these pathways. Excessive development of reactive oxygen species inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase, which in effect stimulates all hyperglycaemic

harm pathways by diverting upstream glycolytic metabolites to these pathways. Also, where the highly potent ROS reaches the seminal antioxidant protection potential, several cascades of reactions can occur which can result in sperm DNA damage and degradation of mitochondrial DNA, then altered sperm parameters and ultimately male infertility (Ahmed, 2005).

5.CONCLUSIONS

In current results, concluded that patients with both obesity and diabetes have a negative impact on semen parameters and fertility than those with healthy control, obesity, or diabetes only. Despite decreasing the semen parameters and fertility in obese and diabetes males, the value of them within the normal ranges of the WHO guidelines. It is evident that in our study, obesity and diabetes have a minor effect and not detrimental to the fertility of males.

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Conflict of Interest

The authors declare no conflict of interest

REFERENCES

ABDULLAH, A. E., MORSI, A. N., ELHASSAN FARAGALLA, M. & ELSAYED, M. 2014. The Association Between Male Infertility And Diabetes Mellitus. *J Pharm Biomed Sci*, 4**,** 1097-1102.

ADA, A. D. A. 2014. Diagnosis and classification of diabetes mellitus. *Diabetes care*, 37**,** S81-S90.

AGBAJE, I., ROGERS, D., MCVICAR, C., MCCLURE, N., ATKINSON, A., MALLIDIS, C. & LEWIS, S. 2007. Insulin dependant diabetes mellitus: implications for male reproductive function. *Human Reproduction*, 22**,** 1871-1877.

AHMED, R. 2005. The physiological and biochemical effects of diabetes on the balance between oxidative stress and antioxidant defense system. *Med. J. Of Islamic Academy of Sci*, 15**,** 31-42.

ALAHMAR, A. T., ALI, Z., MUHSIN, Z. & QASIM, H. 2018. The impact of obesity on seminal fluid in men with infertility. *Middle East Fertility Society Journal*, 23**,** 346-349.

ALI, S., SHAIKH, R., ASHFAQSIDDIQI, N. & SIDDIQI, P. 1993. Serum and urinary levels of pituitary-gonadal hormones in insulin-dependent and non-insulin-dependent diabetic males with and without neuropathy. *Archives of andrology*, 30**,** 117-123.

ALI, S. T. & RAKKAH, N. I. 2007. Neurophysiological role of sildenafil citrate (Viagra) on seminal parameters in diabetic males with and without neuropathy. *Pakistan journal of pharmaceutical sciences*, 20**,** 36-42.

ALLEN, N. E., APPLEBY, P. N., DAVEY, G. K. & KEY, T. J. 2002. Lifestyle and nutritional determinants of bioavailable androgens and related hormones in British men. *Cancer Causes & Control*, 13**,** 353-363.

ALSHAHRANI, S., AHMED, A. F., GABR, A., ABALHASSAN, M. & AHMAD, G. 2016. The impact of body mass index on semen parameters in infertile men. *Andrologia*, 48**,** 1125-1129.

AMIRI, I., KARIMI, J., PIRI, H., GOODARZI, M. T., TAVILANI, H., KHODADADI, I. & GHORBANI, M. 2011. Association between nitric oxide and 8-hydroxydeoxyguanosine levels in semen of diabetic men. *Systems biology in reproductive medicine*, 57**,** 292-295.

ANDERSON, Y. C., WYNTER, L. E., MOLLER, K. R., CAVE, T. L., DOLAN, G. M., GRANT, C. C., STEWART, J. M., CUTFIELD, W. S. & HOFMAN, P. L. 2015. The effect of a multi-disciplinary obesity intervention compared to usual practice in those ready to make lifestyle changes: design and rationale of Whanau Pakari. *BMC obesity*, 2**,** 1-10.

BELLOC, S., COHEN-BACRIE, M., AMAR, E., IZARD, V., BENKHALIFA, M., DALLÉAC, A. & DE MOUZON, J. 2014. High body mass index has a deleterious effect on semen parameters except morphology: results from a large cohort study. *Fertility and sterility*, 102**,** 1268-1273.

BHASIN, S., CUNNINGHAM, G. R., HAYES, F. J., MATSUMOTO, A. M., SNYDER, P. J., SWERDLOFF, R. S. & MONTORI, V. M. 2010. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology & Metabolism*, 95**,** 2536-2559.

BHATTACHARYA, S. M., GHOSH, M. & NANDI, N. 2014. Diabetes mellitus and abnormalities in semen analysis. *Journal of Obstetrics and Gynaecology Research*, 40**,** 167-171.

BIENIEK, J. M., KASHANIAN, J. A., DEIBERT, C. M., GROBER, E. D., LO, K. C., BRANNIGAN, R. E., SANDLOW, J. I. & JARVI, K. A. 2016. Influence of increasing body mass index on semen and reproductive hormonal parameters in a multi-institutional cohort of subfertile men. *Fertility and sterility*, 106**,** 1070-1075.

CASTRO-FERNANDEZ, C., OLIVARES, A., SODERLUND, D., LÓPEZ-ALVARENGA, J., ZAMBRANO, E., VELDHUIS, J. D., ULLOA-AGUIRRE, A. & MÉNDEZ, J. 2000. A preponderance of circulating basic isoforms is associated with decreased plasma half-life and biological to immunological ratio of gonadotropin-releasing hormone-releasable luteinizing hormone

in obese men. *The Journal of Clinical Endocrinology & Metabolism,* 85**,** 4603-4610.

CHANDEL, A., DHINDSA, S., TOPIWALA, S., CHAUDHURI, A. & DANDONA, P. 2008. Testosterone concentration in young patients with diabetes. *Diabetes care,* 31**,** 2013-2017.

CHAVARRO, J. E., TOTH, T. L., WRIGHT, D. L., MEEKER, J. D. & HAUSER, R. 2010. Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertility and sterility,* 93**,** 2222-2231.

COMHAIRE, F. & VERMEULEN, L. 1995. Human semen analysis. *Human reproduction update,* 1**,** 343-362.

DELFINO, M., IMBROGNO, N., ELIA, J., CAPOGRECO, F. & MAZZILLI, F. 2007. Prevalence of diabetes mellitus in male partners of infertile couples. *Minerva urologica e nefrologica= The Italian journal of urology and nephrology,* 59**,** 131-135.

DIAZ-ARJONILLA, M., SCHWARCZ, M., SWERDLOFF, R. & WANG, C. 2009. Obesity, low testosterone levels and erectile dysfunction. *International journal of impotence research,* 21**,** 89-98.

DUBEUX, V. T., RENOVATO, T., ESTEVES, A. C., ANDRÉ, L., DE OLIVEIRA, A. & PENNA, I. A. 2016. The impact of obesity on male fecundity: a Brazilian study. *JBRA assisted reproduction,* 20**,** 137-141.

EISENBERG, M. L., KIM, S., CHEN, Z., SUNDARAM, R., SCHISTERMAN, E. F. & BUCK LOUIS, G. M. 2014. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Human reproduction,* 29**,** 193-200.

ERDEMIR, F., ATILGAN, D., MARKOC, F., BOZTEPE, O., SUHA-PARLAKTAS, B. & SAHIN, S. 2012. The effect of diet induced obesity on testicular tissue and serum oxidative stress parameters. *Actas Urológicas Españolas (English Edition),* 36**,** 153-159.

ESCOBAR-MORREALE, H. F., SANTACRUZ, E., LUQUE-RAMÍREZ, M. & BOTELLA CARRETERO, J. I. 2017. Prevalence of 'obesity-associated gonadal dysfunction'in severely obese men and women and its resolution after bariatric surgery: a systematic review and meta-analysis. *Human Reproduction Update,* 23**,** 390-408.

FUI, M. N. T., DUPUIS, P. & GROSSMANN, M. 2014. Lowered testosterone in male obesity: mechanisms, morbidity and management. *Asian journal of andrology,* 16**,** 223-231.

FURUKAWA, S., FUJITA, T., SHIMABUKURO, M., IWAKI, M., YAMADA, Y., NAKAJIMA, Y., NAKAYAMA, O., MAKISHIMA, M., MATSUDA, M. & SHIMOMURA, I. 2017. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of clinical investigation,* 114**,** 1752-1761.

GAPSTUR, S. M., GANN, P. H., KOPP, P., COLANGELO, L., LONGCOPE, C. & LIU, K. 2002. Serum androgen concentrations in young men: A longitudinal analysis of associations with age, obesity, and race.: The CARDIA male hormone study. *Cancer Epidemiology and Prevention Biomarkers,* 11**,** 1041-1047.

GUIDET, B. & SHAH, S. V. 1989. Enhanced in vivo H2O2 generation by rat kidney in glycerol-induced renal failure. *American Journal of Physiology-Renal Physiology,* 257**,** F440-F445.

HADJKACEM LOUKIL, L., HADJKACEM, H., BAHLOUL, A. & AYADI, H. 2015. Relation between male obesity and male infertility in a T unisian population. *Andrologia,* 47**,** 282-285.

HAMMOUD, A. O., GIBSON, M., PETERSON, C. M., MEIKLE, A. W. & CARRELL, D. T. 2008. Impact of male obesity on infertility: a critical review of the current literature. *Fertility and sterility,* 90**,** 897-904.

HAN, R., MA, J., LIU, W., AN, X., ZHANG, Z.-D. & WANG, S. 2018. Correlation of reproductive hormone levels and seminal plasma oxidative stress with semen quality in obese males. *Zhonghua nan ke xue= National journal of andrology,* 24**,** 419-424.

HSIEH, Y.-Y., CHANG, C.-C. & LIN, C.-S. 2006. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *International Journal of Biological Sciences,* 2**,** 23-29.

IBRAHIM, N. E., RIDA, M. & ABDRABO, A. A. 2018. Evaluation of Semen Quality in Type 2 Diabetes Mellitus Sudanese Patients Compared to Non-Diabetic Subjects. *The Open Clinical Biochemistry Journal,* 8**,** 20-25.

JENSEN, T. K., ANDERSSON, A.-M., JØRGENSEN, N., ANDERSEN, A.-G., CARLSEN, E. & SKAKKEBÆK, N. E. 2004. Body mass index in relation to semen quality and reproductive hormonesamong 1,558 Danish men. *Fertility and sterility,* 82**,** 863-870.

JENSTERLE, M., PODBREGAR, A., GORICAR, K., GREGORIC, N. & JANEZ, A. 2019. Effects of liraglutide on obesity-associated functional hypogonadism in men. *Endocrine connections,* 8**,** 195-202.

JEQUIER, A. M. & CRICH, J. P. 1986. *Semen analysis: a practical guide, Blackwell Scientific Publications; St. Louis, MO, USA. Distributors, USA, Blackwell Mosby Book Distributors.*

JIANG, W., SUN, H., ZHANG, J., ZHOU, Q., WU, Q., LI, T., ZHANG, C., LI, W., ZHANG, M. & XIA, X. 2015. Polymorphisms in Protamine 1 and Protamine 2 predict the risk of male infertility: a meta-analysis. *Scientific reports,* 5**,** 1-11.

KAPOOR, D., ALDRED, H., CLARK, S., CHANNER, K. S. & JONES, T. H. 2007. Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes: correlations with bioavailable testosterone and visceral adiposity. *Diabetes care,* 30**,** 911-917.

KARAOUZENE, N., MERZOUK, H., ARIBI, M., MERZOUK, S., BERROUIGUET, A. Y., TESSIER, C. & NARCE, M. 2011. Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: a comparison of older with young men. *Nutrition,*

Metabolism and Cardiovascular Diseases, 21**,** 792-799.

LA VIGNERA, S., CONDORELLI, R., VICARI, E., D'AGATA, R., SALEMI, M. & CALOGERO, A. 2012. High levels of lipid peroxidation in semen of diabetic patients. *Andrologia,* 44**,** 565-570.

LAZAROS, L., HATZI, E., MARKOULA, S., TAKENAKA, A., SOFIKITIS, N., ZIKOPOULOS, K. & GEORGIOU, I. 2012. Dramatic reduction in sperm parameters following bariatric surgery: report of two cases. *Andrologia,* 44**,** 428-432.

LÉGARÉ, C., DROIT, A., FOURNIER, F. D. R., BOURASSA, S., FORCE, A., CLOUTIER, F., TREMBLAY, R. & SULLIVAN, R. 2014. Investigation of male infertility using quantitative comparative proteomics. *Journal of proteome research,* 13**,** 5403-5414.

LEVINE, H., JØRGENSEN, N., MARTINO-ANDRADE, A., MENDIOLA, J., WEKSLER-DERRI, D., MINDLIS, I., PINOTTI, R. & SWAN, S. H. 2017. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Human reproduction update,* 23**,** 646-659.

LI, C.-J., TZENG, C.-R., CHEN, R.-Y., HAN, B.-C., YEH, C.-Y. & CHIEN, L.-C. 2016. Decline in semen quality in men in northern Taiwan between 2001 and 2010. *Chin J Physiol,* 59**,** 355-365.

LI, W., ZHENG, H., BUKURU, J. & DE KIMPE, N. 2004. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of ethnopharmacology,* 92**,** 1-21.

M. AL-ALI, B., GUTSCHI, T., PUMMER, K., ZIGEUNER, R., BROOKMAN-MAY, S., WIELAND, W., FRITSCHE, H. & AZIZ, A. 2014. Body mass index has no impact on sperm quality but on reproductive hormones levels. *Andrologia,* 46**,** 106-111.

MACDONALD, A., HERBISON, G., SHOWELL, M. & FARQUHAR, C. 2010. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. . *Hum Reprod Update,* 16**,** 293-311.

MACDONALD, A., STEWART, A. & FARQUHAR, C. 2013. Body mass index in relation to semen quality and reproductive hormones in New Zealand men: a cross-sectional study in fertility clinics. *Human reproduction,* 28**,** 3178-3187.

MAKLER, A., ITSKOVITZ, J., BRANDES, J. M. & PALDI, E. 1979. Sperm velocity and percentage of motility in 100 normospermic specimens analyzed by the multiple exposure photography (MEP) method. *Fertility and Sterility,* 31**,** 155-161.

MASCARENHAS, M. N., FLAXMAN, S. R., BOERMA, T., VANDERPOEL, S. & STEVENS, G. A. 2012. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Med,* 9**,** e1001356.

MOHAMMED, M., AL-HABORI, M., ABDULLATEEF, A. & SAIF-ALI, R. 2018. Impact of metabolic syndrome factors on testosterone and SHBG in type 2 diabetes mellitus and metabolic syndrome. *Journal of diabetes research,* 2018**,** 1-8.

MOUSSA, H. N., ALRAIS, M. A., LEON, M. G., ABBAS, E. L. & SIBAI, B. M. 2016. Obesity epidemic: impact from preconception to postpartum. *Future science OA,* 2**,** 1-12.

MUSLIH, R., AL-NIMER, O. & AL-ZAMELY, M. 2002. The level of Malondialdehyde after activation with H2O2 and CuSO4) and inhibited by Desferoxamine and Molsidomine in the serum of patients with acute myocardial infection. *J. Chem,* 5**,** 148-149.

NAJAFI, M., SREENIVASA, G., AARABI, M., DHAR, M., BABU, M. & MALINI, S. 2012. Seminal malondialdehyde levels and oxidative stress in obese male infertility. *Journal of Pharmacy Research,* 5**,** 3597-3600.

OMOLAOYE, T. & DU PLESSIS, S. S. 2018. Diabetes mellitus and male infertility. *Asian Pacific Journal of Reproduction,* 7**,** 6-14.

OMU, A., AL-BADER, M., AL-JASSAR, W., AL-AZEMI, M., OMU, F., MATHEW, T. & ANIM, J. 2014. Antioxidants attenuates the effects of insulin dependent diabetes mellitus on sperm quality. *Bioenergetics,* 3**,** 1-9.

OZATA, M., MERGEN, M., OKTENLI, C., AYDIN, A., SANISOGLU, S. Y., BOLU, E., YILMAZ, M. I., SAYAL, A., ISIMER, A. & OZDEMIR, I. C. 2002. Increased oxidative stress and hypozincemia in male obesity. *Clinical biochemistry,* 35**,** 627-631.

PAL, P., RAJALAKSHMI, M., MANOCHA, M., SHARMA, R., MITTAL, S. & RAO, D. 2006. Semen quality and sperm functional parameters in fertile Indian men. *Andrologia,* 38**,** 20-25.

PELLITERO, S., OLAIZOLA, I., ALASTRUE, A., MARTÍNEZ, E., GRANADA, M. L., BALIBREA, J. M., MORENO, P., SERRA, A., NAVARRO-DÍAZ, M. & ROMERO, R. 2012. Hypogonadotropic hypogonadism in morbidly obese males is reversed after bariatric surgery. *Obesity surgery,* 22**,** 1835-1842.

RADWAN, M., JUREWICZ, J., POLAŃSKA, K., SOBALA, W., RADWAN, P., BOCHENEK, M. & HANKE, W. 2016. Exposure to ambient air pollution-does it affect semen quality and the level of reproductive hormones? *Annals of human biology,* 43**,** 50-56.

RANGANATHAN, P., MAHRAN, A. M., HALLAK, J. & AGARWAL, A. 2002. Sperm cryopreservation for men with nonmalignant, systemic diseases: a descriptive study. *Journal of andrology,* 23**,** 71-75.

SALAS-HUETOS, A., BULLÓ, M. & SALAS-SALVADÓ, J. 2017. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. *Human reproduction update,* 23**,** 371-389.

SAYLOR, P. J. & SMITH, M. R. 2009. Metabolic complications of androgen deprivation therapy for prostate cancer. *The Journal of urology,* 181**,** 1998-2008.

SEAMAN, E., BAR-CHAMA, N. & FISCH, H. 1994. Semen analysis in the clinical evaluation of infertility. *Mediguide to Urology,* 7**,** 1-8.

SENGUPTA, P., NWAGHA, U., DUTTA, S., KRAJEWSKA-KULAK, E. & IZUKA, E. 2017. Evidence for decreasing sperm count in African population from 1965 to 2015. *African health sciences,* 17**,** 418-427.

SERMONDADE, N., FAURE, C., FEZEU, L., SHAYEB, A., BONDE, J. P., JENSEN, T. K., VAN WELY, M., CAO, J., MARTINI, A. C. & ESKANDAR, M. 2013. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Human reproduction update,* 19**,** 221-231.

SINGH, A. K., TOMARZ, S., CHAUDHARI, A. R., SINQH, R. & VERMA, N. 2014. Type 2 diabetes mellitus affects male fertility potential. *Indian J Physiol Pharmacol,* 58**,** 403-406.

SMIT, M., ROMIJN, J. C., WILDHAGEN, M. F., WEBER, R. F. & DOHLE, G. R. 2010. Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. *Fertility and sterility,* 94**,** 1748-1752.

TAHA, E. A., SAYED, S. K., GABER, H. D., HAFEZ, H. K. A., GHANDOUR, N., ZAHRAN, A. & MOSTAFA, T. 2016. Does being overweight affect seminal variables in fertile men? *Reproductive biomedicine online,* 33**,** 703-708.

TAJAR, A., FORTI, G., O'NEILL, T. W., LEE, D. M., SILMAN, A. J., FINN, J. D., BARTFAI, G. R., BOONEN, S., CASANUEVA, F. F. & GIWERCMAN, A. 2010. Characteristics of secondary, primary, and compensated hypogonadism in aging men: evidence from the European Male Ageing Study. *The Journal of Clinical Endocrinology & Metabolism,* 95**,** 1810-1818.

TSAI, E., BOYKO, E., LEONETTI, D. & FUJIMOTO, W. 2000. Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. *International journal of obesity,* 24**,** 485-491.

TSAI, E. C., MATSUMOTO, A. M., FUJIMOTO, W. Y. & BOYKO, E. J. 2004. Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat. *Diabetes care,* 27**,** 861-868.

TSAO, C.-W., LIU, C.-Y., CHOU, Y.-C., CHA, T.-L., CHEN, S.-C. & HSU, C.-Y. 2015. Exploration of the association between obesity and semen quality in a 7630 male population. *PLoS One,* 10**,** 1-13.

WANG, E.-Y., HUANG, Y., DU, Q.-Y., YAO, G.-D. & SUN, Y.-P. 2017a. Body mass index effects sperm quality: a retrospective study in Northern China. *Asian journal of andrology,* 19**,** 234-237.

WANG, L., SOUTHERLAND, J., WANG, K., BAILEY, B. A., ALAMIAN, A., STEVENS, M. A. & WANG, Y. 2017b. Ethnic differences in risk factors for obesity among adults in California, the United States. *Journal of obesity,* 2017**,** 1-10.

WHO 2009. World Health Organisation. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction.Cambridge university press.

WHO 2010. World Health Organization. Laboratory manual for the examination and processing of human semen. Fifth edititon. Geneva 27, Switzerland.

ZHU, J.-Z., DONG, X.-Y., LIANG, J.-J., ZHANG, Z.-Q., HU, X.-Y. & LI, L.-K. 2017. Effects of diabetes mellitus on semen quality in adult men: a systematic review and meta-analysis. *Int J Clin Exp Med,* 10**,** 11290-303.