# Spermatozoal creatine kinase (CK) concentration as an indication of idiopathic obese subfertile males with normozoospermia

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## مستوى إنزيم الكرياتين كاينيز في الحيامين الطبيعية يعتبر مؤشر لمرضى البدانة في حالات العقم الدكوري الغير المفسر

**الخلاصة:** لقد اهتمت الدراسات المتقدمة في مجال التناسلية والعقم إلى اللجوء في تشخيص الخلل الوظيفي للحيمن من خلال استحداث فحوص متطورة لغرض التنبوء والتشخيص والعلاج.لقد أشارت الدراسات الحديثة إلى وجود علاقة وثيقة بين البدانة والعقم بشكل عام والعقم الدكوري الغير المفسر بشكل خاص مع وجود تفاسير عديدة لهذه العلاقة. تشير نتائج هذه الدراسة إلى إن ارتفاع مستوى أنزيم الكرياتين كاينيز في الحيامين الطبيعية يعتبر مؤشر لمرضى البدانة في حالات العقم الدكوري الغير المفسر مما يتسبب في احتمالية قلة الإخصاب.

## Abstract

***Objective***: To determine the mean of spermatozoal creatine kinase (CK) concentration in idiopathic obese subfertile males with normozoospermia and healthy normal fertile control group. And to compare spermatozoal CK concentration between idiopathic obese normozoosperpmic subfertiles and healthy normal fertile volunteers (control group).

**Design**: A prospective study.

***Setting:*** Maternity and Childhood Hospital, Najaf province, during period from April 2006 to January 2007.

***Patients and Methods:*** The present study determined the mean of spermatozoal CK concentration in 47 idiopathic obese male subfertiles with normozoospermia ,their ages ranging between 25-45 years ,and sperm count million ml, motility% , morphology %, vitality% are registered as normozoospermia according to WHO criteria. Fifteen apparently healthy normal fertile were regarded as control group. Traditional semen analyses and sperm parameters were assisted by same examiner. CK concentrations were measured using CK kit after the sperm enzyme was extracted within triton –X. The results of spermatozoal CK concentration was determined and compared between both groups.

***Result(s***): There was a statistically significant differences ($P<0.05$) in active sperm motility % and sperm immotility % in comparison to sperm mean CK concentration in both groups. And sperm mean CK concentration was significantly higher in idiopathic obese male subfertiles with normozoospermia in comparison to control group.

***Conclusion(s):*** Elevated levels of spermatozoal CK concentration in idiopathic normozoospermic obese subfertile males may suggest an indication of sperm oxidative stress status which could play a role in idiopathic normozoospermic obese subfertility and fecundity that warrants more detail studies.

***Recommendation(s):*** *creatine kinase concentration could be use an indication of sperm oxidative stress* in idiopathic obese subfertile males with normozoospermia.

***Keywords:*** **Creatine kinase, idiopathic subfertility, obesity**

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## Introduction

Infertility is common in couples with childbearing age (1). And obesity is therefore considered and associated as one of the main predisposing factor of the higher incidence of male factor of unexplained infertility (2). An individual can be defined as an overweight if their BMI is 25-30kg/m2, and obese if their BMI exceeds 30kg/m2 (3).A combination of an increasingly sedentary lifestyle and unfavorable diet in the western world has resulted in an increase number of overweight and obese children and adults. According to World Health Organization (WHO), approximately 1.6 billion adults were classified as being overweight and 400 million were obese in 2005. Statisticians have predicted that, by 2015, approximately 2-3 billion adult will be overweight and 700 million will be obese (4). Also, gaining attention is the reported decline in semen quality and men reproductive potential over the past 50 years according to study of Carlson et al (5) the quality of semen has potentially declined with the consequent negative effect of poor semen quality of men fertility. In contrast to extensive knowledge of the effects of obesity on female fertility , male factor infertility as a result of obesity has been overlooked ,even after discovery of a threefold increase in the incidence of obesity in patients with male factor infertility, demonstrating the need for greater clinician awareness in this area(6).

Throughout the approximately 50 years, there was no considerable changes in the procedure of semen analysis and , the standard semen analysis has largely remained unchanged .Even though the value of semen analysis has significantly evaded over the period of time due to primarily to the objective nature of its results and luck of standardized training for laboratory personnel as well as presence of insignificant inter and intra-observer variabilities in results (7).Although, many other tests are being introduced which attempt to delve further into specific causes of male subfertility, but never replace the standard semen analysis tests(8).A number of independent studies have indicated that the defective sperm function is associated with elevated levels of creatine kinase ( 9,10).Creatine kinase level in human sperm are an objective biochemical marker of sperm maturity and fertilizing potential(11 ). Interest in CK has been stimulated by studies suggesting that defective sperm function is associated with defect of spermatogenesis that leads to the release of immature spermatozoa from germinal epithelium (12).

Many experts believe that overweight and obesity can be taken to reverse both unhealthy consequences associated with obesity and negative impacts on male infertility (13,14 , 15).The increase in the incidence of obesity has a substantial societal heath impact ,contrasting reports have been published whether overweight and obesity affect male fertility (16).

Cabler's study referred the corresponding decrease in male infertility and fecundity in parallel to obesity, and obesity should be considered as an etiology of male infertility (13). A higher incidence of obesity -associated male subfertility that initiate a direct and /or indirect adverse mechanisms of obesity on idiopathic obese male subfertility and infertility (17,18,19).Creatine kinase is associated with other biochemical markers, namely reactive oxygen species (ROS) and its reflective of oxidative stress (OS) in pathologically abnormal spermatozoa ( 20).As a result of elevated levels of ROS and depressed levels of antioxidant , OS is associated with male infertility (21 ,22 ).A more recent study showed that in normozoosperpmic specimens with a higher percentage of mature spermatozoa ,the CK values were significantly lower (23).

The purpose of this study was to determine the mean concentration of sperm CK of idiopathic obese normozoosperpmic subfertile males.And to compare the result of sperm CK concentration between idiopathic obese normozoosperpmic subfertile males and healthy normal fertile volunteers (control group).

**Patients and Methods**:

This is a prospective study was carried out at Maternity and Childhood Hospital, Najaf province, at period between April 2006 to January 2007. The study consisted of total number 47 idiopathic normozoosperpmic obese subfertile males (barren marriage) of more than one year. All the participated patients were selected to be obese according to their body mass index(BMI) measurements ,more than 30kg/m² in respect to WHO criteria (3) .Patients with proven normal sperm parameters and infertility factor that might interfere with fertility –related origin viz: Hypogonadotropic hypogonadism, varicocele ,cryptorchidisim , venereal disease , leucocytospermia , drug and hormonal therapy ,abnormal sexual function (erectile dysfunction and impotence) and any patient who had difficulties in semen collection by masturbation or coitus interrupts were excluded .

A separate group is consisting of 15 apparently healthy, male volunteers with proven fertility that initiated a successful pregnancy within 1-2 years, regarded as a control group. All scheduled patients in this study accepted with verbal consent, their actual agreement to contribute during the whole period of study.

**Semen collection;**

Samples of semen ejaculate were collected from all married patient by masturbation technique or coitus interrupts after 3-5 days of sexual abstinence. All ejaculate samples were collected in laboratory and brought within 20 min. into a clean aseptic vails.After ejaculation the specimen was placed in an incubator at 37C for 30min to allow liquefaction .All macroscopic and microscopic examinations are carried out according to WHO criteria (24). Quantitation of seminal granular leucocytes in semen specimens were assessed according to Endtz test (25).The sperm pellet was used for a preparation of sperm homogenization buffer for measurement of creatine kinase (CK) concentration.

**Sperm preparation**

All masturbated semen samples liquefied after 30 minutes at room temperature, spermatozoa were separated from seminal plasma by centrifugation at 500 x rpm for 30 minutes. The supernatant was precisely measured by a graduated centrifuge test tube and discarded. Homogenizing buffer added to the pellet fraction. Homogenizing buffer consisted of (11.9 gms of manitol, 4.8 gms of sucrose, 0.09 gms of EDTA) in 250 ml of distilled water adjust the PH to 7.4 with tris-base. Homogenized buffer was kept in refrigerator at 4 C◦. The samples were hand homogenized and were subsequently centrifuged for 10 minutes at 3000 rpm. Cooled 0.9 ml of Triton x – 100 (0.1%) was added to each 0.1ml of pellet obtained from the sample , the samples were centrifuged again at 8000 rpm for half an hour in a centrifuge. The supernatant was used for enzymatic measurements [(26)].

**Creatine Kinase (CK):** Randox creatine kinase with catalogue no : CK 1673, Randox laboratories Ltd , Ardmore , Co. Antrim United kingdom .Enzymatic colorimetric

method was applied to assay CK with spectrophotometer Cecil – 1011 England for all measurements .

**Principle and procedure**

Creatine kinase (CK) utilizes creatine phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH as outline in the coupled enzyme assay shown below. The NADPH produced, is proportional to CK activity and is used to reduce nitroblue tetrazolium (NBT), in the presence of diaphorase, to give the blue / violet color of diformazan which has as an absorption maximum around 560 nm.

Creatine phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

Glucose + ATP $\xrightarrow{HK}$ Glucose-6-p + ADP

Glucose -6- phosphate + $NADP^+$ $\xrightarrow{G6PDH}$ Gluconate -6-p + NADPH

2 NADPH + NBT $\xrightarrow{diaphore}$ difromazan + 2 NAD

*Calculations:*

$$\text{Creatine kinease activity in sample UL} = \frac{\text{A sample}}{\text{A standard}} \text{ x standard conc.}$$

All enzymatic assays and values of CK values were assessed and converted from U/L as fixed on kit instructions to the wanted values $U/10^8$ sperm to be compared to international values.

***Statistical analysis***: Data were analyzed using inbuilt functions within the statistical package of SPSS UK version 10 surreys UK. Least significant diffrence between means at level of significance 0.05 was used.

**Results:**

Table (1) demonstrated a comparison between each sperm variable mean of idiopathic obese normozoosperpmic subfertiles group (N=47) and control group (n=15).The present comparative data revealed that there was a statistically significant differences ($P<0.05$) of sperm mean variables of active sperm motility % and sperm immotility % between idiopathic obese normozoosperpmic subfertiles group and control group. The results were (60.00±2.24 vs 65.61±1.85) and (20.00±1.87 vs 16.92 ±1.74) consecutively.

**Table- 1** Comparison of each sperm variable between idiopathic normozoospermic obese subfertiles (n=47) and healthy normal fertile volunteers (n=15) . Data are presented as mean ± (SEM).

| Sperm variables | Mean ± SEM | | P value |
|---|---|---|---|
| | Idiopathic obese subfertile males with normozoospermia group (n=47) | Healthy normal fertile volunteers (control group) (n=15) | |
| Sperm count $x10^6$/ml | 55.23 ±3.23 | 56.33 ±4.13 | 0.51 |
| Sperm concentration $10^6$ /semen volume | 117.08 ±15.97 | 123.25 ±12.22 | 0.11 |
| Sperm active motility % | 60.00 ±2.24 | 65.61 ±1.85 | 0.01* |
| Sperm sluggish motility % | 19.00 ±1.59 | 17.00 ±1.83 | 0.21 |
| Sperm immotility % | 20.00 ±1.87 | 16.92 ±1.74 | 0.03* |
| Sperm viability % | 61.41 ±2.32 | 63.13 ±2.13 | 0.67 |
| Normal sperm morphology % | 63.46 ±2.96 | 65.38 ±2.56 | 0.37 |
| Abnormal sperm morphology % | 36.53 ±2.96 | 34.61 ±2.56 | 0.37 |

*** Significant value less than P < 0.05**
**Insignificant value P > 0.05**

Table (2) showed a comparison of mean sperm CK concentration between idiopathic obese normozoosperpmic subfertiles group (N=47) and control group (n=15).The results depicted a high statistically significant differences (P<0.05) between idiopathic obese normozoosperpmic subfertiles group and control group .Data were (1.50 ± $6.11\times10^{-2}$ vs. $0.90\pm6.37\times10^{-2}$) respectively.

**Table- 2** Comparison of spermatozoal CK concentration between idiopathic normozoospermic obese subfertiles (n=47) and healthy normal fertile volunteers (n=15). Data are presented as mean ± (SEM).

| Sperm variables | Mean ± SEM | | P value |
|---|---|---|---|
| | Idiopathic obese subfertile males with normozoospermia group (n=47) | Healthy normal fertile volunteers (control group) (n=15) | |
| Creatine Kinase U/$10^8$ | 1.50 ±6.11 $x10^{-2}$ | 0.90 ±6.37$x10^{-2}$ | 0.00** |

**** Highly significant value P < 0.05 when value 0.00 (2-tailed)**

## Discussion

According to World Health Organization (WHO), the prevalence of obesity or morbid obesity in the United States around 41.3% .It is predicted that globally, in the next 5 years, more than 700 million adults will suffer from obesity (27).

Obesity has become an epidemic not only in United state ,but increasingly so in the rest of the world (28).Once considered a problem only in high-income countries ,overweight and obesity are now dramatically on the rise in all countries (29).However, no Iraqi documents are available indicating the incidence of overweight and obese Iraqi people. Besides, to parallel the global increase in obesity , is the reported decrease in male fertility and fecundity (30).The present result in table (1) pointed to a significant differences ($P<0.05$) in mean sperm active motility % and sperm immotility % when the data were compared between idiopathic normozoospermic obese subfertiles group and healthy normal volunteers (control group). The results were (60.00±2.24 VS 65.61±1.85) and (20.00±1.87vs 16.92±1.74) consecutively.

Our data are consistent with other studies (30,31,32,33) since, that patients in this study were idiopathic obese normozoospermic subfertiles .However ,till now no present data are available to link between the adverse effect of obesity in an obese idiopathic males with normozoospermia and male infertility (unexplained infertility ) while, more recent articles elucidated the relation between obesity and idiopathic male subfertility and infertility (13,14 ,16).

The results in table (2) depicted a high significant difference ($P<0.05$) in mean values of spermatozoal CK concentration between idiopathic normozoospermic obese subfertile males group and healthy normal fertile volunteers (control group).The results were ($1.50 \pm 6.11 \times 10^{-2}$ vs $0.90 \pm 6.37 \times 10^{-2}$). No available data are present to compare our results of idiopathic normozoospermic obese subfertiles with other studies. Generally, our findings may interpret the cause of unexplained infertility in idiopathic normozoospermic obese subfertile males due to relationship between obesity and elevated level of spermatozoal CK concentration that could increase the possibility of sperm oxidative stress (OS) in those obese patients which may reflect the cause of unexplained subfertility in patients with normozoospermia. Moreover, recent advances in the field of reproductive medicine have focused the attention of many researchers to consider ROS as one of the mediators of infertility and sperm dysfunction, their excess production results in OS. These findings agree with our findings and supported by many recent studies (20, 21, 23, 34, 35, 36, and 37). On the other hand, emphasizing to the fact that the increase in spermatozoal CK concentration in idiopathic normozoospermic obese subfertiles has occurred relatively in parallel with obesity. In our opinion this possible relationship may correlate inversely with fertilizing potential of spermatozoa, and this is corroborating with our findings and consolidated by another study (34).

Lastly, from this standing point, the present study suggests that it is necessary to focus on the possible effects of obesity as an etiology of male idiopathic subfertility in normozoospermia(unexplained infertility) and the cause of reduced fecundity may accompanied in a concomitance to the elevated concentration of spermatozoal CK that could play a role and give an indication of possible spermatozoal OS effect that reducing fertilizing potential of spermatozoa in idiopathic obese male subfertiles with normozoospermia.

In conclusion, the present study determined that there was a high significant increase in mean of spermatozoal CK concentration in idiopathic normozoospermic obese

subfertile males when compared to healthy normal fertile volunteers, this could play a role in an increase of spermatozoal oxidative stress status that may interfere with fertilizing potential of spermatozoa of idiopathic subfertile obese males with normozoospermia and fecundity that warrants more detail studies.

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