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RESEARCH ARTICLE

IS THE INSULIN RESISTANCE A REGULATOR ON THE LEVELS OF INSULIN AND TESTOSTERONE IN SEMINAL PLASMA IN MEN WITH METABOLIC SYNDROME.

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Abstract

Introduction: The morbidly weight gain is associated with multiple hormonal interactions with potential negative effect on male fertility. It is not clearly understood if visceral adiposity or Insulin Resistance (IR), or both together, is the key mediator for decreasing the testosterone (T) synthesis and activity in men with metabolic syndrome (MS).

The aim: of the study is to establish the influence of IR on the semen levels of Insulin (Ins) and as well as the semen concentration, motility and morphology in men with MS.

Material and methods: A pilot prospective study was done among obese men with IR without knowing fertile problems on age between 20-40 years. According to their BMI the participants were divided into three groups: first group (G_1), of non-obese subjects with BMI $\geq 19.0 \leq 24.9$ kg/m^2, (n_1=4); second group (G_2) of overweight, pre-obese subjects with BMI $\geq 25.0 \leq 29.9$ kg/m^2, (n_2=4) and third group (G_3) of obese subjects with BMI $\geq 30.0 \leq 40.0$ kg/m^2, (n_3=21). Semen samples were collected and assayed for Ins, T and standard semen analysis as well.

Results: Obesity was associated with significantly increased serum insulin levels and IR, but with no significant difference in serum testosterone level. Significantly severe inverse correlations between serum levels of Ins and T, as well as between BMI and serum T were established. Positive correlation between serum and seminal concentration of Ins was found. A moderately strong negative correlation between concentrations of Ins and T in seminal plasma was confirmed. The significant differences in the average sperm concentration and teratozoospermal index were observed by comparison between obese and non -obese men.

Conclusion: This pilot study showed that obesity and IR in general, adversely affects sperm parameters such as decreased sperm motility and increased teratozoospermal index. The pandemic spread of obesity necessitates further studies to clarify the additional links and

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mechanisms that are manifested at an early stage and are related to violations of reproductive function in obese men.

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Introduction:-

The global obesity epidemic is followed by progressively rising incidences of metabolic, cardiovascular, neoplastic complications and shorter life expectancy. The morbidly weight gain has an unfavorable effect on the reproductive system in both gender in combination with negative alterations of the sexual and reproductive health as an essential part of the quality of human life.

The abdominal obesity is characterized with ectopic visceral deposition of white fat tissue and it is linked with insulin resistance (IR), impaired glucose tolerance, type two diabetes mellitus (T2DM), dyslipidemia and cardiovascular diseases.

The classical opinion for the essence of the adipose tissue was dramatically changed in the end in the last century after the discovery of leptin. The previous conception that white visceral fat mass is a passive reservoir of mastocytes for energy storage was converted to the current contrast understanding that the fat tissue is the major endocrine organ with production of different bioactive molecules named "adipocytokines" with their own hormonal activity. Adipocytes together with other bioactive components of the adipose tissue cells play integrative role in hormonal interactions between metabolic and reproductive physiology [1].

Visceral adiposity is associated with multiple hormonal interactions with potential negative effect on hypothalamic–pituitary–testicular axis regulation. Different mechanisms are involved into the genesis of men's infertility in case of visceral obesity and metabolic syndrome (MS). The basics are linked with the synergic effect of some adipocytokines, biomolecules and neuropeptides and their negative impact on the insulin sensitivity, neuroendocrine physiology, hypothalamic function and sex steroid hormone concentrations **[**2].

The induction of hypogonadism in men with MS is not fully elucidated.The viscral fat mass depresses synthesis of testosterone (T) in case of abdominal obesity. Several pathophysiologic mechanisms are associated with lower androgen levels such as: direct suppression of testicular T production by insulin and leptin, rising of estrogen concentrations and attenuated in pulse amplitude and secretion of luteinizing hormone [3]. Some studies suggested that sex hormone-binding globulin (SHBG) may also contribute to metabolic alterations between T and IR. The negative local feedback exists between the T levels and the amount of the visceral adipose tissue which seems to be regulated by keeping the delicate balance between bioavailable T and SHBG.

Physiologically, T has blocking effect on the conversion of the preadipocytes into adipocytes and regulatory effect on the insulin sensitivity. The decreasing concentration of the T could not prevent fat mass growing and aggravates insulin sensitivity. Hyperinsulinemia ,*per se,* directly decreases T secretion by the Leydig cell and impacts in response of the insulin receptors on the surface of Leydig cells [4].

The variance in body weight (increasing and decreasing) in men with MS arecombined with reversible changes in serum insulin levels, SHBG and T concentration. The morbidly weight gain is associated permanently with higher insulin, lower SHBG and lower T levels and has unfavorable effect on male fertility. Some authors confirm inverse association between BMI, insulin sensitivity, T and the sperm parameters in men with MS [5].

Although the effect on obesity on sperm is reported in many studies, some controversial exist referring to the connection between the effect of the IR on sperm characteristics. Du Plessis asserts that male obesity and MS are combined with male hypogonadism and disturbances in semen quality [6]. Some studies have shown association between severity of hyperinsulinemia and sperm count, motility, concentration, morphology and DNA integrity [7] but other studies do not report similar findings [8]. Leisegang et al. first have described the role of insulin and leptin as significant modulators and mediators of the male reproduction. Based on their finding on the presence both hormone in seminal fluid, the authors propose that these hormones could be synthesized and secreted by ejaculated spermatozoa and could have autocrine effect on sperm quality in MS [9].Elsamanoudy at al. report that IR may affect male fertility via inducing effect on the spermatozoa pro-apoptotic genes, proapoptotic proteins, insulin gene expression, DNA fragmentation, and increased seminal glucose. The authors describe existence of a new

spermatozoa stress condition: spermatozoa insulin resistance state as a part of insulin resistance syndrome in MS. Spermatozoa insulin gene expression, seminal plasma insulin and glucose levels are increased in response to spermatozoa insulin resistance stress [10].

The assertion of the existence of spermatozoa stress in state of IR help us to expand the idea of the role of metabolic stress on semen quality in obese men with MS. In a focus of the existing controversial we hypothesed that hyperinsulinemia is the key mediator between abdominal obesity and pituitary–testicular axis and contributes to earlier reduction on male fertility parameters.

The aim of the study was to establish the influence of IR on the semen levels of Ins, T as well as the semen concentration, motility and morphology in men with MS.

Materials/Patients and Methods:-

1. Study population and design:-

We perform the prospective, one-year comparative case-control pilot study in a small population of volunteers with obesity and MS to investigate the role of Ins and T concentrations in semen plasma and to analyze their influence of sperm parameters.

The participants are obese and non-obese volunteers on age between 20-40 years without infertility. The study population has been selected from fitness clubs after advertisements and is based on previous free consultation with endocrinologist.

All participants have been selected according to specific inclusion and exclusion criteria. The main exclusion criteria has been the usage of any drugs such as: anabols, corticosteroids, hormonal replacement therapy with steroids, testosterone, insulin, growth hormone, thyroid hormones, antidiabetic drugs and statins. Participants with a history of some endocrine diseases and diabetes, reproductive tract pathology and infections and with couple infertility or previous infertile problems have not been included as well.

Finally, total 29 men on age between 20-40 years (mean 31.2 ± 0.8 years) have been included in the study.

The participants are divided into three groups according to their BMI: first group (G_1), of non-obese man with BMI $\geq 19.0 \leq 24.9$ kg/m^2, (n_1=4); second group (G_2) of overweight, pre-obese man with BMI $\geq 25.0 \leq 29.9$ kg/m^2, (n_2=4) and third group (G_3) of obese man with BMI $\geq 30.0 \leq 40.0$ kg/m^2, (n_3=21).

The control group (group G_1) are men with normal body weight, BMI ≤ 24.9 kg / m^2. These are healthy volunteers with timely puberty, no known endocrine disease, no prior somatic diseases, no known reproductive problems, no history of erectile dysfunction, aged 20-40 (31,0±7,0 years). The healthy volunteers were selected and directed by a gym, without anamnestic data for usage of anabolic or steroidal drugs.

The study is designed as a case-control type and the results of a normal weight male being compared to the results of an overweight in obese group as follows 1: 1: 3 ratio. The authors believe that the ratio should be 1: 1: 1, but we were unable to motivate a larger number of volunteers to participate in the study.

The study was approved for Local Ethical Committee of the Medical University in Pleven, Bulgaria. All participants have signed an informed concern form before any study activities.

2Methods:-

All included men were hospitalized in University Clinic of Endocrinology and Metabolism in Pleven, Bulgaria for endocrine examination, performing of thyroid ultrasound scan, sample collection and appropriate biochemical and hormonal testing.

Anthropometric data was taken including height (meters) and body weight (kilograms) for determination of the body mass index (BMI). The waist circumference (WC) (cm) was measured on a horizontal plain located between the lowest costal margin and the upper edge of the iliac bone. The systolic and diastolic arterial blood pressure (mmHg) is examined in a sitting position after 5 minute rest.

Blood samples for blood glucose, total cholesterol, HDL-cholesterol, LDL- cholesterol and triglycerides have been performed in a fasting state at 7 am. Total cholesterol, HDL-cholesterol and triglycerides are assessed by the enzyme colorimetric method (GPO- PAP; Biocon® Diagnostik). LDL- cholesterol was calculated by Friedewald formula [11].

The serum hormonal analysis are included: Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL), Testosterone (T), Estradiol (E_2), Cortisol (measured at 6 am and at 10 pm), TSH (thyrotropin), free thyroxin (FT4) and immune-reactive insulin levels. All hormones are assayed by radioimmunoassay technique (RIA).

The *anti-thyroid antibodies* (anti-Tg and anti-TPO) are measured by ELISA kits. Participants with positive autoantibodies are excluded of the study.

A 75 grams *oral glucose tolerance test* (OGTT) was performed with assessment of blood glucose and serum insulin at 0, 60 and 120 minutes in all participants. The interpretation of the results is done by using WHO criteria [12]. Patients with any disturbances in blood glucose levels apart of the normal values are excluded.

The homeostasis model for estimating of insulin sensitivity (HOMA) is calculated by fasting glucose and fasting insulin using the following formula:
HOMA- IR = Fasting glucose (mmol/l) x Fasting insulin (μU/ml)/22.5.The levels of HOMA-IR higher than 2,5 is accepted as an insulin resistant state.

The MS was diagnosed according to IDF criteria [13]. This definition identifies visceral obesity as a key component of MS. The confirmation of diagnosis MS is done by using gender-specific WC cut offs as a mark of central obesity plus any two of the following three parameters: raised triglycerides: ≥1.7 mmol/l; reduced HDL cholesterol: < 1.03 mmol/l or raised blood pressure: systolic BP ≥130 mm Hg or diastolic BP ≥85 mm Hg.

The *semen analysis* is done in Clinical Institute of Reproductive Medicine, Pleven in a private semen laboratory. Seminal fluid is given by masturbation after 2 -7 days of sexual abstinence. In laboratory seminal fluid is divided into two sterile containers for assessments of sperm parameters and seminal Ins and T. Seminal Ins and T were assessed in seminal plasma.

Standard sperm parameters are measured immediately and analyzed according to sperm count, motility and morphology by strict Kruger's criteria using WHO - 2010 manual [14].

Semen samples for hormonal analysis are collected and centrifuged for 10 minutes. The semen fluid is frozen at – 18 ºC and stored for future assessments. Seminal Ins and T are assessed by ELISA kits (commercial test) for quantitative measurement and given by ELIASA reader.

Statistical methods: All analyses have been performed using STATGRAPHICS Centurion XV.I. All data have presented with their mean value and their standards deviations (means ± SD). Comparisons between groups are done using:Independent sample t-test for parametric comparison of the two means, Kolmogorov Smirnov test for a non-parametric comparison and Mann-Withey test for the test median of two groups.Two Way ANOVA for two independents is also used. Two-sided P values ≤0.05 are considered to indicate statistical significant differences. ThePearson (r^2) correlation for measurement the strengths of association between two variables is also done.

Results:

1.Clinical characteristics of the groups:-

The results of data analysis of men with normal BMI are compared with the same results of men with abnormity higher body weight [body weight deviation (BWD)] assessed by BMI, such as overweight men (BMI ≥ 25.0 ≤ 29.9 kg / m^2), aged 31.5 ± 8.3yand men with obesity (BWD ≥ 30.0 kg / m^2), aged 30.1 ± 0.8 y.The comparison between groups have not showed any significant differences about age (P = 0.67), but the mean values of BMI was found significantly different (P = 0.00) between the three groups.

Commonly, 23 participants (82.1%) have three or more clinical or laboratory features of MS.

Low HDL-cholesterol, high triglycerides and WC were the main clinical and laboratory parameters indicating MS. The levels of blood glucose and blood pressure were similar between the groups. The younger age participants are allowed to avoid age-related risk of MS.

2. Lipid prophils:-

The main lipid features indicating MS: significantly lower HDL-Cholesterol [G_2 resp. 1.1± 0,4 and G_3 resp. 0.9± 0,3 mmol/l vs. G_1 1.4 ± 0.1mmol/l); (P= 0.01)] and significantly higher levels of LDL [G_2 resp. 4.3± 0.8 and G_3 resp. 4.9± 1.3 mmol/l vs. G_1 3.2 ± 0.4mmol/l); (P= 0.02)] and triglycerides [G_2 resp. 1.9± 0.1and G_3 resp. 2.7± 0.3mmol/l vs. G_1 1.5 ± 0.2mmol/l); (P= 0.01)] are observed in overweigh subjects and subject with obesity when they are compared with non-obesity subjects, whereas non significantly differences in mean levels of blood glucose were found by comparison between G_1, G_2 and G_3 (Table 1).

Table 1:-Details of the study populations with comparison between the groups

BMI distribution	**Age (years) (mean ±SD**	**Body mass index(kg/m^2) (mean ±SD**	**Patient with MS (≥3 Criteria)**	**HDL-C (mmol/L) (mean ±SD)**	**TG (mmol/L) (mean ±SD)**	**WC (cm) (mean ±SD)**
18-24.9 normal weight G_1 ; n=4	31.0 ± 7.0	22.2 ± 1.5	N=0 (0%)	1.4±0.1	1.1±0.4	93±3.5
25-29.9 Over weight G_2; n=4	31.5 ± 8.3	28.1 ± 1.6	N=2 (9.4%)	1.08±0.2	1.8±0.3	107±7.9
30-40.0 Obese G_3; n=21	33.1 ± 6.9	36.9 ± 4.0	N=21 (72.7%)	0.7±0,9	2.1± 0.2	140±9,4
All patient N=29	32.3 ± 7.0 P = 0.62	33.6 ± 6.5 ***P = 0. 001***	N=23 (82.1%)	1.1± 0.4 ***P = 0. 01***	1.7 ± 0.6 ***P = 0. 01***	114.6 ± 6.5 ***P = 0. 001***

3. Serum glucose and insulin sensitivity:-

All subjects were diagnosed with normal levels of fasting blood glucose (Table 2). The results after *75-gram glucose loading* do not show any pathologic deviations in the levels of blood glucose between G_1, G_2 and G_3 measured at 120 min. Significant differences were found in the levels of blood glucose between G_1 and resp. G_2 and G_3 taking at 60 min. Interestingly, without explanation for us, normal but "flattened" glycemic curve in G_1 was observed. The significant differences in mean insulin levels, measured at 0 and 60 minutes, were found between the three groups [(IRI - 0 min .: n_1 = 7.8 ± 2.1; n_2 = 11.9 ± 8.2 and n_3 = 25.3 ± 12.4 mIU/L; (P = 0.01) and IRI - 60 min: n_1 = 42.4 ± 26.2; n_2 = 68.7 ± 5.2 and n_3 = 166.6 ± 88, 4 mIU/L); (P = 0.01)],whereas no difference was observed in mean insulin levels between the three groups measured at 120 min [(IRI 120 min.: n_1 = 17.8 ± 3.1; n_2 = 22.9 ± 1.2 and n_3 = 23.3 ± 0.4 mIU/L; (P = 0.1)].

Participants from G_1 show a normal value of HOMA-IR index less than 2,5. A progressive raising in HOMA-IR was observed with a significant differences in their value between normal-weight men and obese men, as well as between overweight men and obese men (P= 0.01). Participants with MS had higher insulin basal levels before loading at 0 min [(MS subjects: 19±3.2 vs. non MS subjects: 7.2 ± 2.5mIU/l);(P=0.001] and significantly higher levels after loading, measured at 60 min. [(MS subjects: 168.4± 32.7 vs. non MS subjects: 41.3±12.1 mIU/l);(P=0.001].

Table 2:-Details of the blood serum glucose levels and HOMA- IR index according to BMI

BMI distribution	**Serum glucose 0 min (mmol/l) (mean ±SD**	**Serum glucose 60 min (mmol/l) (mean ±SD**	**Serum glucose 120 min (mmol/l) (mean ±SD**	**HOMA- IR Index**
Normal body weight *[n=4)*	5.1 ± 0.6	4.8 ± 3.1	4.9 ± 1.1	1.7 ± 0.4
Over weight	4.9 ± 0.6	7.9 ± 3.5	6.7 ± 4.4	2.7 ± 1.1

[n=4)				
Obese *[n =21)*	5.0 ± 1.0 NS	7.9 ± 1.8 ***P = 0.09***	6.7 ± 5.2 NS between G_2 and G_3	5.4 ± 2.5 ***P = 0.01***

4. Pituitary-hypothalamic-gonadal axis:-

Table 3 shows all results of the serum hormonal levels for groups with obesity and MS.The serum levels of FSH, LH and PRL between the groups from men with BWD and MS were in the reference ranges. The concentrations of LH are found slightly lower, near to the lower limit in both obese group and MS group but without significant differences between G_1 and G_2 and between non-MS and MS groups. The mean levels of FSH are statistically decreased in MS group. The significant differences in mean PRL levels were found between G_1 and G_2, as well as between G_1 and G_3 (P = 0.009). Men from G_1 and men without MS were found with the lowest prolactin levels. The highest PRL was observed in the overweight group men, nevertheless of its normal value. Interestingly but not surprisingly there was observed significantly higher levels of PRL in men with MS inspite of the fact that the PRL level was in normal reference range.

The mean T levels are below the limit in obese and MS groups. The observed values were not statistically decreased after the comparison between G_3 and $G_1$and G_3 and G_2 resp. Subjects with MS had statistically lower levels of total testosterone, comparable to subjects without MS.

Significant differences in mean morning cortisol levels (at 6am) between the study population were not found. A significant increased levels were observed in evening cortisol (at 10 pm) in obese men and men with MS, although the levels were in range limit. Participants with obesity and MS were found to show equal cortisol levels in the early morning and late evening as well. An assumption that this equilibrium is early mark for a loss of circadian rhythm of the cortisol secretion in the state of IR can be made.

Due to the small number of men, the results of blood estradiol levels are not reliable, according to BMI division. The participants with MS have a tendency to higher levels of E_2.

Table 3:-Hormonal parameters according to presence of obesity and MS

Patient population	**LH (IU/L)**	**FSH (IU/L)**	**PLR (ng/L)**	**Testosterone nmol/L (N=6,0-13,3)**	**Cortisol -6 h (nmol/L)**	**Cortisol-22h (nmol/L)**	**E2 (pmol/l) (102-97 between 20 to 40 years)**
Normal body weight n_1=4	2.5 ± 0.5	5.2 ± 1.9	4.0 ± 0.5	6.6 ± 0.9	239.7±48,6	169.3 ± 52.2	
Overweight n_2=4	2.4 ± 1.3	7.4 ± 8.0	15.9± 4.3	6.5 ± 1.0	241.9 ±96.4	198.8 ±73.5	
Obese n_3 =21	1.6 ± 1.3 NS	4.9 ± 3.3 NS	9.1 ± 3.7 ***P = 0.009***	*5.9* ± 0.7 ***P=0.05***	298.2± 94.3 NS	290.9 ±84.6 ***P = 0.004***	
Without MS	2.5 ± 0.7	5.4 ± 4.6	6.5 ± 1.9	6.9± 0.2	247.5 ±32.5	159 .2±41.8	101.3± 0.5
With MS	1.7 ± 0.1 NS	3.7 ± 0.9 ***P = 0.01***	16.7 ± 0.7 ***P = 0.01***	*5.8* ± 0.5 ***P = 0.04***	298.8± 87.4 NS	290.7 ±72.1 ***P = 0.004***	111. ±10.1 ***P = 0.05***

***5.* Assessment of the pituitary-thyroid axis:-**

The significant differences in the mean TSH blood levels are not found between men in the three groups [TSH: G_1 = 3.0 ± 0.8 mIU/L w G_2 = 4.0 ± 1.2 and G_3 = 4.7 ± 3.0 mIU/L; P = 0.06). The levels of FT4 are non-significantly different between G_1,G_2 and G_3. Obese men and men with MS are found to show normal free T_4 levels and higher TSH levels, in excess of the upper limit of reference range, above 4.2 mIU / L, such as features of subclinical hypothyroidisms.

6.Testosterone levels in seminal plasma:-
The mean T levels in seminal plasma are lower in G_2 and G_3 than G_1, although these differences did not significant. [Testosterone: G_1 = 2.4 ± 1.1 nmol/L; G_2 = 1.4 ± 0.5 nmol/L and G3 = 1.7 ± 0.6nmol/L; (P = 0.06)]. The subjects with MS show trend to significant decreased levels of testosterone in seminal plasma than subjects without MS [Testosterone: MS = 1.6 ± 0.4 nmol/L; vs. non MS = 2.2 ± 0.3 nmol/L; (P = 0.05)].

***7.Insulin levels in seminal plasma*:-**
The significant differences in mean insulin levels in seminal plasma are not found between the non-obese group and group with BWD, although the levels in overweight and obese group were found decreased. [Insulin: G_1 = 8.6 ± 2.0 mIU/l; G_2 = 6.0 ± 2.7 mIU/l and G_3 = 5.0 ± 2.9 mIU/l: (P = 0.07)]. Men with MS have border tendency to significant lower levels of seminal insulin than men without MS [Insulin: MS = 5.6 ± 3.2 mIU/l; without = 7.5 ± 2.7 mIU/l: (P = 0.056)].

8.Semen analysis:-
Sperm concentration is found significantly lower in obese group compared to the first group (P=0.01). Although progressive sperm motility and sperm morphology are decreased in obese group, there are no significant differences between the three groups for the both parameters.

Details of the semen parameters in participants with MS are presented on table 4. Despite the mean values of progressive sperm motility and normal sperm morphology are lower in MS, no significant differences are established when they are compared to the same parameters in non-MS group. Interestingly, the sperm concentration is higher in patients with MS compared with non-MS group. A similar result in sperm concentration is observed when comparing overweight patients with non-obese patients.

It was found that subjects with higher BMI and IR have a significantly negative correlation between FSH [(r^2 = -0.5); (P<0.05)], T [(r^2 = -0.4); (P<0.05)] and sperm motility.

HOMA-IR [(r^2 = -0.5);(P<0.05)], cortisol [at 6 am (r^2 = - 0.5) and 10 pm (r^2 = - 0.5); (P<0.005)] and E2 [(r^2 =-0.5); (P<0.05)] are also negatively associated with sperm concentration in G3 group and MS group.

BMI [(r^2 = -0.4);(P<0.05)] and HOMA IR [(r^2 = - 0.5);(P<0.05)] and morning and evening cortisol (r^2=-0.5);(P<0.05) are also negatively associatedwith normal sperm morphology in obese and MS group.

Participants with MS have higher serum insulin and have lower serum and seminal T together with poorer teratozoospermal index. Negative impact on teratozoospermal index was established between E_2 [(r^2=-0.6);(P,0.001)] and morning and evening cortisol [(r^2=-0.4);(P<0.05)] in the MS groups.

A significant positive correlation was found between FT4 [(r^2=0.6);(P,0.001)] and sperm concentration, motility and normal morphology in obese and MS group.

In overweight patients a significantly negative correlation was found between HOMA-IR [(r^2= - 0.5);(P<0.05)], PRL [(r^2=-0.4);(P<0.05)] and sperm concentration.

Table 4:-*Distribution of the value for sperm parameters*

Patient	**Progressive Sperm motility (A+b %)**	**Sperm concentration (10^6 per ml)**	**Sperm morphology (normal form> 4%)**
Normal body weight n_1=4	48.0 ± 27.2	50.0 ± 27.3	5.2 ± 3.2
Overweight n_2=4	47.0 ± 24.6	224.2 ± 123.6	4.6 ± 2.5
Obese n_3 =21	42.2 ± 16.9 *NS*	*60.2 ± 52.8* *P=0.01*	*3.7 ± 1.8* *NS*
Without MS	47.7 ± 26.8	*79.*± 36.8	*5.3±2,9*
With MS	44.± 20.1 NS	*142.± 87.5* ***P=0.01***	*4.5±2,1* NS

Discussion:-

Obesity, Insulin resistance and BMI:-

The current study confirm the adverse effect of central obesity and IR on the pituitary-hypothalamic-gonadal axis and standard semen parameters. Both IR and higher BMI have a moderate negative effect on the gonadotropic hormones, T and all spermal parameters and correlate negative with them [LH (r^2= -0.4), FSH (r^2= -0.5) T (r^2= -0.6)]. HOMA-IR moderately correlate with PRL and sperm concentration. Total testosterone was found associated with IR, BMI and E_2 and all spermal parameters. It was observed that both morning and evening cortisol negatively associates with T and sperm concentration and normal morphology.

The fact that IR is more strongly and negatively correlated than BMI with the two main components of MS high triglycerides (P=.001) and waist circumference (P=0.01) is somewhat unexpected. It is also surprising that HOMA-IR inversely correlates more with T and E_2 rather than BMI. The follow-up sperm analysis is showing slightly similar negative association between IR, BMI, sperm concentration (r^2 = -0.5) and morphology (r^2 = -0.4). It is not clear whether these deviations would reflect the male fertility, because the observed men do not have any conception trials.

Perhaps the most important finding in that study is the presence of equilibrium in diurnal and nocturnal secretion of cortisol in subjects with MS and proven adverse impact on nightly cortisol on the IR and WC. We may speculate that IR stress in subjects with MS hyperactivate hypothalamic-pituitary-adrenal axis with loss of circadian rhythm of cortisol's secretion and contribute to developing of central obesity as a main features of MS. Apart of the effects of cortisol on visceral adipose tissue it has also been found an unexpected and independently negative association between cortisol and sperm concentration, normal morphology and teratozoospermal index in subjects with MS [15]. Hample and coworkers have reported that serum and seminal cortisol may acts as an antiandrogens by blocking off enzymes involving in testosterone steroidogenesis or like as T receptor's antagonists, after liganding with steroid hormone receptors or trough hypothalamic- pituitary axis [16].

The effect of BMI on sperm parameters has been reported in some scientific studies. Although various pathophysiological mechanisms affecting the gonadal axis have been described, the direct effect of BMI on sperm parameters remains controversial [17]. According to some authors, BMI does not affect the sperm parameters but according to others this mechanism is depending on BMI deviations and third authors report a decrease sperm count, motility, vitality, morphology and DNA integrity [24, 27]. The results of two meta-analysis are also controversial [25,26]

Serum and semen insulin and insulin resistance:-

The results in current study confirm that the serum insulin and HOMA-IR levels are predominantly elevated in obese men and men with MS than men with normal weight and without MS. In obese and MS groups all participants are diagnosed with basal or stimulated hyperinsulinemia. The mean semen insulin levels in obese men are found decreased, but not significantly, whereas the men with MS had tendency to significant lower level of insulin compared with men without MS.

When comparing the serum and semen values of Ins it is found that sperm insulin levels are significantly lower than serum insulin levels measured at 0 minutes in G2 and G3 (P=0.01). After calculation of the differences in serum and semen Ins between G_1, G_2 and G_3 an interesting and unexpected results are observed. Participants from G_1 have higher levels of seminal insulin and are showing negative difference ($Ins_{d)}$; [Ins_d in G_1**= -0.6** ±0.1 mIU/l) whereas mean`s Ins differences in G_2 and G3 are found positive [(Ins_d G_2 =5.9 ±0.6 mIU/l and Ins_d G3 = 20.,3±0.8 mIU/l);(P=0.001)]. Comparison of the same parameters between MS and non-MS groups are showing positive differences in MS group and also negative differences in non-MS group [(Ins_d in MS = 13.3.0 ± 0 mIU/l and Ins_d in non-MS group **-0.3** ± 0.2); (P=0.01)]. A negatively strong correlation is reported between serum and seminal insulin in both obese and MS groups [(G3 r^2=-0.8 and resp. MS gr. r^2=-0.7)]. A moderate negative correlation exists between semen insulin, sperm motility (r^2= -0.5) and sperm morphology (r^2 = -0.3). The high insulin levels in seminal plasma in state of obesity and IR reduces motility and adversely affects the sperm morphology.

The origin of the sperm Ins is not very clear. Schoeller at all.report that Sertoli cells are shown to synthesize and secrete insulin and insulin-like peptides [18]. Another sources of seminal insulin may be the serum insulin which crosses freely the frontier of blood-testicular barrier [19] or may be secreted post-ejaculation in autocrine manner in seminal vesicles and prostate [20. The finding in the current study that non-obese and non-MS participants have

higher sperm than serum insulin concentrations indicates the important role of seminal insulin in normal metabolic state as a sperm mediator with autocrine effect on the sperm quality, sperm cells differentiation and maturation. The negative correlation between serum and seminal insulin in both obese and MS groups show that visceral obesity and IR have unfavorable effect of testicular insulin regulation and spermatozoa parameters [21].

Serum and semen testosterone and insulin resistance:-

When comparing the serum and semen values of T it was found that sperm T levels are significantly lower than serum T levels in all participants (P=0.01). After calculation of the differences in serum and seminal T between G_1, G_2 and G_3 groups it was observed that seminal levels in G_3 is lower than G_2. Participants from G_1 have higher levels of seminal T with negative differences between serum and seminal T whereas those differences are positive in G_2 and G_3 and confirm lower seminal level of T in state of obesity [(T_d G_2 =5.1 ±0.4 mIU/l and T_d G_3 = 4.2±0.3 mIU/l);(P=0.04)]. Comparison in the same parameters between MS and non-MS groups shows also lower seminal level of T in state of IR [(T_d in MS = 4.2 ± 0.3 mIU/l and T_d in non-MS group 4.7 ± 0.4); (P=0.1)]. A negative strong correlation is reported between serum Ins and seminal levels of T in both obese and MS groups [(G_3 r^2=-0.6 and resp. MS gr. r^2=-0.5)]. There is a similar relationship between BMI and seminal T (r^2= -0.4).

The study confirms that endogenous hyperinsulinemia and BMI have a negative effect on seminal T levels. The fact that IR is correlated more negative with seminal T than BMI is somewhat unexpected, but it is not surprising. It is not completely understood for us whether changes in insulin sensitivity *per se*, or both IR and high BMI inversely impact the normal spermatozoa metabolism. It is possible that the existing interaction between various hormones of visceral white fat mass in state of decreased insulin sensitivity causes unbalance in normal testicular homeostasis and spermatozoa maturation.

Thus we confirmed that the semen insulin and testosterone levels and spermatozoa parameters are affected of both IR and central obesity related hormonal-metabolic factors. Leisegang and co-authors report a similar results [9]. In contrast, we do not find any significant differences in seminal insulin and testosterone levels between the two patients groups with BWD, but we report a tendency of reduction in seminal Ins in subjects with MS.

Although the source of seminal insulin is unclear, probably penetrates through the blood-testicular barrier or is secreted into the ejaculate, we accept the biologically importance of seminal Ins in state of IR as a pathophysiological regulator on spermatozoa metabolism. We also speculate that IR condition did not allow adaptation to hormonal variations locally in the testis and did not counteract the effect of various elevated hormones such as insulin, E_2, cortisol, TSH and PRl on spermatogenesis. Aquila and colleagues hypothesize that elevated insulin levels during spermatogenesis may cause insulin resistance in the sperm cells [22].There is biomolecular evidence to support a similar thesis based on the tracking of the intracellular insulin cascade in the spermatozoa by PI3K / Akt to protein kinase B. This metabolic pathway *in vivo* increases the synthesis of nitric oxide and mediates a beneficial effect on ejaculated spermatozoa. In human tissues, the cascade has a negative effect and causes insulin resistance in sperm cells, similar to insulin resistance in other tissues [22].

Insulin resistance and Sperm Parameters:-

The semen parameters showed deviations in morphology and the teratozoospermal index in obese male and in subjects with MS. Interestingly, we do not find any differences in mobility and concentration between the two groups of men with BWD. To investigate the extent to which obesity is associated with a negative impact on the sperm morphology a correlation analysis has been conducted. The results show slightly and moderately significant negative correlations between glucose (r^2 = -0.2) and insulin (r^2 = -0.4) levels before and during OGTT, BMI (r^2 = -0.3), HOMA-IR index (r2 = -0.4), FSH (r^2 = -0.2) and testosterone (r^2 = -0.2).

According to studies, there is no relationship between serum testosterone and sperm concentration [23], while other authors emphasize that testosterone plays a critical role both in sperm maturation and male reproductive function [24]. The influence of metabolic-hormonal changes in obesity on male reproductive function requires further studies.

In this current study, deteriorated sperm quality including decreased motility and concentration is a result of the hyperinsulinemia with increased evening cortisol production, as well as daytime stress and local factors associated with a change in seminal testosterone and insulin concentration. IR is associated with increasing enzyme's aromatase activity, followed by rising peripheral testosterone to estrogen conversion. It was found that endogenous

glucose-dependent hyperinsulinemia trough lowering testosterone levels in semen, modulates sperm function after ejaculation and adversely effects on sperm morphology.

Theoretically we could speculate that IR condition is not allowing any adaptation to hormonal fluctuations locally in testis and is not counteracting the effect of various elevated hormones such as insulin, E_2, cortisol, TSH and PRl on spermatogenesis.

A relatively small numbers of participants is the most important limitation of this pilot study. We intend to perform a future research among a large study population with similar design with extended hormonal and spermal parameters in focus on reproduction.

Conclusion:-

Although the source of seminal insulin is unclear, probably penetrates through the blood-testicular barrier or is secreted into the ejaculate, it is now proven that seminal insulin has different functions on spermatozoa physiology in non-obese and obese conditions. The biologically complexity of Ins resistant state makes it difficult for explanation the existence of the spermatozoa stress.

This study presents relationships between sperm parameters in state of IR and male visceral obesity. The finding that Ins resistance strongly correlates with seminal Ins, seminal T and sperm parameters indicates the IR as a pathophysiological marker in down- regulation on spermatozoa physiology.

Although the significance of changes in semen insulin and testosterone concentrations in state of IR is difficult to explain, this research confirms that IR causes unfavorable hormonal and metabolic effects at the central and testicular levels and could have impact on male reproductive health. The most important finding with clinical-application is the detection of changes in sperm morphology in obese men and in men with MS.

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