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UNIVERSITY OF ZAGREB

SCHOOL OF MEDICINE

Albert Lila

IMPACT OF OBESITY ON OVARIAN RESERVE

DISSERTATION

Zagreb, 2019.

UNIVERSITY OF ZAGREB

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UTJECAJ DEBLJINE NA OVARIAJLNU REZERVU

DOKTORSKI RAD

Mentor:

Prof. Dr Velimir Šimunič

Zagreb, 2019.

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Supervisor:

Prof.dr Velimir Šimunič

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This study has been done in Kosovo Occupational Health Institute in Gjakova, Gynaecology Cabinet and IVF Clinic in Zagreb.

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LIST OF ABBREVIATIONS:

ASRM	American Society of reproductive medicine
AES	androgen Excess society
AMH	anti-Müllerian hormone
AR	androgen recptor
BMI	body mass index
AT	adipose tissue
CT	computerised tomography
MRI	magnet resonance imaging
CVD	cardiovascular disease
CVI	cardiovascular insult
WC	waist circumference
HC	hip circumference
WSR	waist to stature ratio
WHR	waist to hip ratio
RMR	resting metabolic rate
FFA	free fatty acids
WAT	white adipose tissue
BAT	brown adipose tissue
VEGF	vascular endothelial growth factor
CNS	central nervous system
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BMP	bone morphogenetic protein
PPARy	peroxisome proliferator- activated receptor gama
C/EBP-β	CCAAT enhancer- binding protein beta
HOXA	homebox genes

UCP-1	uncoupling protein-1
TG	triglycerides
HSL	hormone sensitive lipase
LPL	lipoprotein lipase
ATGL	adipose triglyceride lipase
NEFA	non-esterified fatty acids
GH	growth hormone
ASP	acylation – stimulating protein
PCOS	polycystic ovary syndrome
VLDL	very low-density lipoproteins
BMR	basal metabolic rate
MS	metabolic syndrome
StAR	steroidogenic acute regulatory protein
SHBG	sex hormone binding protein
HSD	hydroxysteroid dehydrohenase
DHT	dihydrotestosterone
T	testosterone
E	estrogen
E_2	estradiol
P_4	progesterone
TSPO	translocator protein
DHEA	dehydroepiandrosterone
TBF	tumor necrosis factor
IL	interleukine
TGF	transforming growth factor
CRP	C reactive protein
PAI	plasminogen activator inhibitor
FSH	folliculostimulating hormone

LH	luteinising hormone
IR	insulin resistance
AMPK	adenosine monoposhpate activated protein kinase
RBP-4	retinol binding protein
MMP	matrix metalloproteinase
NPY	neuropeptide-y
ACTH	adrenocorticotrophic hormone
POMC	proopiomelanocortin
MCR	melanocyte receptor
AgRP	agonti-related protein
CART	cocaine-amfetamine regulated transcripts
nA	nucleus arcuatus
MSH	melanocyute stimulating hormone
CRH	corticotrophin releasing hormone
DA	dopamine
KISS	kisspeptin
FAI	free androgen index
ECS	endocannabinoil systems
ROS	reactive oxygen species
EDC	endocrine disruptor chemicals
BPA	bisphenol A
ESHRE	European Society for Human Reproduction
FNPO	follicle number per ovary
OV	ovarian volume
AFC	antral follicle count
AF	antral follicle
OHSS	ovarian hyperstimulation syndrome
PCOM	polycystic ovarian morphology

MC	menstrual cycle
OR	ovarian reserve
POR	poor ovarian response
IVF	in vitro fertilization
HR	high response
IGF	insulin like growth factor
GDF	growth differentiator factor
CPR	clinical pregnancy rate
LBR	live birth rate
ART	assisted reproductive tehnologies
OS	ovarian stimulation

1. INTRUDUCTION

1.1 Adipose tissue and obesity

Adipose tissue is an important organ, always present in the human body. Adipose tissue (AT) is quantitatively the most variable body component as it can make up from a few percent to more than 50% of body mass in obese persons. AT sites are also highly variable, ranging from subcutaneous areas and the abdominal cavity to retroperitoneal, mesenteric and muscle depots. There are more than 15 discrete AT depots such as perirenal, orbital, epididymal, omental, popliteal, etc. Approximately 90% of adipose tissue consists of adipocytes (fat cells), which are embedded in stromal connective, endothelial (vascular) and mesenchymal adipocyte precursor cells.
Apart from storing lipids and energy, adipocytes are very active in the secretion of hormones and adipokines used in endocrine and paracrine activities. When energy cannot be supplied directly from the circulation or stored carbohydrates, lipids are mobilised from AT by lipolysis. In adipocytes, triglycerides are broken down into glycerol and free fatty acids, which produces an energy stimulus for organs, as well as the glucogenic substrates in the liver. Metabolic control of AT is regulated by hormones and catecholamines (the sympathicus and insulin). Insulin is the most potent antilipolytic hormone in adipose tissue that controls the anabolic activities of adipocytes (1,2). Adipose tissue is the main energy store in vertebrata and an endocrine controller of energy balance. White adipose tissue maintains energy homeostasis, while brown adipose tissue controls thermoregulation.

The main form of adipose tissue is white adipose tissue that makes up 80-90% of that organ. White adipose tissue performs most of the metabolic and endocrine functions of AT and is richly vascularized and innervated. AT is mostly composed of **adipocytes**, which contain up to 90% of triglycerides, the most concentrated form of energy. Fat cells are incorporated into loose connective tissue that is richly vascularized and innervated. Under normal circumstances, women have more adipose tissue than men (25% vs. 15% of total body mass) due to energy preparations for pregnancy.

Obesity is a multifactorial chronic disease characterized by excessive storage of energy in excess adipose tissue (AT) that is closely related to insulin resistance. Abnormal fat accumulation has become a major global health problem due to increased risks of several chronic diseases and premature mortality. Nurses' Health Study and other major studies have established a correlation between obesity and the risk of metabolic syndrome, cardiovascular disease and mortality.

Worldwide obesity has almost tripled since 1975, reaching 1.9 billion overweight adults in 2016. Out of this number, more than 650 million are obese (2,3). Categories of adult obesity are based upon body mass index (BMI), which correlates with percent body fat. This relatiioship varies among individulas by sex, age and race.
The prevalence of obesity has increased in last 3 decades, so that prevalence of overweight added obese women in numerous europian and western countries is around 60%. The obesity is increasing in women of reproductive age.

Body Mass Index (BMI) is the most common measure to express body fat content in the body of a normal or overweight person. BMI is the ratio of body weight divided by the square of height (kg/m^2) that expresses the area of the body. Such a calculation correlates well with the mass of fat tissue in the body and enables a reliable classification into the following categories:

Table 1. Classification of BMI index by WHO

• BMI < 18.5 kg/m^2		underweight
• BMI	18.5–24.9 kg/m^2	normal
• BMI	25–29.9 kg/m^2	overweight
• BMI ≥ 30 kg/m^2		obesity
-	30–34.9 kg/m^2	class 1
-	35–39.9 kg/m^2	class 2 (severe obesity)
-	≥ 40 kg/m^2	class 3 (morbid obesity).

For BMI in children, the difference is expressed by both gender and age. Most of the world's population lives in countries where obesity is a more common cause of death than malnutrition. In such an obesity pandemic, indicators in children are particularly worrisome. Excessive bodyweight and obesity occur in 41 million children under the age

of 5, and 340 million children and adolescents aged 5–19 (WHO 2016). The prevalence of overweight and obesity in children and adolescents has increased from 4% in 1975 to 18% in 2016, while obesity rates increased from 1% to 8%.

Globally, the average BMI in women increased from 22.1 kg/m^2 to 24.4 kg/m^2, and the incidence of obesity increased from 6.4% in 1975 to 14.9% today. The number of obese women increased 5 times in the last 40 years, with 20% of women being morbidly obese. Interestingly, a rapid increase in the prevalence of obesity has been registered in developed countries, but also in developing countries and many poor populations.
The etiology is seemingly simple, arising from an imbalance between energy intake and energy expenditure. The causes are inadequate nutrition, reduced physical activity, accompanied by genetic and epigenetic effects(2,3,4). Shown below is the prevalence of overweight and obesity in women in selected countries.

Table 2. Differences in obesity prevalence in selected countries

Country	Overweight BMI 25–29.9 kg/m^2	Obesity ≥ 30 kg/m^2
USA	28.5 %	35.7 %
Spain	26.5 %	22.9 %
Albania	27.5 %	9.3 %
Macedonia	33.9 %	21.1 %
Europe	28 %	23 %
Italy	21.9 %	8.2 %
Kosovo ()	**27.7 %**	**23.5 %**

A recent report on the prevalence of obesity in 16 European countries provides somwhat different numbers (5):

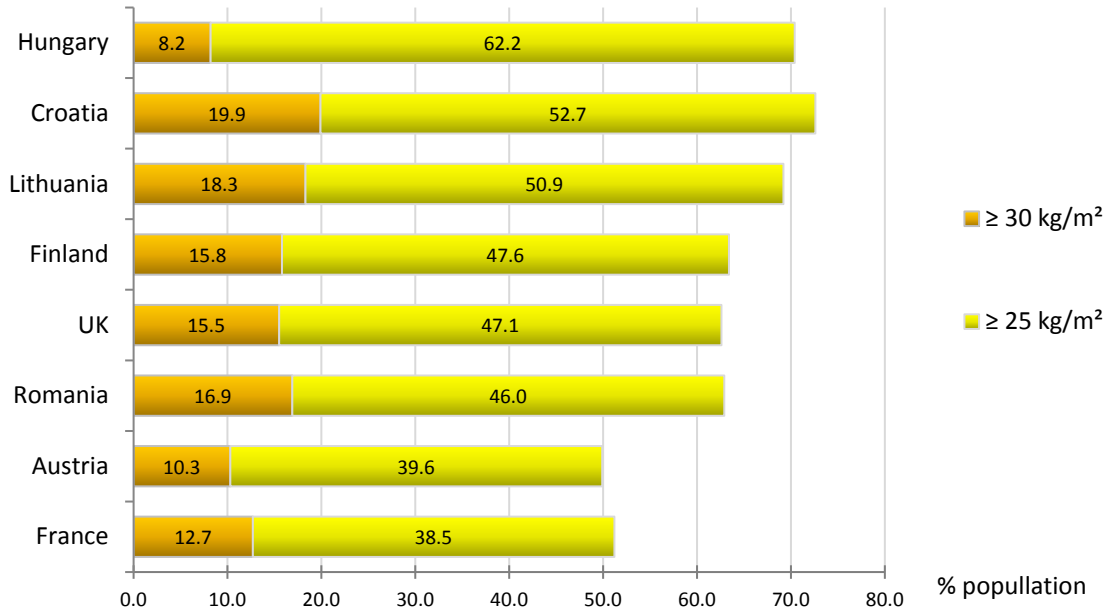


Figure 1. Prevalence of obesity (according to BMI) in some European countries

1.1.1 Anthropometric obesity measurement

Depending on the location of accumulated adipose tissue, we differentiate between subcutaneous and visceral (central) AT. Gluteofemoral fat typically occurs in women (gynecoid fat distribution), while android obesity is characterized by accumulation of abdominal fat. Visceral (abdominal) obesity carries numerous health risks - metabolic syndrome, cardiovascular diseases and some types of cancer. Anthropometric measures for an assessment of general and central obesity include:

a) body mass index (BMI) for general obesity assessment
b) waist circumference (WC)
c) waist to hip ratio (WHR)
d) waist to stature ratio (WSR) - ratio of WC to height
e) hip circumference (HC)
f) body adiposity index (BAI) - ratio of HC to height.

The anthropometric measures listed in b) to f) are indicators of central obesity and thus of cardiovascular risk. The relationship of visceral AT to subcutaneous AT, skinfold measurement, and sagittal abdominal diameter are also used to assess the health risk. Measures of visceral obesity are more precise – dual energy x-ray absorptionometry (DEXA), CT and MRI.

However, in standard clinical practice it is considered enough to measure WC, WHR, HC and possibly WSR. Anthropometric measurement of central obesity is characterized by a higher specificity and sensitivity for the determination of cardiovascular risk (CVD risk) than general obesity measurement (BMI) (2,6,7,8). The cut-off values for central obesity are (1,3,9,10):

Table 3: Normal value of WC and WHR

Measure	Men	Women	
WC Waist circumference	102 cm 94 cm	88 cm 80 cm	WHO DM Society
WHR Waist to hip ratio	0.90	0.85	
WSR Waist to stature ratio	0.5		

WHO – World Health Organization; DM – diabetes mellitus

Approximately half of the women in developed countries have WC > 88 cm (54% of women and 37% of men), which correlates with a high incidence of metabolic syndrome in these populations (> 30%).The degree of cardio-vascular risk (CV) in case of central obesity can be expressed with 3 classes of WHR:

Table 4. Classes of WHR related to CV risk

Risk	Men	Woman
Low	0.95 or lower	0.80 or lower
Moderate	0.96–1.0	0.81–0.85
High	1.0 or higher	0.86 or higher

Numerous studies (Framingham Heart Study, Nurses' Health Study, Buffalo Health Study) have confirmed that obesity increases the risk of CVD (1,7,8). An Australian study conducted on 4487 women aged 20 to 69 has confirmed that anthropometric

measures of central obesity are strongly associated with 10-year CVD risk (1,7). Measures of abdominal obesity are better predictors of CVD risk compared with BMI. Shown below are ORs only for the prediction of 10-year CVD risk(1,9,11).

Table 5. Cardiovascular risk according to anthropometric obesity measurments

	BMI	WC	HC	WHR	WSR
OR (CI)	1.71 (1.59–1.85)	2.12 (1.95–2.29)	1.55 (1.44–1.68)	2.27 (2.08–2.47)	2.35 (2.17–2.56)

All central obesity measurements had a comparable predictive value (9). In several meta-analyses it has been pointed out that WC and WHR are the most acceptable surrogate measures for CVD risk. Women with WHR≥0.88 are 3.25 times more at risk of cardiovascular disease than those with WHR <0.72. BMI only appears to be inadequate for accurate risk prediction.

The composition of the body changes with age. Adipose tissue increases and muscle mass decreases. The increase in AT with aging leads to its distribution towards the abdominal region. These changes are more pronounced in women due to lower basal metabolism (basal metabolic rate). After the age of 20 the resting metabolic rate (RMR) falls by 2–3% every 10 years (total decrease up to 20%). Oxidation of fat, RMR, and energy imbalance alter the body composition with age (12). By comparing anthropometric and advanced (CT, DEXA) determination of AT accumulation, considerable differences were found according to gender, race and age (11).

Today's lifestyle implies a different relationship between energy intake and expenditure. The basal metabolism requires 1500–1600 kcal/day, while physical activities (which are reduced) use up only 500–1000 kcal/day. This means that the total daily need (depending on age) ranges between 2000 and 2500 kcal (1,14).

1.1.2 Distribution of adipose tissue and obesity phenotypes

Adipose tissue is deposited in 5 different regions – depots (12,16,17):

1. **Subcutaneous adipose tissue**, in obesity it can be distributed from head to toe. It has superficial and deep AT regions in the body and gluteofemorally. Their control and functions differ.
2. **Visceral, intraperitoneal AT** affects the omentum and mesentery, and the epiploic appendices. It is drained by lymph and the portal vein, which points to possible pathophysiology.
3. **Retroperitoneal and pelvic AT** is topographically specific but is often categorized as visceral AT.
4. **Pericardial AT** is located around the heart.
5. **Intramuscular AT** is found in the muscles.

It has been pointed out that visceral adipose tissue involves cardiometabolic risk, while other AT sites may have a neutral or even protective metabolic effect.
Regardless of BMI, women mostly exhibit a subcutaneous, **gluteofemoral phenotype** AT called gynecoid AT, which is responsible for the female pear-shaped body shape. Male, android obesity dominated by visceral and subcutaneous abdominal fat is less common. The **android phenotype** gives the body the shape of an apple. **The gynecoid phenotype** occurs in female puberty, where enough energy reserves trigger the hypothalamic-pituitary regulation of the ovarian function, menarche and subsequent cyclicality. In this way, the brain is informed of the readiness for pregnancy.

The uptake of free fatty acids (FFA) and synthesis of triacylglycerides are greater in female gluteofemoral subcutaneous AT compared to abdominal AT. A young woman with a BMI of 22.5 kg/m^2 has about 18 kg of adipose tissue, which amounts to almost 30% of body mass. Only 5% of this mass is visceral AT (). In contrast, men have 12 kg of fat (15% of body mass), of which 10-12% is visceral AT. Different distribution of fat in the gluteofemoral region is induced by the expression of 280 genes that are largely different from those that stimulate abdominal accumulation of fat.

Fat distribution in women is also controlled by ovarian hormones. Estrogens inhibit visceral and abdominal (subcutaneous) adipose tissue and stimulate gluteofemoral AT. Androgens have the opposite effect. Generally, AT distribution depends on androgen and estrogen balance (1,7).

The adipose tissue phenotype also depends on vascularization and innervation. White and brown adipose tissue can produce vascular endothelial growth factor (VEGF), which is a stimulus for neoangiogenesis.

Aging leads to ectopic accumulation of fat in the bone marrow, muscles, liver, which can lead to the dysfunction of these tissues. Also, subcutaneous AT is lost, while the preservation of the visceral depot leads to metabolic disorders. Aging causes reduced capacity to convert preadipocytes into adipocytes, which may increase lipotoxicity. BAT (brown adipose tissue) functionality decreases with age, compromising thermoregulation. BAT atrophy is accompanied by a loss of BAT activity, decrease in adrenergic signalling, and a decline in the mitochondrial function. The consequences are reduced cold tolerance and disturbances in the control of body weight. BAT activity is five times lower at the age of 60 than at the age of 20, promoting age-related weight gain (12). As alcohol affects the oxidation of AT and promotes its storage, excessive consumption is in correlation with obesity.

Regional distribution and redistribution of fat as well as lipolysis are also influenced by adrenaline and cortisol. These hormones support mobilization of abdominal visceral fat. Weight loss and exercise sometimes result in negative regional mobilization of fat, with the loss of AT from deeper depots.

1.1.3 Physiology and pathophysiology of adipose tissue

Adipose tissue (AT) is not a passive storage of fat and energy, but a very active organ with many physiological functions:

- energy storage
- maintenance of energy homeostasis
- regulation of body temperature
- control of the hypothalamic-pituitary axis
- control of gonadal function
- control of reproduction
- endocrine and paracrine functions
- protection of the body from trauma.

In women, AT dictates pubertal changes in the hypothalamus, informing the central nervous system (CNS) about the adequate energy reserve for pregnancy. The composition of the body, with too little or too much AT, reduces the reproductive potential and disrupts the menstrual cycle (1,7,14).

AT is mostly made up of white adipose tissue (**WAT**), from which adipocytes are partially transformed into brown adipose tissue (**BAT**). There are major functional differences between WAT and BAT. It has been established that both types of tissue are metabolically active, with antagonistic functions:

- WAT is the primary energy storage site
- BAT regulates energy expenditure (by generating heat)– adaptive thermogenesis

1.1.4 Differentiation of adipocytes – adipogenesis

The accumulation of adipose tissue is a remnant of evolutionary physiology in terms of adaptation to survival conditions. Endothelial cells of vascular stroma are an unlimited source of adipocyte precursors. Increased caloric intake combined with inadequate expenditure promotes the hyperplasia of adipocytes.

The number of adipocytes grows as a result of stimuli for the conversion of pluripotent mesenchymal stem cells into preadipocytes, which are then differentiated into adipocytes. Fat cells have a high storage capacity for lipids, which significantly changes their volume. Such fibroblast transformation produces a mature (white) fat cell, a lipid droplet surrounded by a thin cytoplasm (with organelles) and a nucleus pressed towards the surface of the cell. The mesenchymal progenitors of adipocytes are distributed in the connective tissue under the skin and in the abdominal mesentery (13,14,15).

Preadipocyte differentiation into fat cells depends on the nutritional status and is possible throughout the whole life. The amount of adipose tissue is therefore determined by the balance between lipolysis and lipogenesis (adipogenesis). Adipogenesis is stimulated by insulin and FFA, while leptin and VEGF participate in angiogenesis.

Excessive intake of calories without adequate energy expenditure promotes adipocyte hyperplasia and adipogenesis, resulting in obesity. Under the influence of numerous signals, pluripotent mesenchymal stem cells are transformed. Since adipocyte precursor reserves are unlimited, the capacity for adipogenesis is high. CNS signals, the sympathicus (adrenergic hormones), insulin, cyclic AMP, and adipokines participate in the mobilization of progenitor cells. Only a balanced stimulus of differentiation activators steers the conversion of stem cells into adipocytes, myocytes, hondrocytes or osteocytes. There are also many genetic and epigenetic factors stimulating adipogenesis (14,15,18).Developmental genes of the homeobox family (HOX), T-box genes and other genes that regulate transcription have a major influence on the occurrence of obesity and distribution of fat. Genes for abdominal fat control are HOX 3/5/6 TBX 5 and for the gluteofemoral phenotype HOXA 10 and SHOX 2. These transcription regulators have an important developmental role in the heterogeneity of adipogenesis and diversity of depots and AT distribution (7,17).

Activation of selective preadipocyte phenotype is triggered by **bone morphogenetic proteins 2 and 4** (BMP 2/4) and **Wnt protein**, which primarily acts a stimulator, but may also inhibit differentiation. Wnt proteins primarily use the canonical pathway in the presence of β - catenin, but the use of noncanonical pathways is also possible. Deviation from differentiation towards adipocytes can ultimately be stimulated by Wnt and β-catenin. Phosphorylation of transcriptional gene factors for peroxiome proliferator activated receptor gamma (PPARγ) type 2 and C/EPB-β activates many adipogenic genes, leading to the generation of mature adipocytes. These transcription factors have multiple functions in adipose tissue (Fig. 2). The variety of their expression also affects the occurrence of different AT phenotypes (15,16,18,19,20).

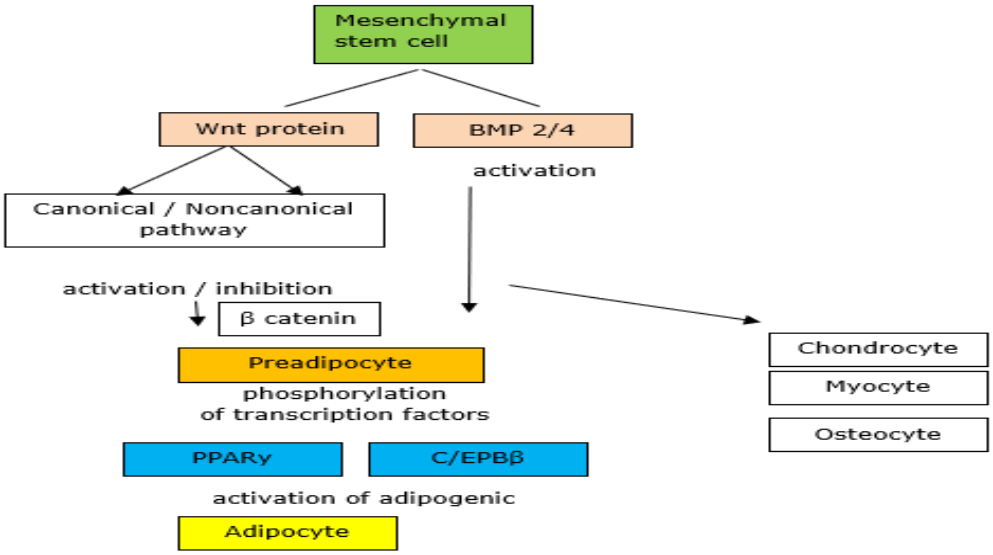


Figure 2. Adipogenesis - differentiation from stem cell to adipocyte (15,20).

BMP - bone morphogenetic protein; PPAR gamma - peroxisome proliferator-activated receptor γ; C/EPBβ - CCATT enhancer - binding protein β

Adipocyte hyperplasia and hypertrophy display significant functional differences. Hyperplasia increases the number of adipocytes, and hypertrophy increases cell size by accumulating triglycerides (Fig.3)

Hyperplastic adipocytes release fewer FFAs and increase insulin sensitivity (20).

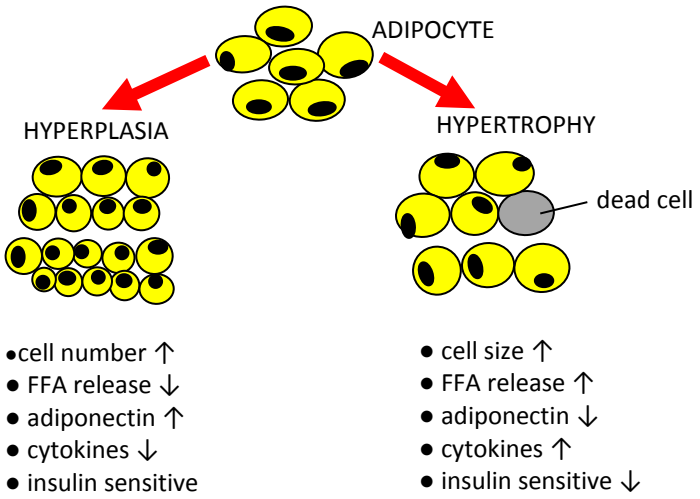


Figure 3. Differences between adipocytes hyperplasia and hypertrophyFFA free fatty acids

A disruption in the signals for adipocyte recruitment and differentiation (adipogenesis) produces dysfunctional hypertrophic adipocytes that are insulin resistant. This can disturb lipolysis inhibition.

WAT plays a major role in the energy homeostasis of the whole body. It regulates lipid metabolism in other organs through its endocrine function. Adipokines, adiponectin and leptin in the liver stimulate the oxidation of FFAs (13,16,18).

WAT contains adipocytes with a large lipid droplet that makes up 90% of the cell volume. Mitochondria are small and few. WAT is an important endocrine/immunological organ because it secretes adipokines and pro-inflammatory cytokines. This allows WAT to have a range of effects, including regulation of appetite, energy and lipid metabolism, control of sensitivity to insulin, etc. (22,23).

Depending on the location, white adipose tissue has several specialized functions. By storing excess energy (in the form of TG), it controls the metabolism of glucose, lipids and overall energy homeostasis. Through endocrine functions, WAT regulates appetite and the reproductive function, while visceral fat plays a role in local and systemic inflammatory processes.

Disturbed adipocyte function, low-grade inflammation and lipotoxicity cause insulin resistance and a disposition to atherosclerosis and hypertension.

The subcutaneous abdominal AT depot and upper body obesity strongly contribute to increased FFA levels (released by adrenergic stimulation), lipolysis and dyslipidemia. The gluteofemoral depot in women and lower body AT remove fat from the circulation and form a long-term energy reserve for all physiological potentialities.

Complex AT functions in metabolism control are responsible for controlling the activities of the autonomic nervous system, endocrine system, feedback mechanisms of autocrine and paracrine secretion of adipokines, leptin function and control of AT vascularization. Diverse AT functions also manage the transfer of pulsations from GH through leptin to FFAs and glycerol.

Triglycerides, esters derived from glycerol, are the main source of energy for all cells. Triglycerides are hydrolyzed via lipolysis into glycerol and free fatty acids, and FFAs participate as an energy substrate, precursors for the lipid cell membrane synthesis and mediators in cell signalling. FFA oxidation in mitochondria liberates ATP and provides energy.

The sympathetic nervous system controls lipid catabolism, and the parasympathicus controls anabolic processes. WAT and BAT differ in terms of function, morphology and molecular structure, but both types are dynamic, pluripotent organs. Environmental conditions may trigger hypothalamic control of white adipocyte conversion into brown adipocytes. This is supported by the existence of BAT islets in the otherwise dominant WAT. Exposure to chronic cold and prolonged β-adrenergic stimulation significantly stimulate the development of such thermogenically competent cells. A transitional form of white to brown adipocytes is **beige adipocytes**. Beige adipocytes are a subtype of BAT dispersed within white adipose tissue (subcutaneous). These UCP1 cells are generated by transdifferentiation of WAT, or from another cell line (13,18).

Thiazolidinedions (insulin sensitizers) stimulate WAT "browning", which gave rise to the idea of obesity therapy. It is undeniable that BAT, apart from the physiological function of adaptive thermogenesis, has an effect towards the leaner phenotype and resistance to obesity (18,19,20,22).

Brown adipose tissue (BAT) exists in newborns, but also in adults, where it helps in adaptation to cold. Metabolically, it is very active in energy homeostasis. The colour of these cells derives from a dark pigment, which is also a reflection of the high density of mitochondria in BAT. BAT is responsible for adaptive thermogenesis (heat production) and oxidation of lipids. Brown adipocytes are smaller than white ones, they contain more small lipid droplets and numerous large mitochondria. On the molecular level, they are characterised by the expression of the **uncoupling protein 1 (UCP1)**, but not of leptin (which is a characteristic of WAT). BAT has a denser network of capillaries and sympathetic nerve fibers (noradrenergic) (13).

Brown adipocytes originate primarily from the same mesenchymal stem cells as myocytes. This means that by origin, they are closer to muscle cells, but without contractility. This also drives their primary function towards lipid catabolism rather than fat storage (which is typical of WAT). A smaller part of BAT can probably be derived from white adipocytes, which is under genetic control. In adults, BAT is distributed in the neck, in supraclavicular, axillary, paravertebral, and mediastinal regions and in the upper abdomen. Such distribution implies a role in the heating of vital organs in their adaptation to cold.

Exposure to cold and feeding increase BAT activity and the expression of UCP1, which is controlled by norepinephrine. Such stimulation is also promoted by the thyroid hormone, insulin, thiazolidinediones and retinoic acid. Glucocorticoids inhibit UCP1 gene expression.

The BAT-based thermogenesis involves non-shivering heat production. Brown adipose tissue on the internal mitochondrial membrane activates UCP1, a protein for oxidative phosphorylation (from ATP synthesis) and thus generates thermal energy.

The basic substrate for thermogenesis is fatty acids released from triglycerides (hydrolysis), the initial signal being noradrenaline stimulation of the β-adrenergic receptor. In response to the cold, norepinephrine binds to β_3-adrenergic receptors in brown adipocytes, which activates adenyl cyclase. Cyclic AMP activates protein kinase, which activates hormone sensitive lypase (HSL) by phosphorylation. This enzyme releases FFAs from triglycerides. One part of FFAs is oxidized, while another part activates UCP-1 (thermogenin) that thermal energy in mitochondria. Norepinephrine stimulates all stages of differentiation and BAT functions. The stimulation of α_2-adrenergic receptors has opposite effects on BAT (20,21,22).BAT also has autocrine/endocrine functions (adipsin etc.), but they are less researched. With an increase in obesity, BAT's ability for thermoregulation during cold adaptation is reduced. There are significant individual differences in adaptive thermogenesis during cold and dieting.

Table 6. The difference between WAT and BAT in metabolic functions

↑ stimulation; ↓ inhibition – protective effect

WAT	Function	BAT
↓	Thermogenesis	↑
↑	Inflammation	↓
↓	Insulin sensitivity	↑
↑	Cardiometabolic effects	↓

BAT has a protective effect on cardiometabolic risks, but also on adipose tissue accumulation. The differences between white and brown adipose tissue are shown in the Table 7. (20,21,22,23).

Table 7: The differences between WAT and BAT

Feature	White fat – WAT	Brown fat - BAT
Function	Energy storage	Thermoregulation - heat production
Morphology	• Large lipid droplet • variable and fewer mitochondria • superficial organelles	• many minor lipid droplets • numerous mitochondria • standard in the cell
Target proteins	Leptin	UCP1
Development	• mesenchymal stem cells transdifferentiation →	• other progenitor cells Myf5+
Location	• gynecoid phenotype • android phenotype	• near WAT • near vital organs
Human effect	• obesity risks • energy homeostasis	• warms the body • suppresses obesity • lower risks
Aging	• increase • redistribution	• drop in quantity • ↓ cold tolerance

The basic physiological functions of WAT include the balance between lipogenesis and lipolysis, i.e. deposition and mobilization of triglycerides, which opens the possibility of storing energy and supplying energy to the periphery. The imbalance of these complex mechanisms can redirect AT to adipogenesis, obesity and a cascade of metabolic, endocrine and reproductive disorders. Acute regulators of activity and adipose tissue are adrenaline and noradrenaline (epinephrine and norepinephrine)

1.1.5 Lipogenesis

Lipogenesis is a process in which glycerol is esterified with free fatty acids and forms triglycerides.The lipogenesis sequence begins in the small intestine with the absorption of FFAs and triglycerides. Through lymph, chylomicrons (microscopic fat particles) enter the veins and liver, where new lipoproteins are formed. Chylomicrons consist of 10% cholesterol and 90% triglycerides. They break down into fatty acids and glycerol and enter adipose tissue, where they are resynthesised into triglycerol and stored in the WAT adipocyte cytoplasm (23,24,25,26,27).

Triacylglycerol (TAG) or triglycerides (TG) are the main lipid reserves in humans. They are synthesized in the endoplasmic reticulum. The deposition of TG into adipose tissue mostly occurs by hydrolysis with **lipoprotein lipase (LPL)**. Fatty acids uptake and esterification into glycerol 3-phosphate are stimulated by insulin. Steroid hormones also act as fat storage regulators. Insulin suppresses the mobilization of fat, i.e non-esterified fatty acids (NEFA) or free fatty acids (FFA) from subcutaneous AT. It has a dual function - it inhibits hormone sensitive lipase (HSL) and stimulates the re-esterification of fatty acids.
Lipogenesis entails the formation of TG in the liver from excessive glucose. This pathway ends with the release of very low-density lipoproteins (VLDLs) into the circulation.

In AT, the most powerful antilipolytic hormone is **insulin**. It regulates a number of anabolic functions of adipocytes and promotes lipogenesis:

- stimulates glucose uptake
- promotes lipoprotein lipase (LPL) enzyme activity on circulating triglycerides leading to free fatty acids uptake
- promotes FFA synthesis
- regulates hormone sensitive lipase (HSL)
- re-esterifies fatty acids into triglycerides
- inhibits lipolysis.

The sympathetic nervous system also stimulates glucose uptake in AT. Adipose tissue undoubtedly participates in the regulation of insulin secretion and glucose homeostasis (22,23,24).
Glucose has 3 functions in adipogenesis and physiology of adipose tissue. These functions include contribution of carbon atoms to create acetyl coenzyme A, hydrogen supply for the reduction and acting as a source of glyceralphosphate. This is important for the re-esterification of fatty acids and their storage in the form of triglycerides.

Lipogenesis is also affected by growth hormone (GH), cortisol, and catecholamine balance. Recently, a local stimulator of fatty acid esterification has been discovered in adipose tissue. With the production of ASP **(acylation-stimulating protein)**, adipocytes independently regulate how much fat is needed. Fatty acids act through the peroxisome proliferator-activated receptor (PPAR). The main transcription factor of lipogenesis and adipogenesis is PPARγ-2, an exclusive mediator of lipid storage in WAT and initiator of energy delivery. It is also required for FFA and perilipine (protein on the surface of lipids in adipocytes) transport. Genes controlled by PPARγ are involved in most lipid metabolism and glucose homeostasis phases. Expansion of fat storage and creation of new adipocytes require neoangiogenesis, which is locally controlled by leptin, cytokine, matrix metaloproteinase and vascular endothelial growth factor (VEGF).
A negative energy balance leads to the reduction in adipocytes and finally to apoptosis (16,20,23,26).
Adipocytes of the gluteofemoral region exhibit higher lipoprotein lipase activity than other fatty depots.

These differences in pathophysiology are partially controlled by estrogen, progesterone and androgens. Patients with polycystic ovary syndrome (PCOS) have a lower expression of LPL in subcutaneous AT as hyperandrogenism leads to the dysfunction of adipocytes. The importance of ovarian hormones in feeding control is supported by evidence that estrogens inhibit emotional and binge eating (occasions for overeating), while progesterone has the opposite effect and stimulates eating dysregulation (28,29,30).

1.1.6 Lipolysis

Lipolysis is a biochemical pathway responsible for the catabolism of triglycerides stored in adipose tissue. TG hydrolysis is possible in all cells and tissues but is primarily inherent and most common in WAT and BAT.

White adipose tissue is the main store of energy in humans. When there is energy demand, fat mobilization occurs, i.e. lipolysis causes triglycerides to break down into glycerol and free fatty acids, which satisfies peripheral energy needs. Lipolysis is performed in 3 steps with the participation of three enzymes (lipases) under the control of the β-adrenergic system (23,26,27):

- **Adipose triglyceride lipase** (ATGL) - or desnutrin
  → hydrolyzes TG to diacylglycerols and FFAs
- **Hormone sensitive lipase (HSL)**
  → hydrolyzes all glycerols
- **Monoglyceride lipase (MGL)**
  → breaks down monoglycerol into glycerol and FFAs.

Epinephrine and norepinephrine together with glucagon stimulate the release of FFAs from triglycerides stored in adipocytes, while insulin is a potent inhibitor of these processes. The modulators of these activities are the growth hormone and cortisol. Adrenergic stimulation activates protein kinase, which phosphorylates perilipin 1 (and 2) and HSL, and the phosphorylation of perilipin further activates ATGL. These are the main steps in TG hydrolysis. Lipolysis is the most active in the visceral AT depot, followed by the subcutaneous abdominal depot. In the conditions of high release of FFAs and glycerol, the liver has on its disposal the elements for the synthesis of gluconeogenesis and lipoproteins. The relationship between FFA and glucose metabolism is negative in both directions. The lowest lipolytic activity is seen in peripheral subcutaneous AT.

In obesity, the functions of adrenergic receptors and afferent signalling to the brain are altered, affecting the amount, function and distribution of AT. Obesity increases FFA

levels and the accumulation of fatty acids and TG in non-adipose tissue (muscles, liver, heart). Ectopic lipids are lipotoxic and compromise the function of insulin.
In the conditions of insulin resistance and low levels of glucose transporter-4, inhibition of lipolysis by insulin is impaired. This contributes to increased levels of glycerol, FFAs and VLDL in the circulation.
Insulin suppresses fat mobilization by HSL inhibition (dephosphorylation) and re-esterification of FFAs.
Lipolysis is strongly stimulated by catecholamines, but stimulation of α_2-adrenoreceptors inhibits lipolysis. Adipose tissue metabolism depends on the nutritional status and physical activity, cold and psychic stimuli (26,27,28).

Energy expenditure involves the basal metabolic rate (BMR), non-shivering thermogenesis, diet-induced thermogenesis and physical activity. BMR accounts for 70% of the total energy expenditure, whereby the brain accounts for up to 20% of total energy expenditure. Another aspect of energy expenditure is adaptive thermogenesis in BAT. Physical activity (moderate) accounts for 20-30% of energy expenditure. For energy, the CNS uses glucose rather than lipids. A normal woman has 18 kg of fat corresponding to 170,000 kcal, which is an energy equivalent enough for 2.5 months. Pregnancy requires at least an additional 400,000 kcal. Understandably, enough AT reserve is a prerequisite for a normal menstrual cycle.

1.1.7 Endocrine and paracrine functions of adipose tissue – adipokines

Adipose tissue maintains extensive communication and is characterized by receptors activated by 3 types of signals:

- endocrine signals – hormones travelling through the circulation
- paracrine signals – adipokines from the neighbouring cells
- neural signals – sympathetic and parasympathetic fibers.

In the neural control of AT, the sympathetic autonomic system (adrenergic) is dominant, while the cholinergic (acetylcholine) system has antagonistic effects. The dominant adrenergic receptors are α_1, α_2 and β_3 receptors. Parasympathetic neurotransmitters

bind to muscarinic and nicotinic cholinergic receptors. The activity of fat cells stimulates norepinephrine and epinephrine (adrenaline), mainly via β_3 adrenergic receptors. Visceral abdominal adipocytes are most susceptible to lipolysis induced by catecholamines. Catecholamines (epinephrine and norepinephrine) and glucagon bind to their receptors on adipocytes, activate adenylate cyclase, and increase cAMP.
The activation of cAMP promotes protein kinase (PKA) that phosphorylates and activates HSL (TG hydrolysis).
Important among endocrine hormone receptors in AT are glucocorticoid receptors involved in the metabolism and distribution of adipose tissue. Androgen receptors have a higher density in visceral fat. Estrogen receptors also exhibit regional differences. They are the most active in the gluteofemoral depot of AT, with estrogens promoting a corresponding subcutaneous phenotype. With the decline of estrogen levels in postmenopause, the distribution of adipocytes shifts to visceral AT. Estrogens and other steroids play an important role in AT metabolism from lipogenesis to lipolysis and affect fat distribution. The metabolism of adipocytes and the pathophysiology of obesity are also affected by vitamin D, xenobiotics and oxysterols. It has been found that many steroid hormones show higher concentrations in AT than in the circulation. Adipose tissue is undoubtedly the most important reservoir of steroid hormones (1,7).

Estrogens induce the "browning" of white adipocytes via ERβ (transformation into BAT) and the emergence of subcutaneous AT in the gluteofemoral region and the upper body. This is associated with less inflammation and lower cardiometabolic risk. Low estrogen activity favours the build-up of abdominal and visceral AT, thereby supporting chronic inflammation and metabolic risks.Estrogens promote gene expression and jointly induce gynecoid adipose tissue. Estrogens and androgens inhibit lipoprotein lipase (LPL) activity (26,28,29).

Androgens and hyperandrogenism are associated with an androgenic, abdominal AT phenotype. Androgen dysregulation (androgen/estrogen balance) has a strong influence on the various phenotypes of obesity and the metabolic syndrome. The availability of estrogens and androgens in peripheral tissues is also affected by the level of sex hormone binding globulin (SHBG) production in the liver. Central obesity has a lower

SHBG due to the production of insulin and androgens. A reduction in SHBG increases the concentration of free testosterone, which is highly typical of central obesity.

1.1.8 Steroidogenesis in adipose tissue

Steroid hormones are synthesized in adipose tissue (de novo) from **cholesterol** or are **converted** from various precursors from the circulation. In adipocytes in which the internal mitochondrial membrane contains the CYP11A1 enzyme, cholesterol is converted to pregnenolone in the presence of steroidogenic acute regulatory protein **(StAR)**. The StAR protein located on the external mitochondrial membrane serves as a transporter (importer) for cholesterol. De novo steroidogenesis is possible in subcutaneous and visceral AT. The expression of the TSPO gene is also involved in the control of cholesterol transport. Both transporter proteins also play a role in adipogenesis. The enzymatic system of adipose tissue further regulates various types of steroidogenesis, secretion of adipokines and lipid metabolism. Further fate of pregnenolone is determined by the presence of 3β hydroxysteroid dehydrogenase (3βHSD) and P450c17 (CYP17A1) enzymes. This can lead to the formation of mineralocorticoids, glucocorticoids and sex hormones, depending on the activity of relevant enzymes (11βHSD, CYP21, CYP19-aromatase, 17βHSD, 5α reductase, steroid sulphatase, etc.) (26,28,29,30).

In addition, adipose tissue can affect the levels of circulating steroids by local conversion of steroids and synthesis of numerous adipokines that affect steroidogenesis in adrenals and gonads through endocrine activity. Apart from the classic pathways of steroidogenesis, adipose tissue is also the site of **oxysterol** synthesis and **vitamin D** storage, which are also involved in the metabolism of adipocytes and lipids. Obesity is associated with disturbances in the levels of these substances.

It has been proven that the gene expression for almost all enzymes involved in the transformation of steroid hormones or pregnenolone occurs in human adipose tissue. Pregnenolone can be converted to progesterone in mitochondria (3βhydroxysteroid dehydrogenase) or exits into the endoplasmic reticulum and is converted to 17α hydroxy pregnenolone (P450C17). These are the starting points for all further steroidogenesis toward progesterone, androstendione, estrone and estradiol, testosterone and DHT

(dihydrotestosterone). The basic enzyme that diverts steroidogenesis (delta 5 to delta 4 steroids) is 3βHSD (type 2), located in the mitochondria and the endoplasmic reticulum. Today, the presence of the P450c17 enzyme in AT has been repeatedly confirmed. There are regional differences in the concentration of enzymes and consequently in the conversion of hormones. In obesity, cortisol in the blood is not increased, but its production is more intensive in visceral adipose tissue (elevated expression of the 11βHSD1 enzyme).

Steroid hormone levels in the circulation enable their conversion to adipocytes. The conversion of these (adrenal and gonadal) hormones and de novo production of steroid hormones make adipose tissue the most active tissue in steroidogenesis.

In adipose tissue, the enzymes P450 aromatase, 17βHSD and 3βHSD convert androstendione, DHEA, DHEA-s, and testosterone into: Regardless of the source of these steroids, the metabolic effects are both local, in adipose tissue, and global with endocrinological effects.

The main part of estrone in the body (E_1), 50% of total testosterone in women, and a variable amount of estradiol (E_2) are produced in adipose tissue. In stromal cells of subcutaneous AT, the expression of P450arom is regulated by glucocorticoids, leptin, and cytokines – interleukin 6 and tumor necrosis factor alpha (TNF-α). The controller of aromatase activity in the breast and subcutaneous AT is prostaglandin E_2. In case of breast cancer, there is increased activity of 17βHSD (type 1,7,12), sulphatase and 4-OH estrone in adipose tissue, leading to local estrogen levels in the breast that are 20 times higher than in the circulation. Type 12 of the 17βHSD enzyme is active in the conversion of estrone to potent E_2. Out of the 14 isoforms of the enzyme 17βHSD family in AT, the expression of types 1, 2, 3, 5,7, 8 and 12 has been reported. Estradiol metabolism is disturbed in adipose tissue associated with breast cancer (1,7,8).

Androgen levels are higher in visceral adipose tissue because of androgenic 17βHSD type 5 activity. Research shows that in adipose tissue androgenic activity dominates over estrogens. In obesity, the production of DHEA and androstenedione is increased.

In sum, steroid activity in adipose tissue is a consequence of precursor supply, local synthesis and hydrolysis (decomposition) of steroid hormones. Local intra-adipose steroid metabolism determines the metabolic and health disorders, as well as the distribution of fat depots. This also applies to the identification of android or gynecoid

phenotype. Androgens play an important role in controlling the distribution of AT and endocrine and metabolic disorders (23,24,25,26).

Exogenously applied hormones are deposited and transformed in adipose tissue, with the potential to change the success of programmed treatment.

Therefore, obesity has a wide potential for the secretion of steroid and sex hormones, which contributes to endocrinological disorders and comorbidity. Endocrine, paracrine and autocrine activity of hormones has been observed in adipose tissue.

In obesity, estrogen production, free estrogen fraction, and estrogen sulphate generation (active estrogen reservoir) are increased. Obesity also changes the balance and the activity of androgens.

1.1.9 Adipokines

Adipose tissue is a large and dynamic endocrine and paracrine organ. White adipose tissue produces proteins and cytokines that transmit signals to control feeding, body weight, metabolism, reproduction, inflammatory disorders, and several other physiological and pathophysiological changes. More than 50 adipokines have been identified so far, but not all of them have defined functions. The first enzyme to be identified was lipoprotein lipase (LPL), which hydrolyzes circulating TG to NEFA. WAT has a complex role in general physiological and control mechanisms, with extensive communication with other tissues and organs. Neither all WAT sites nor all adipocytes produce the entire spectrum of adipokines (30,31,32).

The regulation of adipokine and hormone synthesis is very complex, and depends on the number of adipocytes, supply of precursors, and balance of the autonomic nervous system and local autoregulatory mechanisms. There are no classic feedback mechanisms. In adiposity, the mechanisms for the control of adipokine secretion are different in that they are capable of synthesizing pro-inflammatory and anti-inflammatory proteins. They induce the infiltration of macrophages into AT, which are a source of the tumor necrosis factor (TNF) α and IL-6 (fig 5).

Many adipokines are not exclusive products of adipocytes and are also produced in other tissues.

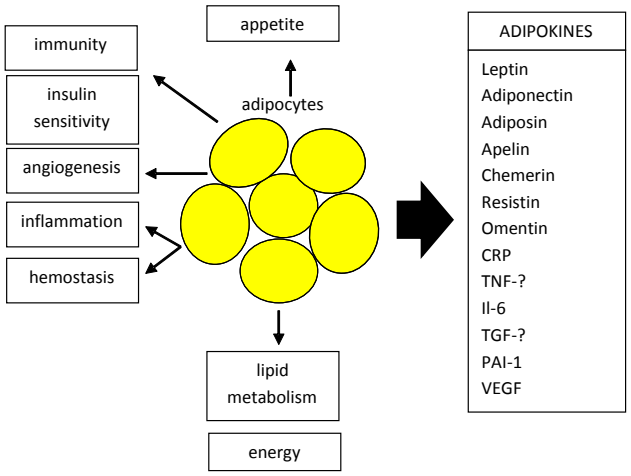


Figure 4. Adipokines and the function of adipocytes, CRP-C reactive protein; TNF - tumor necrosis factor; IL - interleukin; TGF - transforming growth factor; PAI - plasminogen inhibitor activator; VEGF - vascular endothelial growth factor

Leptin

Leptin is a polypeptide hormone, a product of the ob gene (in WAT) with 146 amino acids. Its name derives from the Greek word "leptos" meaning thin, skinny. Women have higher leptin levels and higher pulse amplitudes. Leptin informs the brain about the energy stored in adipose tissue. The leptin gene expression is regulated by hormones, growth factors and cytokines. It is primarily a signal of white AT, minimally of brown AT, but leptin expression also exists in the hypothalamus, pituitary gland, placenta, breasts and gonads. Leptin and the sympathicus are the main links between adipose tissue and the CNS. Estrogens induce leptin production, while androgens suppress it (30,31,32).

Leptin is an indicator of the body's adipose tissue and signals the energy balance. Leptin acitivities are performed through 6 receptors. The production and activities of leptin are proportional to the amount of WAT and stored energy. In such circumstances, leptin secretion and the amount of free leptin increase with obesity. This limit feeding (food intake) and increases energy consumption. Optimally, this would imply weight reduction. However, the chronic excessive intake of food in obesity is more frequently associated with **leptin resistance** and the effect on weight loss is minimal.

When food intake and body fat (energy) are reduced, low leptin levels cause hunger. An excess of AT and energy inhibits the hypothalamus, which reduces appetite, activates thermogenesis and energy expenditure, as well as the sympathetic tone. Low leptin on the other hand signals an increased need for energy. Leptin is secreted in a pulsatile manner, normally every 60 minutes.

Leptin plays an important role in controlling the hypothalamic-pituitary axis and the reproductive system. It stimulates the release of GnRH and LH pulsatility and is also involved in folliculogenesis (1,7,33,34).
In sum, **leptin** activities include:

- signals the energy status to the CNS
  - reduces appetite and thermogenesis or
  - increases appetite and energy reserves
- influences gonadotropic functions and puberty
- influences the function of gonads
- stimulates angiogenesis
- immunomodulation
- stimulates hematopoiesis and platelet aggregation
- thermogenesis.

Obesity is associated with an increase in the levels of leptin, which is a sensitive marker for the metabolic syndrome and cardiovascular risks. Essentially, leptin is anorexigenic. Increase in leptin expression is stimulated by sex hormones, glucocorticoids, cytokines, and some inflammatory toxins. Catecholamines reduce the circulating leptin levels. Insulin stimulates leptin, and both hormones have a lipostatic role.

Adiponectin

Adiponectin is a protein hormone with 244 amino acids like the TNF superfamily. It is produced by adipocytes in WAT, and binds to 3 receptors (AdipoR1, AdipoR2 and T-cadherin) to affect the cell function of enzymes (AMPK). Adiponectin concentrations are lower in men, obesity, android obesity, diabetes and people with coronary heart disease

(CHD) and hypertension. Adiponectin has several positive effects, with a strong anti-diabetic effect (30,31):

- increases insulin sensitivity
- increases insulin activity in the liver
    - reduces hepatic production of glucose
    - thereby lowering blood glucose
- increases oxidation of fatty acids and lowers triglyceride levels
- anti-inflammatory effects – increases IL-10 production
- stimulates lipolysis and inhibits the synthesis of cholesterol, triglycerides and adipogenesis (increases LPL)
- increases the production of nitric oxide (NO) and promotes vasorelaxation
- has anti-atherogenic properties
- concentration of adiponectin is low in patients with breast, endometrial, colon, and prostate cancers. It limits cell proliferation and stimulates apoptosis. Although it is undoubtedly present, the role of adiponectin in reproduction has not been fully defined. Lower levels of adiponectin in women with polycystic ovary syndrome (PCOS) are associated with insulin resistance.

Resistin

Resistin is an adipokine (cytokine) produced in adipocytes and macrophages. This dimeric protein is a mediator of insulin resistance and appears to be related to obesity. When resistin is elevated, AMPK (adenosine monophosphate activated protein kinase) activity is reduced, which affects cellular energy homeostasis. Low resistin levels are associated with elevated AMPK activity and a reduction of gluconeogenesis, and glucose production in the liver. Therefore, resistin is an important controller of glucose metabolism with the following effects (29,30):

- increases insulin resistance
- blocks insulin signals
- increases hepatic production of glucose
- controls adipogenesis
- inhibits the differentiation of preadipocytes

- participates in chronic inflammation and immunomodulation
- participates in central feeding control.

The relationship between resistin, reproduction and PCOS is still being investigated. However, it has been established that resistin levels are elevated in obese women with PCOS. Resistin increases P450c17 activity in theca cells.

Omentin and chemerin

Omentin is an adipokine that is mainly produced in visceral adipose tissue (omentum etc.). It stimulates glucose uptake in fat cells. Its concentration in the circulation is lower in obese persons, i.e. when BMI and leptin are elevated. It is also lower in people with diabetes and obese female patients with PCOS and insulin resistance. Metformin increases omentin levels.

Chemerin is an adipokine (chemokine) involved in the control of adipocyte development and metabolism. Its levels increase with obesity, metabolism of fat and glucose, DM, and inflammation. Insulin up-regulates chemerin, exacerbates glucose tolerance and insulin resistance.

It seems that both adipokines may be involved in the function of granulosa and theca cells of the follicle.

Other adipokines

Adipose tissue (adipocytes) as well as numerous other tissues and cells produce a variety of other cytokines and cell metabolism control factors characterized by autocrine and paracrine activities.

Complement-like factors are adiposine and acylation stimulation protein (ASP), the factors of adipogenesis or lipolysis autocontrol. **Inflammatory cytokines** are circulatory and local inflammatory markers, pro-inflammatory and acute-phase proteins. Their importance is reflected in the fact that obesity is a **low-grade inflammatory condition**. The inflammatory condition in obesity may cause insulin resistance, hyperlipidemia and metabolic syndrome. It remains unclear whether obesity is a result or a cause of inflammation. Inflammatory markers are generated in several ways. They are produced

in other organs; adipose tissue finalizes liver products (CRP) or they are self-produced with adiposity in adipocytes (or in infiltrated macrophages) (fig 6).

Visceral adipose tissue has a higher capacity to produce TNF-α, CRP and IL-6 (26,30).

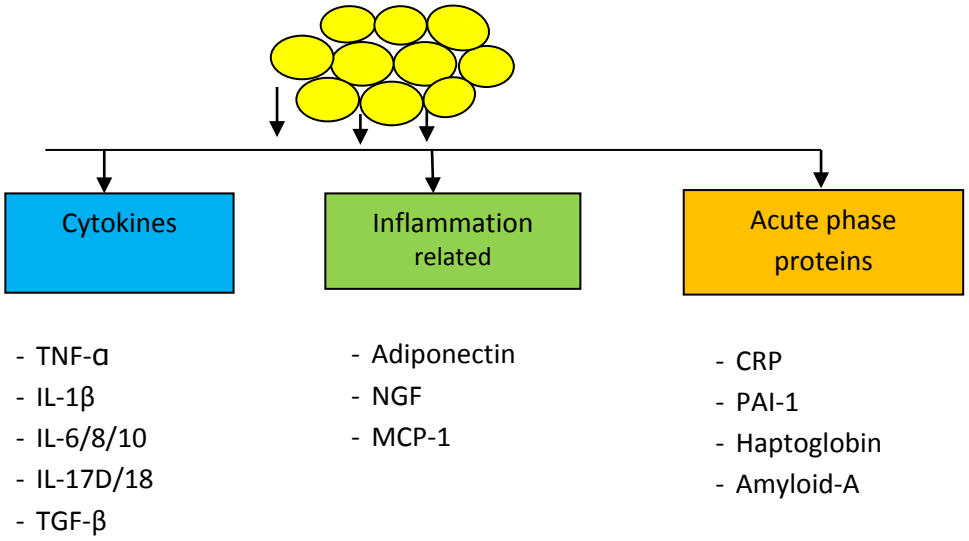


Figure 6. Inflammatory cytokines potentially produced in AT , TNF - tumor necrosis factor; IL - interleukin; NGF - nerve growth factor; MCP - monocyte protein; CRP - C reactive protein; PAI - plasminogen activator inhibitor

Tumor necrosis factor alpha (TNF-α) is a product of WAT and a powerful autocrine and paracrine adipocyte regulator. It is elevated in obesity and inhibits the signalling of insulin receptors and leads to insulin resistance. It is the main regulator **of IL-6 synthesis** and haptoglobin, and controls apoptosis. In addition to its local effects, IL-6 also acts centrally because it regulates the energy balance in the hypothalamus (along with leptin). Elevated BMI and WHR are accompanied by an elevation in the abovementioned cytokines. In obesity, IL-8 and IL-18 production is increased.

Plasminogen activator inhibitor-1 (PAI-1) is an important factor in maintaining hemostasis. Its production in WAT has been proven in obesity. It is also associated with a tendency to atherogenesis, diabetes, and cardiovascular risk, which accompany obesity. The stimulation of the PPAR gamma nuclear receptor (by thiazolidinediones) inhibits haptoglobin, TNF-α and leptin.

Whether obesity is a state of chronic inflammation can be seen from an elevated production of C-reactive protein (CRP) in the liver. Elevated levels of high sensitivity CRP (hsCRP) are a surrogate marker for the expression of visceral obesity and PCOS.

Neurotrophin (NGF) is produced in adipocytes, and is important for maintaining neural sympathetic connections, and for immune and inflammatory processes. The main stimulator for NGF expression is TNF-α.

The following activities are common to TNF-α, IL-6 and CRP are:

- increase in vascular inflammation
- proatherogenic action
- reduction of insulin sensitivity and signalling
- insulin resistance
- prodiabetic effects
- they are elevated in abdominal obesity
- 30% of IL-6 in the circulation is from AT
- there is mutual production control
- they are potent adiponectin inhibitors.

Visfatin has proinflammatory and proatherogenic effects. It causes vascular dysfunction and stimulates angiogenesis.

Vaspin is an adipokine with beneficial effects. It improves insulin sensitivity and reduces ROS. It reduces food intake.

Retinol binding protein-4 (RBP-4) increases insulin resistance and glucose intolerance (30).

AT products include adipokines functioning as vascular cell adhesion molecules – 1 (VCAM-1), collagen, fibronectin, matrix metalloproteinase (MMPs 1/7/9/10/11/14/15), as well as products for the synthesis of prostaglandin (cox pathway), nitric oxide (NO), renin-angiotensin system and tissue inhibitors of MMPs (TIMPs).

BAT primarily produces paracrine factors – vascular endothelial growth factor (VEGF), NGF, FGF-2 angiotensinogen and NO, which enables more vascularization and sympathetic stimulation of brown adipocytes and enhances the positive effects of BAT.

BAT also produces adiposine, which inactivates ASP and contributes to reduced adipogenesis. This occurs through paracrine effect on white adipocytes.

The increase in inflammatory adipokines in obesity is considered as a local response of WAT to hypoxia. Growth of AT depot with weaker vascularization and expression of hypoxia-inducible factor-1 (HIF-1) may stimulate the production and activity of proinflammatory cytokines.

Gastrointestinal hormones in energy control

In addition to adipokines, which are **anorexins** involved in energy homeostasis and feeding (leptin, adiponectin, resistin), there are also digestive tract hormones involved in the central control of hunger and appetite. These are peptide hormones of the stomach and the intestines – **orexins** and **anorexins** (37).

Ghrelin

Ghrelin is a peptide consisting of 28 amino acids, named after its growth hormone release stimulating function. It is produced mainly in the stomach (70%), the intestine and the pituitary gland. Apart from participating in feeding control and energy metabolism, it is active in the stomach and pancreas. The most important are its central functions regulating energy homeostasis in the hypothalamus. Its levels increase during hunger and in lean persons, stimulating appetite and food intake by modulating neuropeptide-Y (NPY) functions. Its levels and activity are high in starved and anorectic persons, and low in obesity and postprandially. Ghrelin inhibits the expression of kisspetin, which inhibits GnRH and LH pulsatility. It stimulates the secretion of the growth hormone, prolactin and ACTH. Ghrelin has orexigenic effects both in the metabolism and in reproduction. It is an important link between peripheral energy balance, CNS and reproduction.

Ghrelin and leptin are the main coordinators between reproduction, body composition and energy metabolism. Ghrelin inhibits and prevents reproduction in conditions of energy deficit, whereas leptin has the opposite effect. Ghrelin is the only known hormone to stimulate appetite.

Peptide YY i oxyntomodulin (OXM)

Gut hormones, enterokins, (PYY) and OXM have different effects compared to ghrelin. Like leptin, they are anorexigenic and inhibit food intake through the hypothalamus. In response to a meal (filled intestines), they function as anorexins. They are suppressed during hunger and inhibit food intake by 40%. PYY also exerts effects on the reproductive axis and the development of puberty. Food intake and a filled ileum stimulate PYY release.

Obestatin

Obestatin is a stomach hormone with 23 amino acids. Its effects on central and reproductive functions are undefined. Its activities seem to be like those of ghrelin.

Glucagon-like peptide I, insulinotropic polypeptide and cholecystokinin are gut hormones that also seem to balance food intake and reproduction.

Cholecystokinin is produced in the duodenum and jejunum. It reduces food intake.

Enterostatin, bombesin and amylin are enterokines with anorectic effects.

1.1.9.1 Neurophysiology and neuropathology of adipose tissue and obesity

The brain is an important regulator of energy homeostasis, used to coordinate reproductive functions. The hypothalamus and the dorsal medulla are the main sites that receive and integrate peripheral energy storage signals and feeding behaviour. In normal conditions, an optimal energy reserve in AT is a physiological stimulus for timely puberty, menarche and ovulatory menstrual cycle. Obesity is a result of a chronic disorder of energy homeostasis accompanied by changes in the peripheral metabolism, resulting in a hormonal, metabolic and inflammatory imbalance (32,33,34,35).

With food intake, glucose, fatty acids and amino acids are hydrolysed to ATP, CO_2, water and heat. Feeding behaviour and all accompanying sensations are mutually dependent in the CNS.

The periphery affects the brain with signals from three directions.

- humoral factors – hormones
- metabolic factors – metabolic components
- autonomic nervous system – two-way system.

The hypothalamus contains centers for hunger (appetite) and satiety, with numerous links and signals that affect these functions. and make them directly involved in any potential disorder, from anorexia to obesity. The functions of hypothalamic centers are coordinated by both peripheral signals and other CNS regions through important pathways: hypothalamocortical, hypothalamolimbic, endocannabinoid pathways, n. vagus and reproductive centers. Thus, serotonin and allopregnenolone have a positive effect on appetite, while cortical releasing hormone (CRH), IL1/6 and TNF- α have a negative (inhibitory) effect.
Four basic hormones control energy homeostasis through the hypothalamus: leptin, insulin, ghrelin and PYY (34,35,36,38,41).

Leptin regulates physiological processes in two ways. In case of an optimal body weight, leptin is an indicator of the body's AT reflecting the balance between energy input and expenditure. In case of increase or decrease in BMI, leptin signals energy imbalance. In obesity, it encourages cessation of food intake with a tendency of AT loss, while a fall in energy reserves signals to the brain that energy is needed (low leptin). Therefore, by regulating fat and body weight, leptin has a crucial role in energy expenditure or build-up. In obesity, leptin, in synergy with insulin, inhibits appetite and food intake in the hypothalamus and increases energy expenditure and thermogenesis, as well as the sympathetic tone. Hunger and leanness reduce leptin activity resulting in energy conservation, increased appetite, and parasympathetic activity. In chronic obesity conditions, "leptin resistance" develops.
In controlling energy homeostasis and obesity, leptin and insulin use the same pathway in CNS (phosphatidylinositol 3-kinase), which is the basic anorexigenic enzyme (fig 7, table 8).

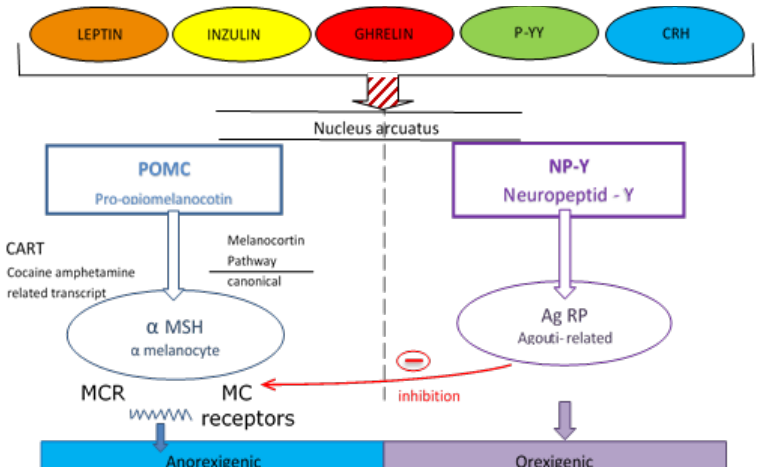


Figure 7. Central control of appetite in the hypothalamus, The inhibitory pathway AgRP-MCR is unidirectional; MCR melanocyte receptor

In nucleus arcuatus (n.A.) and n. paraventricularis of the hypothalamus leptin has neuromodulatory effects on two systems: **POMC (pro-opiomelanocortin)** with the melanocortin pathway (α MSH), and **neuropeptide Y (NPY)** agouti-related protein (AgRP). Leptin also suppresses the expression of endocanabinoids, stimulates α MSH and cocaine- and amfetamine-regulated transcripts (CART). All these actions reduce appetite and food intake. Increased or low leptin (and insulin) can stimulate or inhibit energy accumulation (food intake) through anabolic and catabolic pathways. These actions are shown in Table 8:

Table.8. Different hormonal activities in feeding control

↑ high; ↓low; MCR- melanocyte recptor; CART- cocaine amfetamine regulated transcripts

Anorexigenic catabolic pathways	Orexigenic anabolic pathways
• POMC • αMSH • CRH • TRH • CART • IL-1β • Inhibition of MCR (low) • Leptin ↑ • Insulin ↑ • Enterokin ↑	• NP-Y • AgRP • Ghrelin • Leptin ↓ • Insulin ↓ • Inhibition of MCR (high)
Negative energy balance	Positive energy balance

With the loss of MSR function (αMSH receptors), morbid obesity develops (5% of obese population). Obesity is associated with chronically high neuropeptide Y (NPY) levels.
Leptin and insulin circulate at the levels determined by AT and energy balance. In the CNS and the hypothalamus, they inhibit orexigenic activity and promote catabolic pathways of reduced food intake and increase energy expenditure. In leanness (loss of AT), these two hormones stimulate anabolic signals that promote food intake and energy saving. They inhibit catabolic pathways. The intake of food triggers satiety signals, and leptin/insulin regulate the size of the meal. In the described synergism, the role of leptin is more important for definitive effects in the CNS (34,38,39,40).

Neuropeptides are the most powerful anabolic effectors that stimulate food intake, while inhibiting energy expenditure. At the same time, they promote lipogenic enzymes in adipose tissue and the liver. Anabolic signals (orexines) are also produced by agouti-related proteins. In contrast, catabolic signals in the CNS are POMC, melanocyte-stimulating hormone alpha (MSH), CRH, tyrotropine-releasing hormone (TRH), CART and interleukin-1 beta. They all promote a negative energy balance, with the melanocortin system having the most powerful effect (40,41,42).
Simply put, leptin and insulin stimulate catabolic pathways and inhibit anabolic signals.

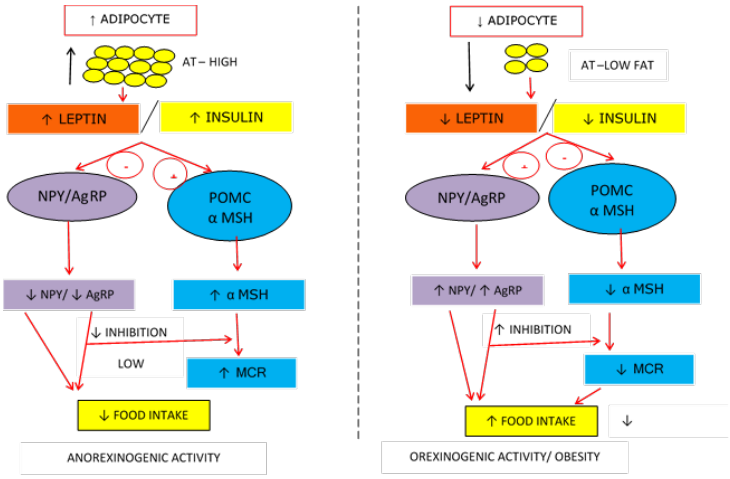


Figure 8. Adipose tisue signalling and feeding behaviour. Balance of αMSH and AgRP activity regulates appetite; Insulin and leptin are appetite suppressors; AT - adipose tissue

High leptin/insulin levels inhibit NP-Y/agouti-related peptides and stimulate the POMC melanocortin pathway. This reduces appetite and increases energy expenditure. Also contributing to negative energy balance control is weak (or no) inhibition of MSR through AgRP activity (fig 8).

Low leptin/insulin levels arising from leanness and AT and energy deficits have an opposite effect. NP-Y/AgRP is stimulated, MCR inhibition is increased and the POMC system is inhibited. Food intake increases while energy is preserved (34-42).
Adipose tissue and energy homeostasis signals are linked through the hypothalamus of the lymbic system with the control of the reproductive axis.

Adipose tissue reserves, and energy reserve signals are prerequisites for a timely course of female puberty, menarche and ovulation cycle. The menstrual cycle is established only with optimal body weight (≈ 48 kg) and the desired body composition (AT i.e. BMI ≈ 19-21 kg/m^2). Excessive undernutrition (BMI < 18) or obesity (BMI > 30) send signals to the CNS that seriously disrupt ovulation and the cycle (30).
Taste centers and the cognitive CNS are involved in feeding control. **Dopamine** (DA) neurons in the corpus striatum and the amygdala evaluate food characteristics and visual effects of food (enjoyment).

Estrogens modulate AgRP effects and MCR expression and thus affect food intake. The inhibition of NP-Y and stimulation of αMSH neurons are variable estrogen functions during the cycle. Peripheral hormones and adipokines have a different effect on the hypothalamus in conditions of high or low estrogens.
Low estrogen status makes numerous signals inhibitory. It has been shown that estrogens in women influence AT distribution (with adipocyte genes), so that AT is increased with their depletion. Increased estrogen levels in periovulation reduce the daily intake of food, and RBM increases in the luteal phase (29,30).

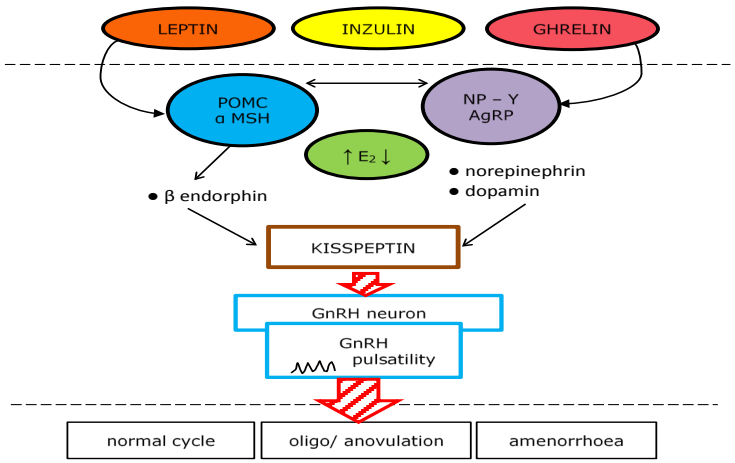


Figure 9. Relationship between adipose tissue, food intake and GnRH neurosecretion

In maintaining energy homeostasis, adipose tissue and adipokines are directly (CNS) and indirectly (ovarian and endocrine functions) involved in the control of the reproductive axis. Via the hypothalamus, leptin controls the development of sexual organs and sexual maturation. Adipose tissue signals control POMC and neuropeptides, and their further secretion is dependent on estradiol levels, i.e. the activity of the estrogen receptor beta (Erβ) in n. arcuatus. The effects of leptin, NP-Y, insulin and ghrelin are stimulatory in the presence of estrogens, and inhibitory in their absence. Lack of adipose tissue, lack of food and energy deficit will result in low leptin. NP-Y stimulation and POMC inhibition have an impact on the dysregulation of the kisspeptin system, resulting in the reduction of neurosecretion and pulses of the gonadotropin-releasing hormone (GnRH) (FSH/LH are affected).

The expression of **kisspeptin**, the primary controller of GnRH secretion, depends on the nutritional status and adipokines. Additional relays between leptin and GnRH hormones are kisspeptin, glutamate and brain opiates as GnRH neurons lack leptin receptors (fig 9).

Kisspeptin, the central controller of pulsatile neurosecretion of GnRH, depends on normal energy balance in multiple ways. Obesity, elevated androgen levels and insulin resistance inhibit the concentration of kisspeptin, and consequently that of GnRH. KISS1R receptors are located on GnRH neurons but are expressed elsewhere in the

brain and peripheral tissues. The neuropeptide kisspeptin is active in adipose tissue, liver, pancreas and the ovaries. It has been found that kisspeptin is an intermediary neuropeptide that links the metabolic status and reproductive functions, as a mediator of leptin activity. Leptin and estrogens promote the expression or transcription of kisspeptin-54. In conditions of leptin deficiency (in response to a negative energy balance) or leptin resistance, kisspeptin activity declines. Kisspeptin disorder can directly or indirectly affect the metabolism, obesity, and energy and glucose homeostasis. The effect of leptin on GnRH neurons can bypass the KISS1 pathway; in this case, signals are transmitted via glutamate (34,35,38,39).

Obesity, high BMI and WC are negatively correlated with FSH and LH. Morbid obesity suppresses LH and its pulsatility. Also, elevated BMI, insulin resistance and elevated androgen levels (FAI) decrease (inhibit) kisspeptin neurosecretion. These changes disrupt the menstrual cycle, from ovulations to amenorrhea.

1.1.9.2 Endocannabinoid regulation

The endocannabinoid system (ECS) is a molecular system that maintains homeostasis by autocrine and paracrine activity. It is present in many organ systems and tissues, such as the nervous system, adipose tissue, and the immune system. Endocannabinoids suppress or limit cellular response to numerous signals. Each deviation and potent response of the cell exceeding physiological limits activates ECS, which tries to re-establish homeostasis. The endocannabinoid system consists of 3 components (34,35,40):

- **endocannabinoids** – molecules released from lipids as needed – anandamide and 2 AG
- **cannabinoid receptors** – CB1 high levels in CNS and CB2 throughout the body
- **metabolic enzymes** – destroy ECS molecules once used (limiting effect).

Among others, ECS is engaged in the following important activities:

- CNS functions in neurosecretion – retrograde suppression of overactive neurons
- control of the peripheral nervous system
- control of homeostasis and chronic inflammation in adipose tissue
- control of inflammation and autoimmune response – in chronic inflammation, limits the intensity and duration of the response and suppresses and limits cellular signals.

In a special way (neuromodulators), ECS links brain activity to the health of organ systems. Among other neural functions, this system is involved in controlling appetite. ECS molecules are not neurotransmitters, but intracellular lipid messengers that inhibit excitation signals.

CB1 concentrations in the hypothalamus are high, and the regulation (suppression) of these receptors in the brain reduces appetite and participates in thermoregulation. In adipose tissue and the liver, endocannabinoids control the energy metabolism. ECs also have a known psychotropic effect, also exhibited by marijuana.

The endocannabinoid system interacts with the orexinergic system in the joint control of cognitive and physical energy functions. ECS induces a fall in cAMP levels and increases mitogen-activated protein kinase (MAPK).

In this chapter, it is important to point out that ECS regulates appetite, and leptin activity, increases dopamine, and increases taste quality. It also controls the function of adipocytes and modulates insulin sensitivity. As already mentioned, this system is an important neuro- and immunomodulator. It is believed to regulate endometrial receptivity and implantation.

1.1.9.3 Epigenetic effects and obesity

Epigenetic changes alter the function of genes without changing the DNA sequence. These changes to genes and transcription are caused by external environmental factors or developmental issues (42,52).

Epimutations change gene expression as well as the differentiation and function of the cell. They are frequently a normal occurrence but can also be abnormal (1,7,40,52).

Functional changes of the genome without in intervention in the nucleotide sequence occur in several ways:

- DNA methylation
    - hypomethylation
    - hypermethylation
- histone modification
    - repressive marks
    - active marks
- Non-coding RNA (micro RNA)
- Methylation of mitochondrial RNA (mRNA)

The selective and inherited alteration of chromatin, with modified gene transformation, changes the cell function. Epigenetic mechanisms are triggered in the following circumstances:

- exposure in utero (during development, childhood)
- aging
- food
- pollutants – environmental factors and chemicals
- obesity.

Epigenetic modifications can be inherited through both mitosis and meiosis, but the definition also extends to uninheritable alterations of transcriptional potential. The cell takes a different, epigenetic phenotype (with changed imprinting, gene silencing, etc.) Epigenetic changes mostly occur throughout a person's life, but transgenerational transfer (via sperm or oocyte) to the first or second generation is also possible.

Epigenetic changes are frequently caused by DNA damage. External DNA modifications affect gene activity or inactivity. Physical inactivity and the intake of food impact the risk for obesity by changing the FTO (fat mass and obesity) gene. Congenital or acquired epimutations in neurotransmitter canonical pathway genes in the hypothalamus lead to an imbalance in energy expenditure and to obesity.

Obesity occurs through a combination and interference of genetic and environmental factors. It is assumed that obesity is 40-70% heritable, with many genetic reasons for obesity and fat distribution. As there are no precise interpretations of genetic obesity, the importance of numerous epigenetic effects is increasingly emphasized.

Research has clarified three types of connections:

- epigenetic effects of obesity – lipotoxicity
    - inherent mutations
- transgenerational – vertical effects
    - intrauterine, effects on oocytes and sperm
- environmental stressors – effects of pollutants
    - and endocrine disruptors.

Obesity triggers epigenetic disorders associated with comorbidity and reproductive dysfunction. Adipose tissue dysfunction and lipotoxicity involve a state of chronic inflammation that promotes oxidative stress. This alters the synthesis of adipocyte hormones and cytokines. Tumor necrosis factor α (TNF-α), IL-6, CRP and matrix metalloproteinases, i.e. chronic inflammation, release the **reactive oxygen species (ROS)**. Such disorders lead to epigenetic modification of a series of enzymes, damaging intracellular energy metabolism. Epigenetic changes to genes modify chromatin (DNA damage) in somatic cells and gametes. Alterations can therefore be transferred transgenerationally, vertically, through the epimutation of sperm or oocytes and induce inherited obesity or other disorders in the first or second generation (generations F1 and F2).

Exposure to maternal obesity or undernutrition in pregnancy increases the risk and predisposition of the child for obesity and hypometilation of genes that promote adipocyte differentiation. Child development in suboptimal intrauterine conditions increases the risk for diseases later in life (51,52,53,54).

Epigenetic modifications of genes can program the phenotype of adults if they induce changes in placenta function, foetal development, organ function and epigenome that regulates energy balance (POMC, NP-Y, dopamine, norepinephrine, etc.).

A disturbance in epigenetic regulation is present in many diseases: atherosclerosis, obesity, autoimmune and vascular diseases.
Reproductive functions are very sensitive to environmental conditions such as nutrition, climate and chemical pollutants, which can cause epimutations. Epigenetic reprogramming is an important co-factor of PCOS (methylation disorders in 40 genes) and hyperandrogenism. In hyperandrogenism, epimutations of the peroxisome proliferator-activated receptor γ (PPARγ) gene have been identified, which is a significant transcription factor for the differentiation and function of adipocytes (42,52).

Epimutations can be related to **endocrine disruptor chemicals (EDC)** that interfere with the endocrine system and hormone activity. All organ systems that depend on hormone control can exhibit a disruptor-induced dysfunction. ED has been proven to be related to obesity, diabetes, reproductive disorders and several diseases. The most sensitive to numerous environmental pollutants, toxins and chemicals are the earliest stages of embryogenesis and cell differentiation, as well as gametes. Food is the most common form of exposure to pollutants. There is a close correlation between epigenetic changes to gene transcription and the effects of endocrine disruptors, many of which directly affect lipoprotein lipase function, feeding control, energy homeostasis, obesity and insulin resistance. Environmental chemicals disrupt signalling in estrogen, androgen and thyroxine receptors (or imitate endogenous hormones).

There are thousands of environmental toxins, including among others (42,47,50,51,52,53,54):

- xenoestrogens – imitating estrogens
    - biphenyls (PCBs)
    - alkylphenols
    - bisphenol A (BPA)
- pesticides (DDT, endosulfan)
- phthalates (cosmetic products, sprays)
- phytoestrogens
- heavy metals, paints.

The endocrine disruptor BPA increases the transcription of estrogen receptors, promotes adipogenesis transcription factors (PPAR, C/EBPα) and significantly increases adipogenesis. Different ingredients of plastic, pesticides, insecticides and cosmetic products accumulate in adipose tissue and affect the metabolism and production of adipokines. The expression of genes encoding IGF-1 and LPL is also increased. BPA disrupts estrogen activity via ERs. Endocrine disrupting chemicals play a role in lipogenesis, obesity, insulin secretion, and insulin resistance. Even the sharp increase in the prevalence of obesity in the last 40 years is associated with frequent exposure to EDC. Some EDCs directly affect adipocytes and promote adipogenesis, while others interfere with gametogenesis and reduce fertility in men and women. It has been established that BPA is accumulated in the body of 95% of people. Pesticides, BPA and perfluorinated chemicals (protection of materials) have been proven to have an obesogenic effect directly via PPARγ or indirectly by disrupting thyroid and adrenal hormones, as well as an epigenetic effect on DNA methylation and metabolism of mitochondria. Intrauterine exposure to EDC leads to the transgenerational transfer of epigenetic markers up to generation F3.

Chemicals that can alter adipocyte differentiation, lipid metabolism and promote obesity are called **obesogenic chemicals**. They may include endocrine disruptors, drugs and other chemicals impairing and disrupting:

- metabolism of adipocytes
- production and conversion of steroids
- central control of energy balance.

Neuroendocrine effects of obesogens directly affect the control of reproductive functions. Some of the more common obesogens are: bisphenol-A, sweeteners, nicotine, arsenic, some psychiatric drugs (SSRIs), other xenobiotics, antidiabetics, stilbestrol etc.Factors that lead to obesity undoubtedly affect reproductive dysfunction. The microenvironment promotes epigenetic modification of gene expression and enzyme activity, causing changes in DNA methylation and histones. Environmental toxins (stressors) and diet lead to obesity, while dysregulation of adipocytes (and their signalling) causes reproductive disorders (42-51).

1.2 Relationship between obesity and polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, with a frequency of 7% – 25%, depending on the diagnostic criteria. Symptoms and signs of this syndrome change with age (56,57,58,59) shown in table 9.

Table 9. Symptoms, signs and risks of PCOS during life, EDC- endocrine disruptor chemicals; IUGR- intrauterine growth retardation; IR- insulin rezistence; HA- hyperandrogenism; ROS- reactive oxygen species; MetSy- metabolic syndrome; IGT- glucose intolerance; T2D- typ 2 diabetes; CVD- cardiovasculardiseases; CACS- coronary artery calcification score; AUB- abnormal uterine bleeding; OSA- obstructive sleep apnea

PCOS during life

Symptoms and signs changes

Fetus • in utero	Iuvenile period→ pre-puberty	Adolescence	Reproductive age	Peri and postmenopausis
- Epigenetic reprogramming - EDC • androgens - Hypoxia • IUGR • Macrosomia	• Catch-up growth • Adipose tissue • IR • Premature - adrenarche - pubarche	• Weight gain • Visceral ob. • IR-SHBG • Oligo/anovulation • HA - acne -seborrhoea - Hirsutism • ROS- oxidative stress	• Obesity • Dyslipidemia • Anovulation • Infertility • Met. Sy • IGT • Perinatal pathology • Depression	• Central ob. • T2D • CVD • CACS • Hypertension • Endometrial polyp • Endometrial hyperplasia • AUB • Endometrial cancer • OSA

Simunic 2017

There are significant geo-epidemiological differences in the prevalence of PCOS, and in the expression of individual phenotypes in different populations. The criteria for PCOS diagnosis were discussed for many years, as US expert societies considered that hyperandrogenism (HA) was a mandatory factor for diagnosis. Today, the extended Rotterdam criteria (2003) with the subsequent European and American Reproductive Society Consensus (ESHRE/ASRM) are widely accepted. The diagnosis of PCOS requires that two of three criteria are met: ultrasonographic polycystic ovary morphology (PCOM), ovarian dysfunction (OD–oligoovulation/anovulation), and hyperandrogenemia and/or hyperandrogenism (HA) (table 10).

Table 10. Criteria and classification of PCOS, PCOM- polycystic ovarian morphology; OD- ovulation disfunction; HA- hyperandrogenism

PCOS Classification

	Criteria	PHENOTYPE			
		A PCOM-OD-HA	B OD-HA	C PCOM-HA	D PCOM-OD
PCOM	PCOM-ovaries - morfology - US	Polycystic	Normal	Polycystic	Polycystic
OD	Ovulation-disfunction - oligo/anovulations	Anovulations	Anovulations	Normal cycle	Anovulations
HA	Hyperandrogenism Hyperandrogenemia	Severe	Severe	Severe	Mild
	Insulin	High	High	High	Normal
	Syndrome expression	Classic PCOS Severe		Moderate PCOS	
	Risks	High		Moderate	Mild
	Prevalence	62 %	8 %	15 %	15 %

Legro 2013/2014

Based on the presence of these disorders (PCOM/OD/HA), we distinguish between four PCOS phenotypes: phenotype A, B, C and D. The most common are phenotypes A and B (70%), which are considered 'classic PCOS', with strongly expressed hyperandrogenism and hyperinsulinemia, and the highest metabolic and reproductive risks (58-64).

In adolescence, and in normal circumstances, the occurrence of cycle disorders, PCOM (50%) and acne (about 30% of the population) is more frequent, and it is not recommended that PCOS is diagnosed until two years after menarche if all three criteria are expressed. High levels of the anti-Müllerian hormone (AMH) also contribute to the diagnosis.Recently, there are more and more recomendations to use high AMH values as valid finding for PCOS (65-69).

PCOS in peri- postmenopause is diagnosed based on anamnesis from the reproductive period, and relative hyperandrogenism (1,7,57).Diseases that imitate indicators of this syndrome should be excluded before any final PCOS diagnosis. Those may be congenital adrenal hyperplasia, Cushing syndrome, hyperthecosis, ovarian and adrenal tumors, hyperprolactinemia and severe insulin resistance, as well as androgenic drugs, whose incidence among all hyperandrogenism causes is 5-7% (1,7,57,59).

There are ongoing debates about the significance of PCOM (and phenotype D) and whether it should be included in the syndrome. Such occult PCOS involves mild changes and minimal metabolic risks, but the finding is significant to reproductive endocrinologists considering the difficulties in infertility treatment and risks for the ovarian hyperstimulation syndrome (OHSS). PCOM is more frequently expected to occur in women with infertility. Detailed work-up will identify mild biochemical and hormonal disorders, and a higher sensitivity of follicles to FSH can be expected. AMH is a surrogate marker for PCOM and may be a discriminatory finding for hypothalamic anovulation and hyperprolactinemia (which have PCOM in 30-50% of cases) (64-77).

Formerly, the traditional PCOM criterion was the ultrasound finding of more than 12 antral follicles (AF 2-9 mm) in one or both ovaries. With improved precision and resolution levels of today's ultrasonographic devices, a count of more than 25 AF (20 AF) per ovary (follicular number per ovary - FNPO) is proposed as the new criterion. This type of precise diagnostics requires the use of a vaginal probe (≥ 8 MHz), a multiple section scan, a grid section system, or an automated sono-AVC (56,59).
Additional ultrasonographic criteria for PCOM (ovarian morphology) are ovarian volume (OV) > 10 ml, stromal volume > 7ml, and ovarian area > 5 cm^2. The ratio of ovarian stroma and ovarian area is higher than 0.32.

In classic PCOS, antral follicles with a diameter of 2–5 mm dominate, and antral follicular count (AFC) is 5 to 10 times higher than in the normal population. The count of small 2–5 mm AFs strongly correlates with HA, while those with a diameter of 6–9 mm is less strongly correlated (table 11).

Table 11. Ovarian reserve findings in different populations

Population	Follicle number(per ovary)	AMH pmol/L	Ovarian volume (ml)
Normal	12-20	10-20	5-9
PCOM	22-30	40-60	10-12
PCOS			
-ovulatory	25-30	30-50	13-22
-anovulation	35-80	60-100	
-amenorrhoea	> 70	> 80	

According to these criteria, in our (peri-Mediterranean) region the incidence of PCOS is 15-22%, and in infertile patients the incidence is even higher. The question whether PCOS confers a predisposition to obesity or vice versa remains unanswered.

Obesity and adipose tissue dysfunction are closely related to the polycystic ovary syndrome (PCOS). It has been established that 40-50% of obese women develop PCOS, while 60% of PCOS patients are obese. Women with a pronounced syndrome have more adipose tissue in the upper body, with expressed abdominal (visceral obesity).

The following obesity and PCOS relationships have been established (70-75):

- the prevalence of PCOS in obese women is 30-40%
- obesity in 50-75% of women with PCOS
- abdominal (central) distribution of obesity in 50-60% of women with PCOS
- gynecoid distribution of AT in only 30% of women with PCOS; most women exhibit abdominal (visceral) obesity.
- lean women with PCOS may have an increased amount of visceral fat
- 70-90% of obese women with PCOS exhibit insulin resistance (IR)
- 30-50% of lean (normal) women with PCOS have IR
- chronic inflammation occurs in both obesity and PCOS
- WC > 88 cm in 83% of PCOS patients.

There are studies pointing to a high prevalence of AT disorder in PCOS patients (75,77):

- 85% of women with PCOS are overweight or obese (USA, Australia)
- 83% of PCOS patients in the United Kingdom have a WC > 88 cm (abdominal obesity)
- 65% of abdominal obesity (visceral) is accompanied by PCOS in Italy and USA

Prevalence and clinical expression of PCOS are predominantly induced by obesity. In a meta-analysis of 21 studies, the prevalence of obesity in PCOS patients was 61% (75). The risk for PCOS increases by 9.2% for each BMI unit (BMI above 25) (73). There is evidence that elevated BMI is associated with more frequent and severe abnormalities of the cycle and more pronounced hyperandrogenism. There is no doubt that high BMI and visceral obesity increase insulin resistance (IR) with known consequences. The molecular mechanism of insulin resistance in obesity (and type 2 DM) is different from that in PCOS patients. In PCOS patients, insulin sensitivity is primarily exhibited in skeletal muscles, while hepatic IR is exclusive to obesity (with or without PCOS) (77,78).Both syndromes act in synergy to promote IR and the metabolical syndrome expression.

Obesity, PCOS and hyperinsulinemia significantly affect the reproductive function. Different PCOS phenotypes carry different risks, which are highest with hyperandrogenemia and oligomenorrhea/amenorrhea. Metabolic and reproductive risks are further increased by adiposity. Such phenotypes are also the main risk factors for glucose intolerance, gestational and type 2 diabetes mellitus (DM), dyslipidemia and hypertension (78-86).

Defective insulin signalling in PCOS consists of a 30% decrease in insulin receptor (IR) phosphorylation in adipocytes, elevated serine phosphorylation in fibroblasts in 50% of patients and lower phosphoinositide-3 kinase activity in muscle cells. Hyperinsulinemia is the main cause for PCOS, but a synergistic effect of insulin on steroidogenesis in ovarian theca cells has also been recorded. Adrenal steroidogenesis in PCOS contributes to hyperandrogenemia. Visceral adipocytes are involved in local

steroidogenesis as they possess the enzymes 3βHSD, 17βHSD, 11βHSD and aromatase (87,94).

Visceral adipocytes exhibit a higher sensitivity to lipolysis, i.e. in PCOS the activities of protein kinase A and hormone sensitive lipase are increased. In PCOS adipocytes have an increased capacity for steroidogenesis and stronger conversion of androstendione to testosterone. Visceral adipocytes can convert cortisone into metabolically active cortisol. Increased testosterone and cortisol additionally stimulate central obesity and IR. As the production of SHBG is reduced, levels of free T and free E_2 in the circulation increase.

Adipokine disorder can occur in PCOS. Leptin levels and leptin resistance can be higher. There is an increase in the levels of TNF-α, IL-6 and PAI-1, which are involved in the paracrine activity of adipocytes, as well as ovarian and adrenal stimulation.

In obese and PCOS patients, the production of adiponectin is lower, which also plays a role in IR. Resistin is significantly elevated in these patients, especially in case of anovulation, which is associated with the paracrine control of insulin resistance. Resistin also increases the activity of P450C17 in theca cells, which is an additional incentive for ovarian contribution to hyperandrogenism.

Yildiz and Azziz (73) found the impact of obesity on the prevalence of PCOS to be minimal. However, they found the average BMI in the PCOS population to be 37.3 kg/m^2. The prevalence of obesity (BMI≥30 kg/m^2) in their PCOS population was 74%.

In obesity and elevated BMI there is a higher incidence (with PCOS) of menstrual irregularity (oligomenorrhea/amenorrhea), together with elevated levels of total testosterone (T), free T and free androgen index. At the same time, the levels of sex hormone binding globulin (SHBG) in these patients are lower. Some researchers point to a more frequent occurrence of ovarian cysts in obese patients.

In PCOS patients, obesity is associated with (68,70,71,72,80):

- elevated insulin and insulin resistance (IR)
- elevated cholesterol and LDL, lower HDL
- elevated blood pressure
- central obesity – lower SHBG and higher T, higher IR
- findings of association with hirsutism are not consistent
- hepatic IR
- central obesity has a higher lipolysis rate, and therefore elevated free fatty acid levels in the portal vein.
-

Hyperandrogenism is a disorder that is inherent to PCOS and is further modified and amplified by insulin resistance.According to the current classification of syndromes and reproductive indicators, we distinguish between:

- **polycystic ovary syndrome (PCOS)**, where the above-mentioned phenotypes are expressed
- **polycystic ovary morphology (PCOM)**, where only polycystic ovaries are expressed.

In PCOS, insulin exhibits synergism with the luteinizing hormone (LH) in stimulating the synthesis of ovarian androgens. Insulin also increases the sensitivity of granulosa cells to follicle-stimulating hormone (FSH), which increases the number of antral follicles. They are kept in a state of **arrest** by increased levels of intraovarian androgens and high AMH. For this reason, all circumstances including hyperinsulinemia may result in PCOS. Abdominal obesity (and obesity) induce the secretion of adipokines, which have a direct effect on the ovaries and the adipocyte metabolism. Hyperandrogenism is a result of androgen synthesis in the ovaries, adrenal gland and adipose tissue. Increased androgen levels and environmental factors (abdominal and general obesity, insulin resistance) result in PCOS.

Chronic exposure to elevated androgens (in utero) alters gene expression and leads to the PCOS phenotype. In this way, increased androgens and androgen-induced obesity become a circulus vitiosus for PCOS (64,66,69,80).

The risk of infertility is 3 times higher in obese women and 15 times higher in women with PCOS.

The main reproductive risks in obesity and PCOS are:

- anovulation
- impaired folliculogenesis, oogenesis
- non-receptive endometrium
- multiple pregnancies (with treatment)
- early miscarriages
- obstetrical risks; gestational DM; hypertension; preeclampsia; delivery
- neonatal risks

1.3 Obesity and ovarian reserve

Ovarian reserve is the overall capacity of the ovaries for healthy folliculogenesis and oogenesis.Ovarian reserve is determined by several types of factors:

- **age**
- **genetic factors**
- **epigenetic effects**
  - obesity
  - PCOS
  - environmental endocrine disruptors
- **diseases**
  - endometriosis, inflammation, tumors
  - malignant
  - autoimmune
- **ovarian surgery**
- **treatment**
  - radiation
  - chemotherapy
  - other.

Of the many tests used to determine ovarian reserve (OR), those routinely applied are: basal follicle-stimulating hormone (FSH) level, anti-Müllerian hormone level (AMH) and number of small antral follicles in reserve (AFC – antral follicle count). Additional OR

markers are ovarian volume and levels of inhibin B and estradiol. Ovarian reserve markers are not only a powerful predictor of fertility, but also of infertility treatment success. Today they are an indispensable indicator of the reproductive potential. Findings on the qualitative value of ovarian reserve markers are inconsistent because age diminishes the competence of oocytes which can be present in sufficient numbers. Tests consist of biochemical analysis and ultrasonographic ovarian evaluation. Age and epigenetic factors have the highest impact on ovarian reserve (81-100).
Characteristics of the menstrual cycle (MC) may serve as a simple indicator of ovarian reserve and fertility. Short MC (≤ 23 days), shortening of the cycle, and increasingly scanty periods usually indicate a drop in or low ovarian reserve (101-105).

The **level of basal FSH,** a pituitary glycoprotein hormone, is determined on day 2 or 3 of the cycle (in case of long-term oligomenorrhea/amenorrhea the day is not important). Values above 10 IU/L indicate lower OR, while levels ≥ 18 IU/L are an indicator of primary ovarian insufficiency and inability to conceive. In addition to basal FSH, blood estradiol (E_2) levels are also determined, supplementing the findings about cycle quality. Basal E_2 ≥ 70 pg/ml is an unfavourable sign for reproduction and accompanies increased FSH levels (1,7,100,101).

Anti-Müllerian hormone (AMH) is a representative of the TGF-β superfamily. Produced by the granulosa cells of preantral and small antral follicles (2-7 mm), its functions are both autocrine and paracrine. It controls the recruitment and growth of antral follicles with primarily inhibitory effects. AMH inhibits recruitment, FSH activity, and aromatase. It is an important controller of steroidogenesis and the selection-dominance mechanism in folliculogenesis. The AMH levels vary minimally throughout the cycle, with the pill or ovulation stimulation. Therefore, it is today considered as the most reliable marker of ovarian reserve. It is a measure of the quantity of oocyte reserve (follicles), but not its quality. It is a reliable predictor of success and risks of ovulation stimulation (OS), as well as of female fertility. It works via Type 1 and Type 2 receptors with 3 transmembrane domains. As an expression of the functional reserve of the ovary, AMH is an indicator of follicles that can react to FSH. Also, AMH synergizes with LH and insulin in stimulating androgen production. Its normal serum levels are 12-20 pmol/L.

There are 4 generations of AMH laboratory tests, which can deliver different results (± 20%): DSL, Beckman coulter I and II, Elecsys (Roche). Today, the best (and compatible) are BCII and Elecsys (108,109,110).

Antral follicle count (AFC) is the sum of follicles displayed by the ultrasound with a mean diameter of 2 to 10 mm in both ovaries. This measure has a good inter-cycle reliability with similar results in experienced diagnosticians. The administration and evaluation of the test changes with technological advances and resolution of modern US devices. That is why today AFC ≤ 9 is considered low, while the count of 10-20 antral follicles of that size is normal. AFC is a quantitative, rather than a qualitative measure of follicles (oocytes).

The expected ovarian response to ovulation stimulation is determined based on AMH and AFC ovarian reserve markers. The response may be:

- poor ovarian response (POR)
- normoresponse (NR)
- high response (HR)

Ovarian reserve markers are neither a reliable predictor of definitive fertility nor infertility treatment. These markers are directly related to natural fertility indicators and all negative effects on the ovaries. Evidence of poor ovarian reserve (POR) is associated with a small number of oocytes, poor response to treatment, frequent discontinuation of treatment and low success rate and live birth rate (fig 10).

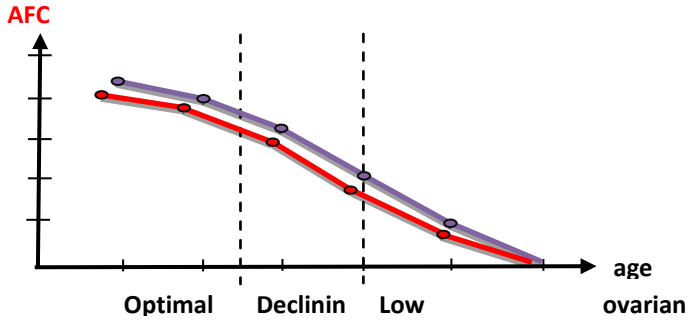


Figure 10. Ovarian reserve with aging, AFC - antral follicle count; AMH - anti-Müllerian hormone

AFC and anti-Müllerian hormone are good PCOS markers as numerous antral follicles in reserve exhibit particularly high values in patients with this syndrome.

Interestingly, most analyses show that obesity is negatively correlated with AMH, while in PCOS/PCOM the anti-Müllerian hormone is significantly elevated. AMH is also higher with elevated androgens. Obese patients with PCOS also have lower AMH than normal weight patients. The highest values of ovarian reserve markers are found in PCOS patients with amenorrhea, hyperandrogenism and normal BMI (see table 12).

Table 12. Approximate values of ovarian reserve markers and incidence in infertile population (some groups overlap in terms of prevalence), **POR** - poor ovarian response; **HR** - high response; **PCOM** - polycystic ovary morphology; **PCOS** - polycystic ovary syndrome

Characteristic Patients	FSH IU/L	AMH pmol/L	AFC	oocyte number IVF	Prevalence
Normal	4-8	9-20	9-20	5-14	40 %
POR	8-11	≤ 8	≤ 8	≤ 3	16 %
HR	3-5	> 20	> 20	≥ 15	15 %
PCOM	3-5	30-60	20-30	≥ 20	15 %
PCOS	2-4		> 25	≥ 25	30 %
● normal cycle		50			
● oligomenorrhoea		80			
● amenorrhoea		≥ 100			
Obesity	4-8	10-20	10-20	4-12	30 %

We used the most frequently cited data in literature to illustrate in the figure the findings concerning ovarian reserve markers in the infertile population of women (81,88,90,93,97,101,102,106).

Obesity - consequences and comorbidity

The described pathophysiological changes of adipose tissue and adiposity have a strong effect on energy imbalance, metabolism and reproductive capacity. Today, obesity is on the rise as the most important factor of cardiometabolic risk and mortality. The relationship between complex AT functions and control centers in the CNS and peripheral organ systems and tissues undoubtedly exerts influence on energy homeostasis, metabolism, immune system and gonadal function.

It has been shown that there is a close association between obesity and the metabolic syndrome (MS). The metabolic syndrome, i.e. energy imbalance, affects the control of the cycle and reproduction. The onset of puberty is delayed by excessive leanness and accelerated by obesity. The disturbed gonadal function further stimulates MS. Poor energy homeostasis affects the production of steroids and ovarian peptides, which hinder the function of adipocytes and induce insulin resistance (43,44,45,46,47).

Metabolic syndrome and other health risks in obesity

Nearly 300,000 people die annually in the US due to obesity. Adiposity comorbidity includes diabetes, dyslipidemia, cardiovascular disease, hypertension, obstructive sleep apnea and some types of cancer. Generally, excessive BMI has a strong impact on health (83,101,107).

Metabolic syndrome (MS) has several definitions (according to the WHO and professional associations) depending on which component of the disorder is emphasized. According to the WHO, it is a combination of the following:

- presence of diabetes mellitus (DM), elevated blood glucose, glucose intolerance or insulin resistance, and 2 of the following additional criteria:
- abdominal obesity (WHR > 0.90 men; > 0.85 women) or BMI > 30 kg/m^2
- elevated triglycerides
- low levels of high-density lipoprotein (HDL)
- albumin excretion (proteinuria)
- hypertension.

Other definitions emphasize elevated BMI, WC and most importantly **visceral obesity.** Specific health risks include cardiovascular disease and type 2 DM. Also associated

with MS are hyperuricemia, fatty liver, PCOS, erectile dysfunction and acanthosis nigricans. Global prevalence of the metabolic syndrome is 20-30%.

The relationship between visceral obesity, white adipose tissue (WAT) and its dysfunction, and general and liver insulin resistance (IR) has already been explained. Central and peripheral energy homeostasis disorder, and adipokine imbalance (leptin, adiponectin, resistin) induce IR and hyperinsulinemia. Insulin and adipokines steer ovarian steroidogenesis towards androgens, which provide in that closed circuit an additional stimulus for IR. Hyperinsulinemia reduces SHBG production in the liver, resulting in higher free testosterone and estradiol levels. High insulin levels impact the imbalance of TG transforming enzymes (LPL, HSL) and expression of PPARγ, which leads to visceral lipogenesis. The brain modulates the peripheral lipid and glucose metabolism and is therefore important for MS development (87,94,101).

Pathophysiological changes in adipose tissue further disturb and amplify PCOS. Apart from contributing to IR, PCOS intensifies menstrual cycle disorders and leads to oligoanovulation. Absolute and relative elevation of estrogen (estrone and free E_2) levels without counteracting progesterone effects creates a risk for pathological changes of the endometrium - polyps, hyperplasia and endometrial cancer.

The described changes of visceral adipose tissue induce accelerated atherogenesis and cardiovascular diseases This risk is indicated by the inflammatory marker CRP (high sensitivity CRP is determined). Low adiponectin is a silent indicator of visceral adiposity and the risk for atherosclerosis and MS, i.e. cardiometabolic risk. Dysfunctional adipose tissue with adipokine and FFA metabolism disorder leads to ectopic fat accumulation in the muscles, liver and epicardium.

All the described changes may cause early coronary heart disease (CHD) and stroke. In metabolic syndrome, the risk of type 2 DM, which is considered a complication of MS, is five times higher. The prevalence of metabolic syndrome in female patients with CHD is above 50%.

1.4 Obesity and reproduction

Obesity has a strong negative effect on human reproduction, equally in men and women. Obese women have elevated risk of subfecundity and infertility, especially due to frequent association with polycystic ovary syndrome. Obesity affects the dysfunction of all fertility levels: natural, induction of ovulation, in vitro fertilization (IVF), donation programs. It has been found that reduced fertility is more pronounced with excessive BMI, central obesity and more severe PCOS forms. Obesity and hypogonadism reduce male fertility (109-123).

Interpretations of the pathophysiology of obesity (and PCOS) distinguish between central and peripheral disturbances that impair fertility.

The hypothalamic control of energy homeostasis and reproduction includes the following disturbances in obesity:

- elevated leptin and insulin levels have a negative effect on the relationship between POMC (αMSH)/neuropeptide (NP-Y-AgRP) and kisspeptin
- estrogen-androgen imbalance affects stimulation or inhibition of GnRH neurons
- the concentration of kisspeptin is negatively correlated with BMI, insulin resistance and FAI
- ghrelin suppresses the expression of KISS1 and inhibits the secretion of LH (GnRH)
- LH activity (pulsatility) is decreased by obesity and increased by PCOS
- adipokines, insulin, CRH and steroid hormones additionally disrupt the functions of brain opiates, dopamine, autonomic nervous system, and endocannabionid system
- disturbed neurosecretion of GnRH translates into gonadotropin dysfunction (level, isoforms, activity, pulsatility)
- this results in pubertal disorders, menstrual cycle disorders, oligoanovulation and potentially amenorrhea.

Increased leptin and endocannabinoid system activities have a negative effect on the central control of reproduction.

Peripheral disturbances that impair fertility in obese (and PCOS) female patients are:

- lower (or disturbed) FSH and LH activity in obesity and PCOS (↑LH and pulses)
- negative correlation of BMI, visceral fat, waist circumference with FSH and LH
- effect of insulin and adipokines on liver (SHBG, IGFBP-1) and ovarian (follicles) function
- reduced SHBG and increased testosterone levels in central obesity
- impaired folliculogenesis due to elevated androgen production (ovary, AT) and elevated free testosterone, leading to anovulation and follicular arrest
- reduced oocyte biosignals (GDF-9 and BMP-15), elevated IGFBP-4
- AMH and inhibin B: lower in obesity, significantly elevated in PCOS
- positive correlation of AMH with insulin resistance, free T, cholesterol
- inhibition of steroidogenesis, oocyte maturation and endometrial transformation affected by elevated leptin levels in the ovaries
- compromised endometrial receptivity caused by obesity and PCOS
- follicle functions undoubtedly affected by obesity and PCOS
    - differentiation, growth, steroidogenesis, gametogenesis
- changed hemostasis factors and increased tendency to thrombosis (venous and arterial) in obesity and PCOS.

Adipose tissue affects steroidogenesis disorders in all sources of production and the bioavailability of steroid hormones. As already described, obesity is accompanied by a dysfunction in the conversion of prehormones to androgens and estrogens in adipocytes. There is also a direct effect on steroidogenesis in follicular granulosa and theca cells, and a reduction in the peptide transporter (SHBG) of steroid hormones in the circulation.

Obesity is characterized by hypersecretion of LH and elevated LH/FSH ratio, as well as increased pulse frequency with reduced amplitudes. This is particularly pronounced in obese persons with PCOS. Increased insulin reduces SHBG, ovarian IGF binding

protein-1, and increases the levels of ovarian androgens. Also, elevated TNF-α (with obesity) is an independent inhibitor of SHBG (1,7,40,110).

Central and peripheral disturbances in obesity and PCOS interfere and are complemented by other epigenetic effects. This results in several consequences for reproductive capacity (Fig 11 and 12). Intrauterine impacts of obesity, endocrine disruptors and transgenerational transfer of abnormalities should also be considered. Since mother's obesity may cause child macrosomia, there is a 25% higher risk for childhood and adolescent obesity. Intrauterine exposure to androgens can cause intrauterine growth retardation (IUGR), later catch-up growth of the child, juvenile visceral obesity and hyperandrogenism. Obesity and endocrine disruptors are characterized by the ability of vertical, transgenerational, transfer of foetal gamete damage. Elevated androgen and AMH levels in utero cause subsequent development of PCOS phenotypes.

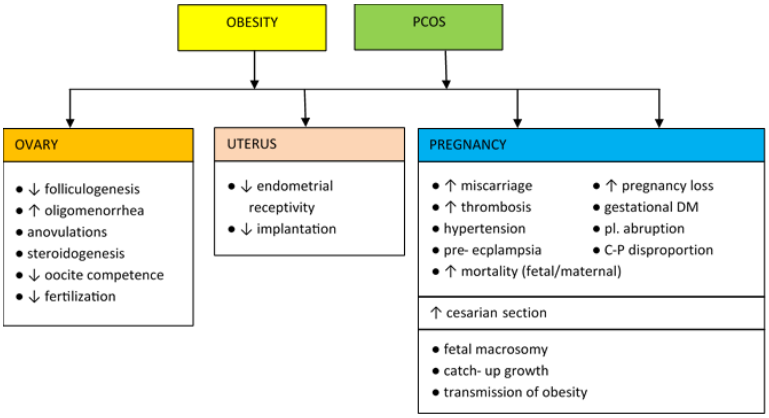


Figure 12. Influence of obesity on reproduction (women)

Obesity and PCOS in the mother increase the likelihood of the daughter later developing hyperandrogenism and PCOS phenotype.

Menstrual cycle abnormalities are common in obesity, central obesity and PCOS. The amount of adipose tissue is associated with events in female puberty. Obesity induces early onset of menarche and PCOS can be the primary and long-term cause of menstrual cycle inhibition. Irregular cycles occur more frequently in obese adolescents

compared to those with normal weight (54% vs. 19%). Obese women have longer cycled, and 40% have cycle and bleeding disorders. Women with oligoamenorrhea have higher BMI, WC and higher insulin levels compared to women with normal cycles. Obesity and IR are associated with earlier menarche and anovulation and disturbed luteum function. Obese adolescents are at a significant risk of infertility later in life (OR 2.84 CI 1.59-5.10). The Nurses' Health Study reported that in 2527 women a correlation was found between anovulatory infertility and elevated BMI (> 26 kg/m^2) with a relative risk of 1.7-2.7 depending on the degree of obesity. In the same population (study), a significantly higher risk of anovulatory infertility was found in obese women (RR 3.1 CI 2.2-4.4) than in women with normal BMI (1,7,50,109,110).

A Danish study found that each increase in WHR by 0.1 units results in the fecundity rate drop by 30% (HR 0.70 CI 0.56-0.88). Other studies also point to reduced fecundity (probability of pregnancy in a single cycle) in obese women – OR 0.69 and OR 0.82 (CI 0.72-0.95) compared to normal BMI. Time to pregnancy is also longer in obese women (longer than 1 year) OR 1.32 (CI 1.26-1.37) (50,110).

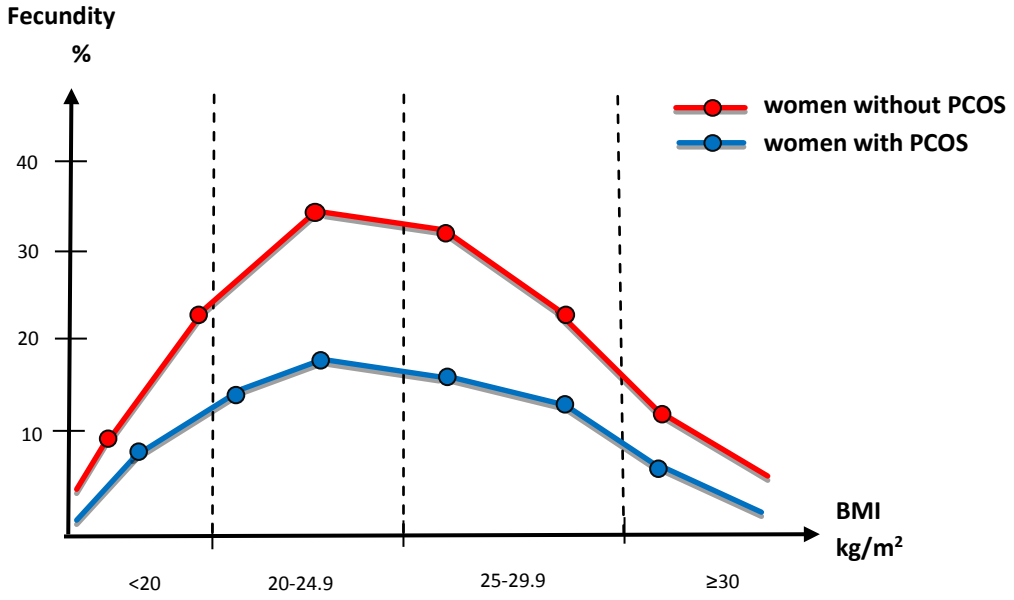


Figure 13. Body mass index and fecundity in normal and PCOS women(Fecundity - probability of pregnancy per month), Central obesity (WHR> 0.85 / WC≥90 cm) significantly reduces fecundity – OR 0.80 (CI 0.59-1.0).

Pregnancy in obese women, regardless of whether it is natural, through ovulation induction or in vitro fertilization (IVF), is more frequently terminated by **early miscarriage**. Most studies show that after anovulation treatment, or in vitro fertilization, obesity increases the risk of miscarriage:

OR 1.69 (CI 1.13- 2.51) Norwegian study

OR 1.53 (CI 1.27-1.84) Meta-analysis Maheshwari 2007

OR 1.67 (CI 1.25-2.25) Meta-analysis Metwally 2008

OR 2.7 (CI 1.5-4.9) ET 1 blastocyst / Rittenberg 2011

OR 1.31 (statistically significant) Meta-analysis of 33 IVF studies / Rittenberg 2011

These studies compared obese patients with those with normal BMI who were treated in the same way (50,110,111,112).Obese IVF patients exhibit relative gonadotropin resistance.

There is an extensive literature that has consistently confirmed the negative effects of obesity on **oocyte quality**, which has been consistently supported by extensive literature. Obese patients treated with IVF have several intra-follicular disorders, elevated insulin, triglyceride, inflammatory marker and androgen levels, which change the function of the mitochondria and endoplasmic reticulum, granulosa and theca cells as well as the signalling of oocyte maturation. Such changes are more frequently accompanied by apoptosis, immature oocytes and aneuploidy (125-140).
The fact that in obesity (BMI> 40 kg/m^2) the chance of IVF pregnancy is reduced by 50% is also an indication of lipotoxicity to follicles and oocytes (116,124).

In obese IVF patients, ovulation stimulation takes longer, the dose of gonadotropin is higher, the cancelled cycles rate is higher and less oocytes are aspirated. Adiposity and dysfunctional AT are believed to accumulate hormones, while leptin changes the FSH response and paracrine activities (IGF system, AMH, BMP) that control ovarian steroidogenesis and oocyte maturation. Adipose IVF patients have smaller oocytes and significantly fewer metaphase II oocytes resulting in lower fertilization rates. Some researchers believe that obesity additionally impairs the development of the embryo, which in the blastocyst stage has fewer cells and disrupted TG, glucose and amino acids catabolism, more frequently leading to embryonic arrest. These findings are not

consistent because more comprehensive IVF cycle studies have demonstrated a minimal effect of obesity on oocytes and embryos. Today, however, an increasing amount of research shows that an BMI > 25 (30) is related to a significant reduction (-10 to 15%) of the fertilization rate, which is a surrogate marker for oocyte quality (126,127).

The effect of obesity on **endometrial receptivity** is also unclear due to inconsistent study findings. It has been found that obesity is associated with disturbed expression of endometrial implantation genes. This includes defective decidualization and placental dysfunction, as well as evidence of a negative correlation between BMI and endometrial leukemia inhibitory factor (LIF). In obese patients, implantation disorders and repeated miscarriages are a consequence of abnormalities in leptin and insulin signalling and elevated estrogens in obese patients. Hyperinsulinemia lowers local glycodelin and IGFBP-1 levels.

Obesity also increases pro-inflammatory cytokines, which negatively affect implantation. Controversies over the significance of the relationship between obesity and endometrial receptivity have been resolved by steering research towards the oocyte donation model. This allows to establish whether obesity has an effect on oocyte/embryo or the endometrium. In 2656 cycles with donated oocytes, a lower ongoing pregnancy rate (OPR) was found in obese recipients of quality embryos (36.1% vs. 45.2% OPR). In a donor program with 22,000 patients, Provost et.al. (116). found that obesity in recipient patients was linked to reduced clinical pregnancy rates (CPR) – OR 0.78 (CI 0.69-0.87). In case of morbid obesity, live birth rate (LBR) was 40% lower (OR 0.64 - CI 0.51-0.81), and pregnancy loss rate was twice as high (15.9% vs. 8.6%). It has been concluded that obesity has a detrimental effect on embryo-endometrial dialogue. Bellver et al. (118,127). did not establish that obesity reduced the quality of the embryo, but the results of 9587 first ovum donation cycles showed that obesity progressively reduced implantation, CPR and LBR. Obese recipients have significantly lower LBR (27.7% vs. 38.65) - OR 0.73 (CI 0.66-0.80), while the incidence of early miscarriage is the same. In surrogate procedures, LBR is 15% lower in obese recipients (133,134).

The relationship between **obesity and embryo** can be significant if it is assumed that excessive adipose tissue negatively affects oocyte maturation. In this regard, findings on the reduction of embryo quality are inconsistent. Approximately the same number of studies that supports either assumption is available, but evidence is weak, and it cannot be concluded that obesity affects the quality of embryos. It has been found that cryopreservation of embryos is twice as frequent in patients with normal weight than in obese patients (22.7% vs. 10.7%). Retrospective analysis of 6500 IVF cycles has not shown differences in embryo quality and freezing possibilities between obese and normal BMI patients. However, IVF in obese patients seems to create embryos of inferior quality, that formation of the morula is quicker, and that the blastocyst has lower cellularity. Negative effects on the embryo are exerted by leptin and FFA lipotoxicity. There is no correlation between obesity and embryo aneuploidy (125,126,123).

Results of assisted reproductive technologies (ART) in obese patients are inferior to those in women with normal BMI. There is ample evidence that lower LBR is associated with higher BMI. Ovulation stimulation (OS) in adipose women (8145) undergoing IVF is characterised by a need for higher FSH doses, follicular asynchrony, higher cancellation rates, fewer oocytes. Higher doses and OS duration are explained by higher FSH threshold, absorption and metabolism of gonadotropins (relative gonadotropin resistance). There is a positive correlation between obesity and the amount of gonadotropin, with obese patients needing on average 771 IU of FSH more than normal weight patients (114,121,124,133).

OS success is lower if WHR is higher (OR 0.60; CI 0.40-0.89), and in case of high BMI, ovulation rates are significantly lower - OR 0.44; CI 0.31 to 0.61. With each unit of BMI increase above 29 kg/m^2, the rate of spontaneous pregnancy is decreased by 4%.

In terms of lower IVF success in obese patients, findings are consistent (though not entirely). In two retrospective studies on 3586 and 2660 women, a significantly lower LBR was found in obese women (BMI > 30 kg/m^2) - OR 0.73 (CI 0.57-0.96) and OR 0.75 (CI 0.57-0.98). Central obesity (WHR > 0.8) significantly lowers CPR, i.e. by 58%. Meta-analysis of 33 IVF studies determined that obesity reduces the success rate -

CPR and LBR (RR 0.84). A recent study of 4609 women shows that an BMI above 30 kg/m^2 statistically reduces LBR by 37%, while an BMI ≥40 kg/m^2 reduces LBR by 68% (133,134,137).

In a study with 45,163 ART embryo transfers, Luke et al. (132). found that the freqency of the failure to achieve pregnancy is significantly higher in obese women. Failure to achieve pregnancy increases with increasing BMI - the probability of failure increases by 48% to 64% depending on obesity category.

Obesity and PCOS occur together in 50-70% of patients, and as emphasized earlier, syndrome expression is increased with obesity. For this reason, obese women with PCOS exhibit a more severe syndrom phenotype with corresponding consequences. These patients have a high prevalence of menstrual cycle disorders (78%). Reproductive disorders are especially pronounced in PCOS patients with central obesity. Ovulation stimulation is compromised and more difficult, withdrawal rates are higher, and probability of ART success is reduced. There is a high risk of miscarriage – OR 3.05 (CI 1.45-6.44) due to combined pathophysiological disorders of obesity and PCOS. Anovulatory PCOS is associated with a more pronounced visceral obesity – average waist circumference is 93 cm (7 cm more than in PCOS patients with ovulation). Obese patients with PCOS have a 77% lower chance of pregnancy after ET than lean PCOS women (OR 0.23; CI 0.08-0.68). The odds ratio for live birth rate is similar (OR 0.29). Obesity combined with PCOS reduces the risk of ovarian hyperstimulation syndrome (OHSS). Morbid obesity further reduces CPR in PCOS patients (134,135,137).

Congenital abnormalities are more common in children of obese mothers. Extensive epidemiological studies have shown that children of obese women have a more frequent incidence of heart conditions, disorders in the development of the neural tube, palate, and front abdominal wall as well as multiple anomalies. Absolute risk for these defects is low (127,134).
Fetal abnormalities are associated with maternal metabolic disorders.

There is uniform evidence that a reduction in AT or body weight significantly improves metabolic and reproductive indicators. Traditionally, a reduction in body weight by 5% is believed to improve fertility, but there is still disagreement in that respect (136,138).

2.HYPOTHESIS

Through this prospectivestudy, it has been proposed to do comparation and evaluation of theimpactof overweight andobesity of women's in the reproductive age(≥23 y ≤ 38 y), in ovarian reserve and their reproductivefunctions, making comparisonbetween the two observed groups with (BMI 25-29.9 kg/m^2 and BMI ≥30 kg/m^2),with control group BMI 18-24.9 kg/m^2, measuring values ofhormones such are FSH, LH, Estradiol, AMH, Testosterone, SHBG, TSH, HbA1C, on day 3 of menstrual cycle and by ultrasound measuring of AFCand ovaries-volume's.

Hypothesis: The **o**varian reserve markers are disturbed more frequently in overweight and obesity group of infertile women than in infertile women with normal BMI **and t**he standards and procedures for estimation of ovarian reserve in infertile women will be simplified for using in Kosovo health care conditions.

3. AIMS AND THE PIRPOSE OF THE RESEARCH

In line with the hypothesis the aim of the study is evaluation of the impact of overweight and obesity in ovarian reserveand doing comparison between three observed groups with different BMI, changes in the values of hormones levels of and measurement of ovaries-volume's and AFC, in follicular phase of menstrual cycle in infertile women's of Kosovo population and based on the results of the comparison, the model of decision making for treatment of infertile women will be developed.

SPECIFIC OBJECTIVES

- To do comparison between three observed groups with different BMI, changes in the values of hormones levels of and measurement of ovaries-volume's and AFC, in follicular phase of menstrual cycle in infertile women's of Kosovo population.

- Based on the results of the comparison, the model of decision making for treatment of infertile women will be developed

4. MATERIALS AND METHODOLOGY

This is a prospective cross-sectional cohort study (observational) on 182 women participants recruited during 2,5 years in infertility clinics in Kosovo (n=126) and Polyclinic IVF Zagreb (n=56). Primarily, we analysed 268 infertile patients who came on first visit and workout, but complete results we were able to collect for 182 women. Ethical boards of clinics involved in this investigation approved the study.

All participants were informed about the study and signed the written consent form for participating in it.

4.1 Observed outcomes measures.

In a desire to research the impact of obesity on the ovarian reserve and some of the indicators of fertility, all women are grouped in 3 categories according to body mass index (BMI):

Table 13. Three groups of patients according to BMI

I group	normal BMI	18,5 – 24,9 kg/m^2	–	64 patients
II group	overweight	25 - 29,9 kg/m^2	–	56 patients
III group	obese	≥ 30 kg/m^2	-	62 patients

We also investigated some effects of morbid obesity (BMI ≥ 40 kg/m^2) on the indicators of fertility. We have also analysed the incidence of central (abdominal) obesity and its impact on the ovarian reserve.

In the researched population of infertile women there are **52 patients** with PCOS - 15 patients with normal BMI; 16 PCOS patients were overweight, and 21 PCOS patients were obese. We performed **anthropometric measurements** on all patients, analysed the characteristic of the menstrual cycle, indicators of lowered fertility and detailed hormonal analysis.

We connected these findings with indicators of ovarian reserve and determined the effect of obesity on the analysed hormones.

Women with normal BMI, without PCOS, were the control group.

4.2 Inclusion criteria

Infertile women aged 23 to 38 years, with BMI from 18,5- more than 30 kg/m^2. Primary or secondary infertile PCOS women regardless BMI. Participants with poor response on infertility therapy, and patients with metabolic syndrome and glucose intolerance risks. Secondary infertile women with previous spontaneous abortions or deliveries.

4.3 Exclusion criteria

Women younger than 23 and older than 38 years. Infertility because of uterine anomalies, tubal factor, severe endometriosis, genetic and autoimmune diseases. Women with bariatric surgery, and patients with ovarian or uterine tumours, general endocrine diseases (pituitary, adrenal, thyroid) or diabetes mellitus type 1. Patients infertile because of husbands (partners) azoospermia. Also, a patients on hormonal medications were excluded (except those with subclinical hypothyroidism).

4.4Definitions and measurement

4.4.1 Infertility

According to World Health Organization (WHO- human reproduction programme 2016):
Infertility is disease of reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility prevalence is 15-17%.
We distinguish primary infertility when pregnancy is not achieved and secondary infertility after history of at least 1 pregnancy.
Fecundability and fecundity is the probability of achieving pregnancy or giving birth in one menstrual cycle (normal is 20% or 10% respectively) subfertility is a term of lessened fertility which prolongs the time to pregnancy (TTP).
All indicators of female fertility depend on the age of the woman.

4.4.2 Questionnaire

All patients filled out a previously designed questionnaire regarding personal, familial and reproductive history, prior to getting examined and treated.
They also answered the questions:

- obesity, diabetes and PCOS in the family
- when they gained weight
- characteristic of menstrual cycle
- previous pregnancies
- what was their body weight when they conceived
- how much time has passed until they achieved desired pregnancy
- what was the excess of weight left after giving birth
- start and prominence of acne andhirsutism
- other eventual signs of hormonal disorder.

4.4.3 Ovarian reserve

Ovarian reserve is the overall capacity of the ovaries for healthy folliculogenesis and oogenesis. The main background is adequate number of antral follicles 2-10mm in diameter which is capable for recruitment.

Table 14. Markers of ovarian reserve are:

	Normal	Low
• antral follicular count – AFC	9-20	≤ 8
• antimüllerian hormone AMH (pmol/L)	9-20	≤8
• ovarian volume OV (ml)	5-9	< 5

Secondary biomarkers are FSH ≥ 11IU/L, and serum estradiol higher than 70 pg/ml.

4.4.4 Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) was diagnosed according to Rotterdam extended criteria. The diagnosis of PCOS requires two out three criteria:

1) ultrasonographic polycystic ovarian morphology (PCOM)
- more than 12 antral follicles (AF 2-10 mm in diameter) in one ovary
- ovarian volume ≥10 ml
2) ovarian dysfunction (OD)
- oligo/ anovulation
3) hyperandrogenaemia and (or) hyperandrogenism (HA)
- biochemically high serum androgens
- calculations of HA by free androgen index (FAI)

orhirsutism (according to mod F-G score)
- acne

Additional criterion could be high AMH value (≥ 35 pmol/L).

4.4.5 Ultrasonography

Ultrasonographic (US) transvaginal examinations was performed by 3 experienced reproductive subspecialists using modern US machines with vaginal probe ≥8 MHz (General Electric LOGIQ E6/8). The ovarian scan analysis was made on 3 sections scans, or by automated sono - AVC. In brief, it was measured and calculated AF, and length high width of the ovaries x 0,523 was formula for ovarian volume.

4.4.6 Menstrual cycle and ovarian dysfunction

Subject were asked to report average menstrual cycle length in the last year, and oligomenorrhoea was defined as cycle length more than 35 days.
Anovulation (or oligo-anovulation) were diagnosed by basal body temperature, repeated gynaecological and ultrasound examinations, LH estimation, and low luteal serum progesterone values.
Amenorrhea was defined as loss of menstrual cycle for longer than 3 months.

Heavy menstrual bleeding was diagnosed by periods longer than 7 days, clots in menstrual blood, use of more than 8 sanitary pads per day, blood analysis.

4.4.7 Anthropometric obesity measurement

All participants were grouped according to body mass index (BMI) in 3 groups (as it was mentioned). The physical measurements were:

- height (cm), weight (kg), BMI (kg/m^2)
- waist circumference (WC)
- hip circumference (HC)
- waist to hip ratio (WHR)
- waist to stature (height) ratio (WSR)

Body mass index was for general obesity assessment, and WC, WHR and WSR are measures of central (abdominal, visceral) obesity. The cut-off values (abnormal) for overweight, obesity and central obesity are:

Table 15. Cut-off abnormal values of WC, WHR and WSR

Measure	Cut-off values	
BMI (kg/m^2)	25-29.9 Overweight	≥ 30 obese
WC (cm)	88	
WHR	0,85	
WSR	0,50	

BMI is defined as persons weighted in kilograms divided by square of his height in meters (kg/m^2)

Waist circumference was measured after full expiration, from the front at the narrowest point between the rib cage and iliac crest (the smallest part of waist).

Hip circumference was measured from the side at the maximal extension of buttocks (distance around the largest part of hips). Measurements should always be strictly horizontal, using a non-expansible tape measure.

For both measurements, the WHO STEPS protocol recommends that the subject stands with arms at the sides, feet positioned close together, and weight evenly distributed across the feet (WHO, 2008b)

WHR was calculated by dividing waist circumference by hip circumference.

The aim was to assess the associations between general and central obesity anthropometric measures with hormonal production and ovarian reserve.

4.4.8 Hormonal analyses

On the 2nd to 4th menstrual cycle day serum was analysed for folliculostimulating hormone (FSH), luteinising hormone (LH), prolactin (PRL), estradiol (E_2) total testosterone (T), antimüllerian hormone (AMH) and sex hormone binding globulin (SHBG) using assays and instrument from Beckman Coulter Inc. or Roche (by medical equipment ROCHE Cobas- Elecsys E411), by ECLIA methodology, which consist by taken intravenous of 3ml of blood, waiting for 30 minutes and then separated serum was used.

ECLIA is the Novel Electrochemiluminescence (ECL) technology and provides superior analytical performance. Increased sensitivity means that extremely low levels of antigen, as well as subtle changes in levels, can be detected. The very wide measuring range facilitates cost and time efficient testing by reducing the need to dilute and repeat samples). Total testosterone was mostly assayed by imunochemiluminescence method. If needed progesterone (P_4) was analysed in the mid luteal phase.

Free androgen index (FAI) was calculated according to formula FAI= total testosterone (nmol/L) x100/ SHBG (nmol/L). Normal values (our laboratories) for hormones in women are shown in table 10.Test HbA1C have been performed by ROCHE Cobas integra 400 plus by turbidimetric inhibition immunoassay

Table 16. Normal values of hormones and FAI

Hormone	Normal values
FSH (IU/ L)	5-10
LH (IU/ L)	5-10
PRL (IU/L/ µg-L)	100-500/ 4-23
AMH (pmol (L)	9-20
E_2 (pg/ ml)	30-70
T (nmol/ L)	0,20- 1,80
SHBG (nmol/ L)	18-144
FAI	1-4

We have analysed the impact of obesity and PCOS on hormonal activity of ovaries and fat tissue. Also, the associations of central obesity with ovarian reserve and hyperandrogenaemia.

4.4.9 Hyperandrogenism

Hyperandrogenism is the possible indicator of the more severe phenotype of PCOS, high ovarian reserve and reproductive risks. Signs are hirsutism, acne, male type baldness (android alopecia).Hirsutismwas graded base on modified Ferriman- Gallwey scale (m FG score), a cut-off for increased hairness was taken according to recommendations (m FG>7).

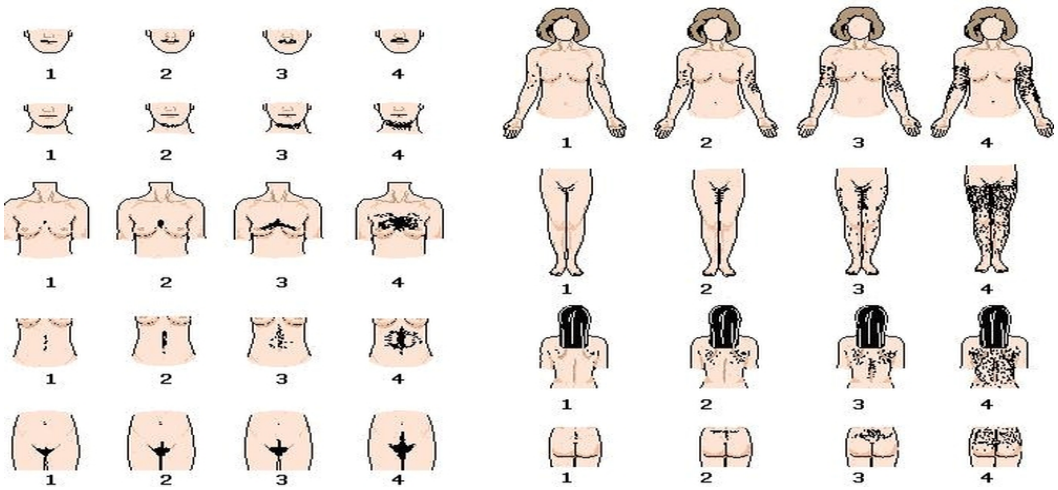


Figure 14. Modified Ferriman- Gallwey score (57)

Acne, oily skin and hair (scalp) are signs of **hyperandrogenism.** Acne were characterized as mild, moderate and severe (Allen) and alopecia, according to Ludwig scale. **Hirsutism** (**hyperandrogenism**) is expressed in 10-15% of normal women and in 60-70% PCOS patients.

4.5Statistical analysis:

Statistical analysis were performed by using statistical package SPSS 17.0. Data are reported as mean ± standard deviation.

Student's *t* test/ANOVA was used to compare means of parametric data and Man-Whitney test/ Krusckal Wallis test to compare non-parametric data. Chi square test for analysis of categorical outcomes.

For statistical significance the value of the factor alpha is ≤ 0.05.

5.RESULTS

Following the study inclusion criteria, a work-up was performed on the first 268 long-term infertile female patients who came for their first visit to the (tertiary) infertility clinics in Kosovo (Priština, Gjakova) and Zagreb (Poliklinika IVF). A full work-up was possible in 182 women (from the same ethnic group), of which 126 in Kosovo and 56 in Zagreb. The research took place over a period of 2.5 years, with the same criteria used to collect patient demographics, anthropometric measurements, ultrasound biometry and hormone analyses. Polycystic ovary syndrome (PCOS), inevitably associated with female infertility and obesity (Table 11), was diagnosed in 52 patients. All patients were classified in three groups according to body mass index (normal BMI - 64 patients, overweight - 56 patients, and obese - 62 patients) and their results were compared. Results were classified and analyzed according to PCOS status into non-PCOS and PCOS patients (n=130 and 52, respectively). The primary objective was to investigate how patient characteristics were associated with ovarian reserve (Table 17). Results are presented as mean ± standard deviation, with the prevalence (%) in each investigated group (% within group, % of total).

5.1 Patients characteristics and distribution

Depending on body mass index, patients were categorized into group I, II or III. These groups were analyzed on the basis of two basic characteristics. Non-PCOS patients were affected only by obesity, while PCOS patients exhibited both PCOS and obesity.

Table 17. Incidence and categorization of participating women

Patients	All	Non PCOS	PCOS
I Group **BMI 18,5-24,9 kg/m^2**	64 (35,16%)	49 (37,69%)	15 (28,84%)
II Group **BMI 25-29,9 kg/m^2**	56 (30,77%)	40 (30,77%)	16 (30,77%)
III Group **BMI ≥ 30 kg/m^2**	62 (34,06%)	41 (31,54%)	21 (40,38%)
TOTAL	182 (100%)	130 (71,43%)	52 (28,57%)

It should be noted that this prevalence results from the selection of patients as we recruited the first 60 patients from each BMI group who underwent full work-up.

Of the total number of patients (n=182), 1/3 had normal BMI, 1/3 were overweight and 1/3 were obese. In the population of long-term infertile female patients, **71.43%** were classified according to growing body mass index, while **28.57%** were diagnosed as PCOS. Such prevalence is expected in an infertile population. The incidence of obesity is predictably the highest in PCOS patients (**40.4%**). Overweight and obesity prevalence in infertile women is 62.3%, and 71.1% in PCOS patients. BMI range was between 18.6 and 49.6 kg/m^2.Overweight and obesity prevalence is shown in Table 12. (see also Table 18)

Table 18. Prevalence of patients with abnormal BMI

Patients	**All** **n= 182**	**Non PCOS** **n= 130**	**PCOS** **n= 52**
Overweight	56	40	16
Obese	62	41	21
TOTAL	118 (64,83%)	81 (62,31%)	37 (71,15%)

Among infertile patients without PCOS, **62.3%** are overweight and obese, while a higher (abnormal) BMI is found in **71.1%** PCOS patients. Occurrences were compared within BMI groups, as well as non-PCOS and PCOS patients. Non-PCOS patients exhibited impacts only of obesity, while PCOS patients exhibited impacts of both that endocrine disorder and of obesity.A body mass index < 20 kg/m^2 was found in 8 women (4.4%), and morbid obesity (BMI ≥ 40kg/m^2) in 10 patients (5.5%).The highest obesity incidence is in PCOS patients (40.4%).

Demographic characteristics of patients in all three groups of different weight and BMI were the same and are shown in Table 19.

Table 19. Demographic characteristic in patients (n=182) distributed by BMI

	BMI		
Parametar	**I Group normalweight n=64**	**II Group overweight n=56**	**III Group obese n=62**
Age- years mean/ range	32,20 24- 38	31,09 23- 37	32,16 24- 38
Height (cm) mean/SD	165,7 6,77	164,3 7,49	164,7 6,67
Weight (kg) mean/SD	62,6 7,37	72,9 8,07	92,7 14,36
BMI kg/m^2	22,7	26,9	34,1
Previous pregnancy/ birth n/ %	6 (9,4%)	12 (21,4%)	12 (19,3%)
Primary infertility n/ %	52 (81,3%)	36 (64,3%)	38 (61,3%)
Infertility duration(years) mean/ range	4,11 1-11	3,80 2-10	4,65 1-17
Previous gynaecologic surgery n/ %	7 (10,9%)	6 (10,7%)	9 (14,5%)
Male subfertility n/ %	19 (29,7%)	13 (33,9%)	16 (25,8%)
Hypothyroidism n/ %	4 (6,2%)	4 (7,1%)	5 (8,1%)
PCOS n/ %	15 (23,4%)	16 (28,6%)	21 (33,9%)

Average patient age was also approximately the same, as well as average infertility duration (around 4 years). Only 38 patients (20.9%) had previously received intensive infertility treatments, out of whom 8 with in vitro fertilization (IVF). In 25% to 33% of couple's male subfertility was a cofactor of infertility, but this prevalence was disregarded due to the selection according to BMI, while couples with total male infertility were excluded from the study. Primary infertility is the most frequent in the normal weight population (81.3%), which is significantly more frequent than in patients with an abnormal BMI (61.3 - 64.3%). Around 20% of overweight and obese patients had already given birth.

The differences between weight and BMI by patient groups are predictably statistically significant ($p < 0.001$), as that was the principle of sample creation (Fig. 15).

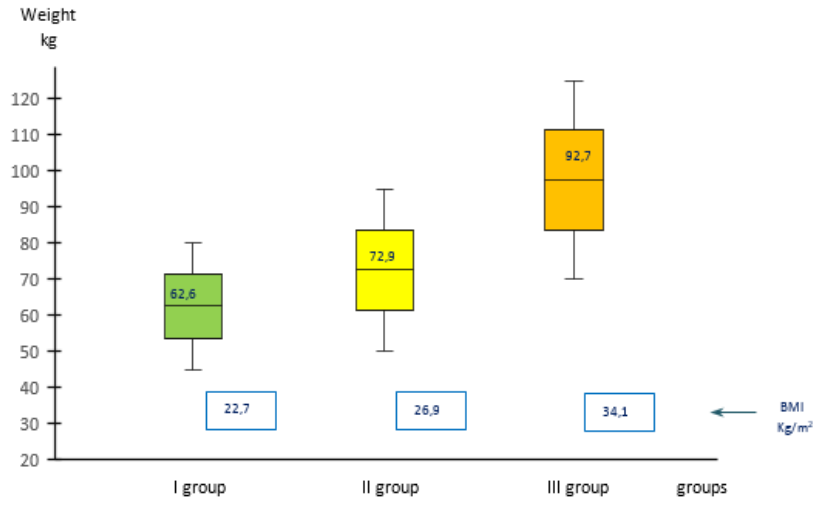


Figure 15. Group differences for weight and BMI (mean)

The incidence of polycystic ovary syndrome in normal weight patients is 23%, compared to 29% and 34% respectively in overweight and obese patients.

Of the 30 patients who had previously given birth and were now examining secondary infertility, 15 had been overweight or obese before pregnancy, and 11 were normal weight, but have later developed post-pregnancy obesity (body weight increase of 10 - 16.5 kg).

NPar Tests

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
Weight kg	<25	64	42,06	2692,00
	25-30	56	81,57	4568,00
	Total	120		

Test Statistics[a]

	Weight kg
Mann-Whitney U	612,000
Wilcoxon W	2692,000
Z	-6,208
Asymp. Sig. (2-tailed)	,000

a. Grouping Variable: Group

Other anthropometric measurements and central obesity

In order to explore the relationship between central (abdominal) obesity and general and hormonal characteristics of women in this study, the measurements for waist circumference (WC), hip circumference (HC), waist to hip ratio (WHR) and waist to stature ratio (WSR) were analyzed. The patients in BMI groups had a similar WC. However, WC significantly increases with increased BMI, which makes the difference between WC in overweight and obese patients and WC in normal weight patients statistically significant ($p < 0.001$).

Table 20. Waist- circumference in participating patients (mean and SD)

	Waist circumference (WC) cm		
Patients	**All**	**Non PCOS** n= 130	**PCOS** n= 52
B I Group n= 64 SD	**71,66** 7,58	**70,57** 7,0	**75,20** 8,56
M II Group n= 56 SD	**78,4** 7,16	**77,62** 7,06	**80,8** 7,23
I III Group n= 62 SD	**95,52** 11,71	**94,24** 11,27	**98,00** 12,43
TOTAL n= 182 SD	**81,87** 13,55	**80,20** 13,14	**86,03** 14,11

• **BMI** body mass index • **PCOS**- polycystic ovary syndrome

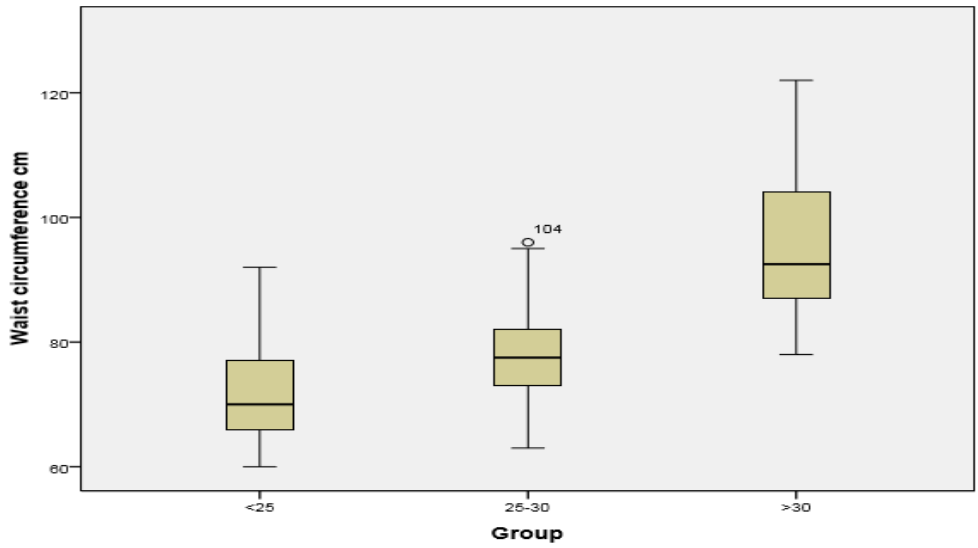


Figure 16. Waist circumference (all patients) in 3 BMI groups

Obese PCOS patients have the highest WC, but without a statistically significant difference compared to non-PCOS women. WC value distribution in study groups is also presented in a histogram.

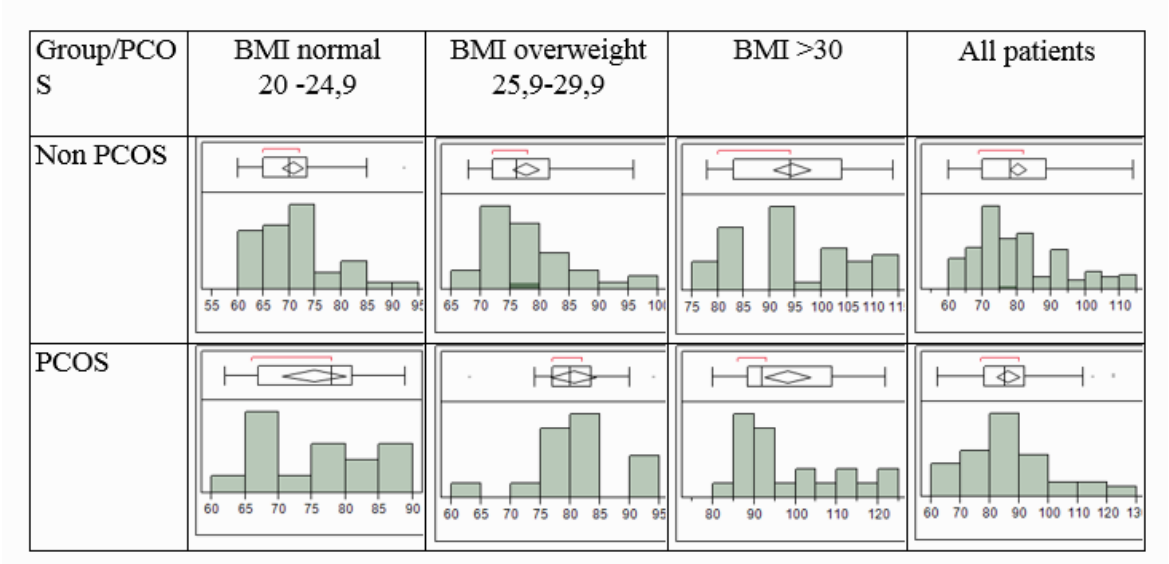


Figure 17. WAIST circumference histogram of distribution

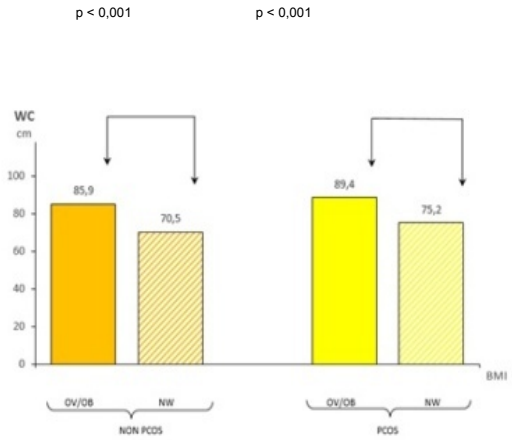
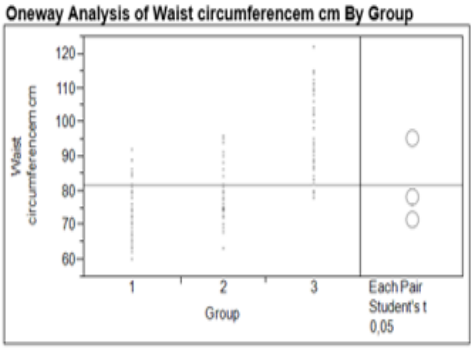
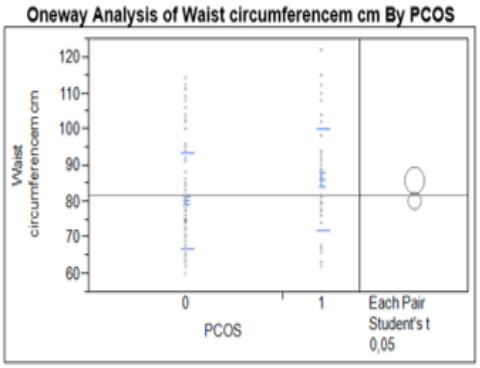


Figure 18. WAIST circumference differences according to BMI

• **NW-** normal weight • **OV-** overweight • **OB-** obese



$H_0: \mu_3 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_3 - \mu_1 \neq 0$		23,85988	<,0001*	
$H_0: \mu_3 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_3 - \mu_2 \neq 0$		17,06970	<,0001*	
$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_2 - \mu_1 \neq 0$		6,79018	<,0001*	



$H_0: \mu_1 - \mu_0 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_1 - \mu_0 \neq 0$				
		5,830769	0,0088*	

Waist circumference greater than 88 cm is a reliable indicator of central obesity in women, which represents an additional metabolic and reproductive risk.

Table 21. Prevalence of central obesity in two populations (according to WC> 88cm)

	Waist circumference > 88 cm		
Patients	**All**	**Non PCOS**	**PCOS**
B I Group	**3 (4,7%)** n= 64	**1 (2,04%)** n= 49	**2 (13,3%)** n= 15
M II Group	**8 (14,3%)** n= 56	**4 (10,1%)** n= 40	**4 (25,0%)** n= 16
I III Group	**42 (67,7%)** n= 62	**26 (63,4%)** n= 41	**16 (76,2%)** n= 21
TOTAL	**53 (29,1%)** n= 182	**31 (23,8%)** n= 130	**22 (42,3%)** n= 52

Central obesity (WC > 88 cm) was recorded in 53 patients (29.1%), of which 23.8% were non-PCOS patients and 42.3% patients with PCOS. More than 60% of obese patients also exhibit abdominal obesity, while as many as 76.2% of obese PCOS patients have WC > 88 cm.

According to that parameter, the prevalence of central obesity is statistically significantly higher in PCOS patients who are overweight or obese.

Inter-BMI-group prevalence of central obesity is similar, without statistically significant differences, while normal-weight patients have a low incidence of visceral obesity (2.04% and 13.3% respectively).

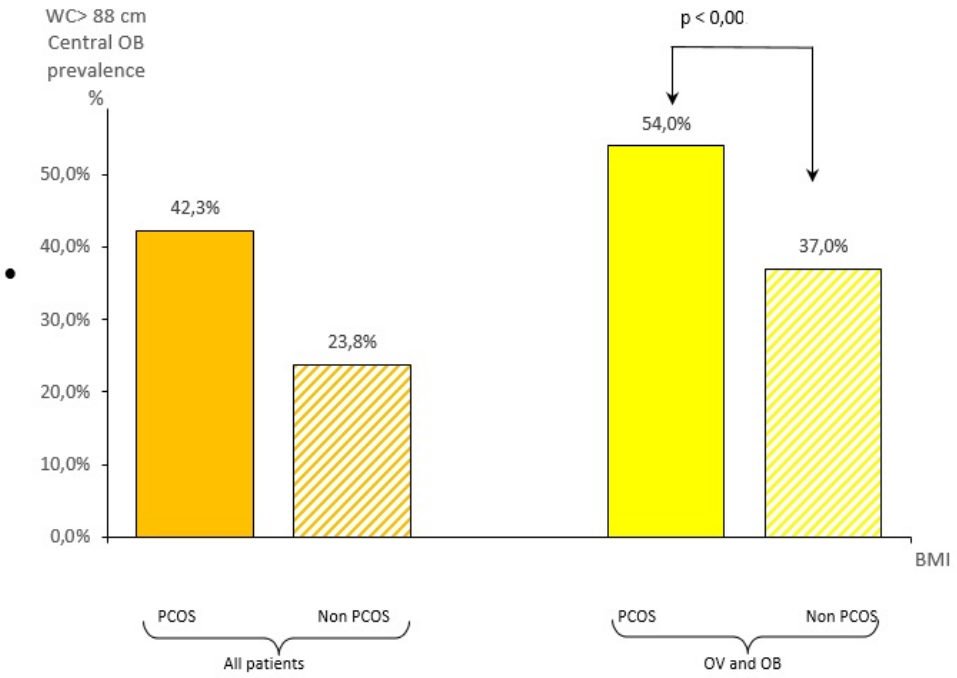


Figure 19. Prevalence of central obesity in PCOS and non PCOS populations

Hip circumference (HC) is similar, without significant differences within BMI groups. Obese women have the highest HC, which is statistically significantly higher than HC in normal weight patients.

Table 22. Hip circumference in all participating patients (mean and SD)

	Hip circumference (HC) cm		
Patients	**All**	**Non PCOS** **n= 130**	**PCOS** **n= 52**
B I Group n=64 SD	**94,6** 6,13	**94,1** 5,79	**95,2** 7,37
M II Group n= 56 SD	**102,2** 6,22	**100,3** 6,50	**108,3** 5,46
I III Group n= 62 SD	**112,5** 8,88	**111,6** 7,24	**113,5** 11,59
TOTAL n= 182 SD	**103,1** 9,55	**102** 7,66	**105,7** 12,10

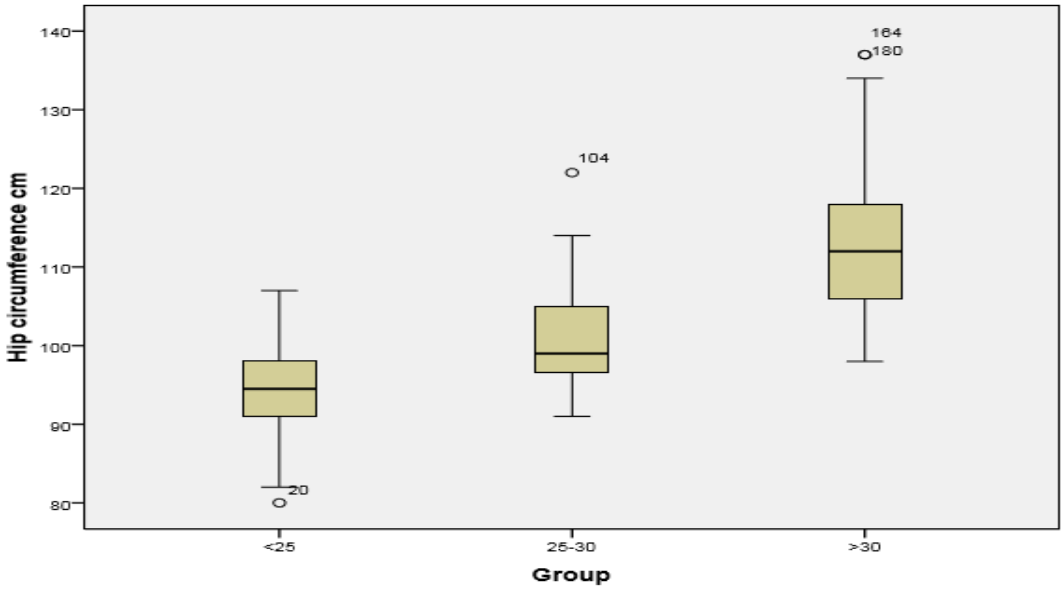
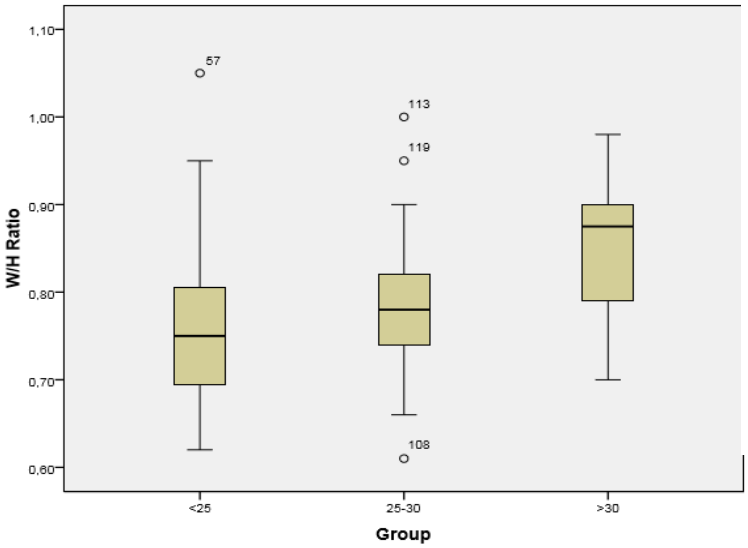


Figure 20. Hip circumference in all patients by BMI groups

In addition to using WC, central obesity is often diagnosed using the waist to hip ratio (WHR). Cut-off values recommended for women is 0.85 or even 0.80. The normal upper limit of 0.85 was used, as most frequently recommended by the WHO. Normal average WHR values were observed in the normal weight and overweight groups, while in obese patients the ratio was pathological. Obese patients (BMI > 30 kg/m2) more often exhibit abdominal obesity, i.e. a higher WHR.

Table 23. Waist to hip ratio in participating populations (mean and SD)

	Waist to hip ratio-WHR		
Patients	**All**	**Non PCOS** n= 130	**PCOS** n= 52
B I Group n= 64 SD	**0,76** 0,08	**0,75** 0,06	**0,79** 0,1
M II Group n= 56 SD	**0,78** 0,06	**0,77** 0,05	**0,79** 0,08
I III Group n= 62 SD	**0,85** 0,07	**0,84** 0,07	**0,88** 0,05
TOTAL n= 182 SD	**0,80**	**0,79**	**0,81**



Ranks

	Group	N	Mean Rank
Waist circumference cm	<25	15	14,03
	25-30	16	20,50
	>30	21	39,98
	Total	52	
Hip circumference cm	<25	15	12,53
	25-30	16	24,59
	>30	21	37,93
	Total	52	
W/H Ratio	<25	15	20,53
	25-30	16	20,25
	>30	21	35,52
	Total	52	

Figure 21. Waist to hip ratio in 3 BMI groups

In the obese group, non-PCOS women have border-line WHR, while patients with PCOS have a pathological WHR (0.88). When these values were compared to the normal-weight group, the difference was statistically significant. Central obesity has a high prevalence in obese women and is almost exclusive to obese women with PCOS (66.7%). Obesity (BMI ≥ 30kg/m^2) in PCOS patients significantly contributes to central obesity. Further, central obesity prevalence is statistically significantly higher in obese PCOS patients.

Table 24. Incidence of central obesity according to WHR higher than 0,85

	WHR > 0,85		
Patients	**All**	**Non PCOS**	**PCOS**
B I Group	**7 (10,9%)** n= 64	**4 (8,2%)** n= 49	**3 (20,0%)** n= 15
M II Group	**7 (12,5%)** n= 56	**4 (10,0%)** n= 40	**3 (18,7%)** n= 16
I III Group	**30 (48,4%)** n= 62	**16 (39,1%)** n= 41	**14 (66,7%)** n= 21
TOTAL	**44 (24,2%)** n= 182	**24 (18,5%)** n= 130	**20 (38,5%)** n= 52

Central obesity incidence is most accurately shown if we include in the calculation all patients with one or both indicators: waist circumference > 88 cm and/or waist to hip ratio > 0.85. Another good indicator is a WSR ratio (waist to stature (height)) higher than 0.50.

Table 25. Prevalence of central obesity according to high WC and WHR

	Central obesity WC > 88 cm and (or) WHR > 0,85		
Patients	**All**	**Non PCOS**	**PCOS**
Overweight	**11 (19,6%)** n= 56	**6 (15%)** n= 40	**5 (31,2%)** n= 16
Obese	**47 (75,8%)** n= 62	**28 (68,3%)** n= 41	**19 (90,4%)** n= 21
TOTAL	**58 (49,1%)** n= 118	**34 (41,9%)** n= 81	**24 (64,9%)** n= 37

WC- waist circumference; **WHR**- waist to hip ratio

An analysis of these two parameters shows that 90.4% of obese PCOS patients also exhibit central obesity, which is statistically more significant than in obese non-PCOS patients. There is also a statistically significantly higher incidence of abdominal obesity in obese than in normal weight participants. According to these criteria almost all PCOS patients (90.4%) exhibit central obesity.

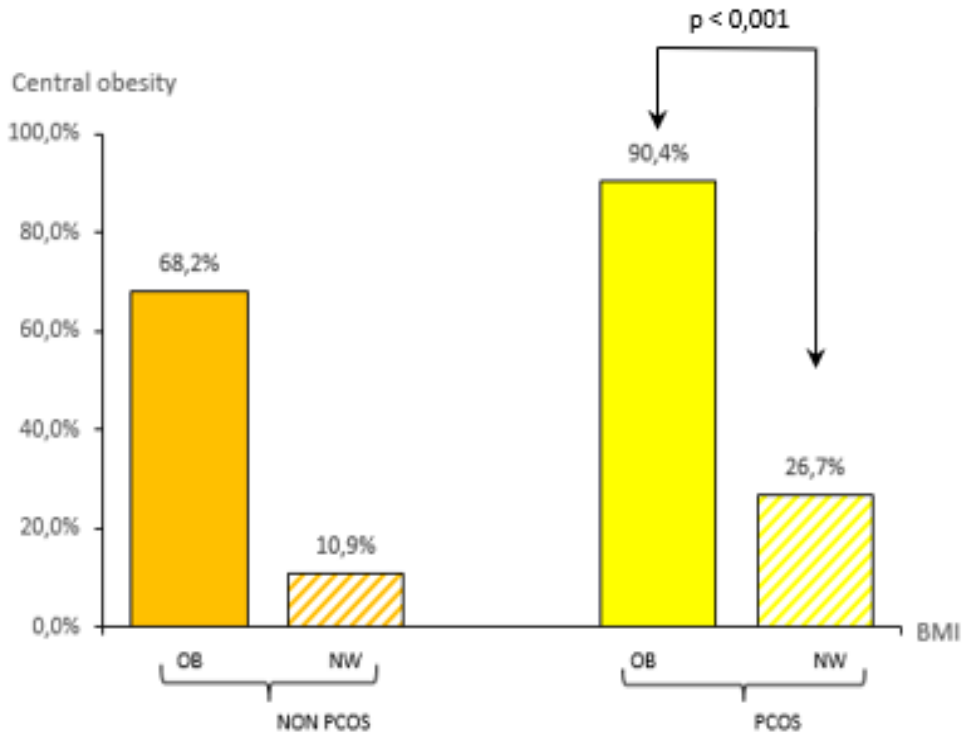


Figure 22. Prevalence of central obesity according to WC/ WHR

Normal weight PCOS patients exhibit 2.5 more central obesity than women with the same BMI, but without PCOS.Waist to stature ratio (WSR) is the ratio of waist circumference to height. When this ratio exceeds 0.50, it is an indicator of central obesity.

Table 26. Waist to stature ratio in participating patients (mean)

	Waist to stature ratio, WSR*		
Patients	**All**	**Non PCOS**	**PCOS**
B I Group n= 64	**0,43**	**0,43**	**0,45**
M II Group n= 56	**0,47**	**0,46**	**0,48**
I III Group n= 62	**0,57**	**0,56**	**0,58**

- **Value > 0,50 sign of central obesity**

WSR shows a similar incidence of central obesity. Both populations of obese women (PCOS and non-PCOS) have a high prevalence of WSR > 0.50, which is an important indicator of abdominal obesity. Obese PCOS patients have a high WSR in 95.2% of the cases, which is statistically significantly higher than among overweight and normal weight participants.

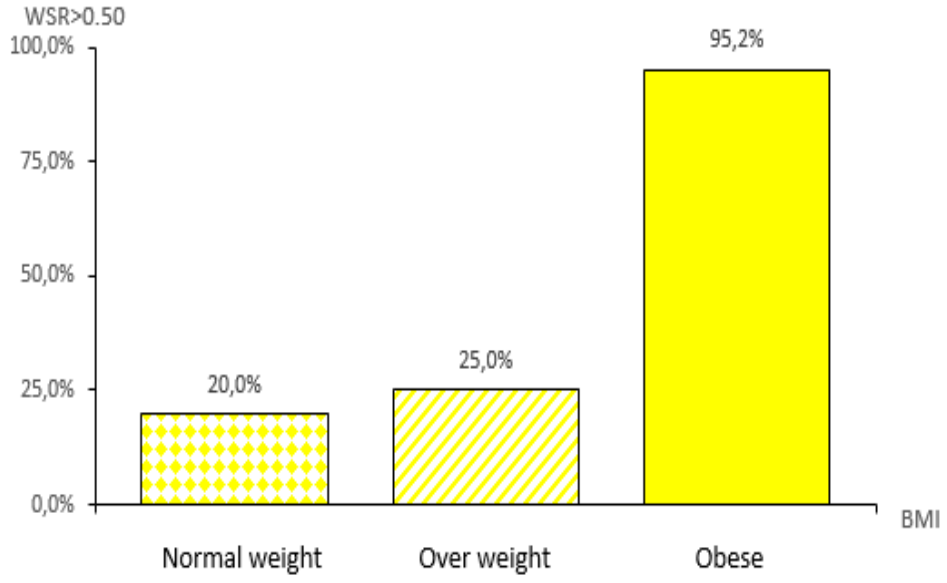


Figure 23. Waist to stature ratio > 0,50 in PCOS patients

5.2 Menstrual cycle characteristics

We explored a possible effect of obesity and PCOS on some characteristics of the menstrual cycle and menstrual bleeding in the participating patients. The onset of the first menstrual cycle (menarche) is shown in Table 27.

Table 27. Age of menarche in participating patients (mean and range)

	Menarche (year) age		
Patients	**All** n= 182	**Non PCOS** n= 130	**PCOS** n= 52
B I Group range	**12,40** 10,5-14,5	**12,20** 10,5-13,5	**13,06** 11-14,5
M II Group range	**12,80** 10-15	**12,07** 10-13,5	**13,50** 12-15
I III Group range	**12,90** 10,2-15,5	**11,60** 10,2-13,0	**13,94** 12-15,5

In the studied population, menarche occurred between the ages of 10 and 15.5 years. No primary amenorrhea was recorded in the participating patients. A trend of early menarche (at the age of 11.6) was observed with obesity, while it is a known fact that PCOS postpones menarche. In the PCOS population, menarche occurred at an average age of 13.5 years in overweight women, and at the age of 13.9 in obese women. These differences exhibited no statistical significance.

The difference (in terms of menarche) between obese (BMI>30 kg/m2) non-PCOS and PCOS patients is statistically significant.

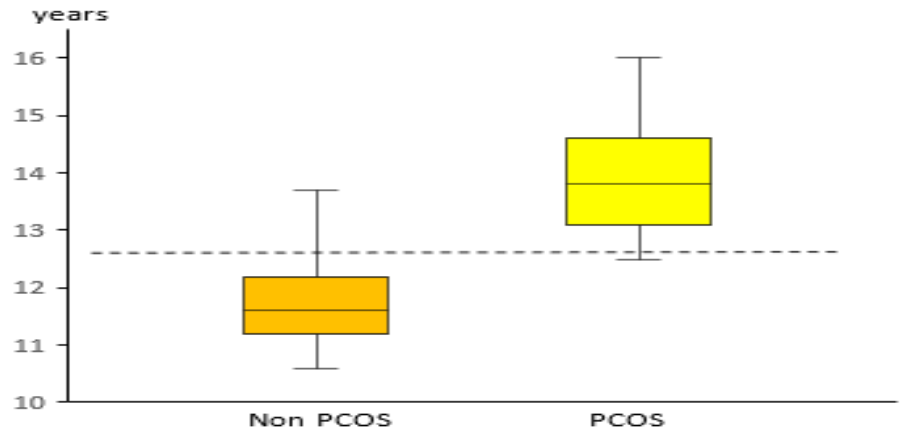


Figure 24. Difference in menarche age in obese patients

The duration of menstrual bleeding (MB) widely varied, from 2 to 8 days. The longest menstrual bleeding occurs in PCOS patients (BMI>25) withan average of almost 6 days (5.8 days). In general, the duration of MB is longer in obese than in normal weight women.Obese and overweight PCOS patients have statistically significantly longer MB than normal weight women.

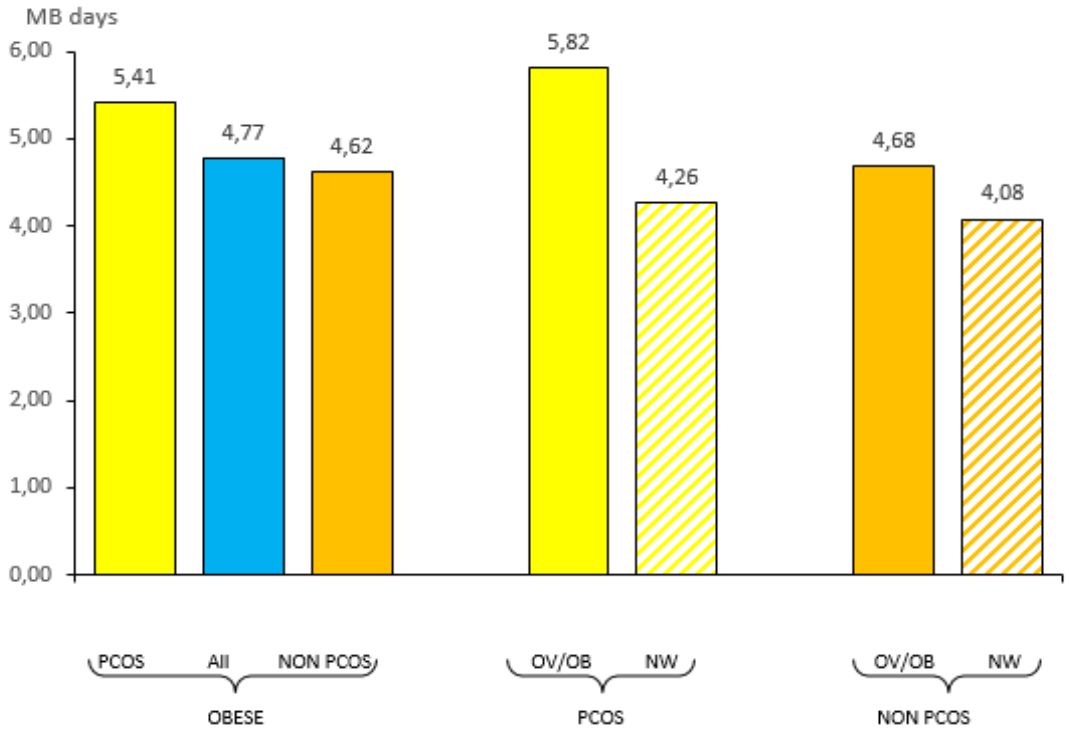


Figure 25.Menstrual bleeding duration (days) in participating women, NW- normal weight; OV- overweight; OB- obese

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
bleeding days	<25	64	53,75	3440,00
	>30	62	73,56	4561,00
	Total	126		

Test Statistics[a]

	bleeding days
Mann-Whitney U	1360,000
Wilcoxon W	3440,000
Z	-3,254
Asymp. Sig. (2-tailed)	,001

a. Grouping Variable: Group

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
bleeding days	<25	15	11,70	175,50
	25-30	16	20,03	320,50
	Total	31		

Test Statistics[b]

	bleeding days
Mann-Whitney U	55,500
Wilcoxon W	175,500
Z	-2,730
Asymp. Sig. (2-tailed)	,006
Exact Sig. [2*(1-tailed Sig.)]	,009[a]

a. Not corrected for ties.

b. Grouping Variable: Group

Irregular cycles are defined as prolonged cycles, oligomenorrhea lasting from 35 to 199 days, uneven and unstable cycles, anovulation. Amenorrhea is defined as absence of menstruation longer than 199 days (≈6 months). More severe cycle disorders are associated with more serious hormonal disorders and insulin resistance.

Obesity and PCOS are characterized by irregular cycles, with 62 occurrences (34.1%) in all patients, and significantly more in PCOS patients than in the population of only obese women (69.2% vs. 20.8%).

Table 28. Irregular menstrual cycles in non PCOS and PCOS populations (n and %)

	Irregular cycles n (%)		
Patients	**All** n= 182	**Non PCOS** n= 130	**PCOS** n= 52
B I Group n= 64	**18 (28,1%)**	**9 (18,4%)** n=49	**9 (60%)** n=15
M II Group n= 56	**17 (30,3%)**	**8 (20%)** n=40	**9 (56,2%)** n=16
I III Group n= 62	**27 (43,5%)**	**10 (24,4%)** n=41	**17 (80,9%)** n=21
TOTAL	**62 (34,1%)**	**27 (20,8)**	**36 (69,2%)**

In obese PCOS patients, 80.9% had irregular cycles and 85% had anovulatory cycles. This is statistically significantly more frequent than in the normal weight population (60% compared to 46.7%).

On the other hand, anovulation was identified in 39% of patients who were only obese (BMI > 30 kg/m^2). As many as 35 (67.3%) PCOS patients had anovulatory cycles.

Further, 5 non-PCOS patients reported amenorrhea (3.8%), while the incidence of amenorrhea accompanying obesity was 3 (7.3%). In the PCOS population, 9 patients reported amenorrhea (17.3%), mostly among obese PCOS participants 5 (23.8%). Anovulation was also identified in these patients.

Table 29. Anovulatory cycles in participating patients (n and %)

	Anovulations (%)		
Patients	**All** n= 182	**Non PCOS** n= 130	**PCOS** n= 52
B I Group n= 64	**17 (25,6%)**	**10 (20,4%)** n=49	**7 (46,7%)** n=15
M II Group n= 56	**22 (39,3%)**	**12 (30%)** n=40	**10 (62,5%)** n=16
I III Group n= 62	**34 (54,8%)**	**16 (39,0%)** n=41	**18 (85,7%)** n=21
TOTAL	**73 (40,1%)**	**38 (29,2%)**	**35 (67,3%)**

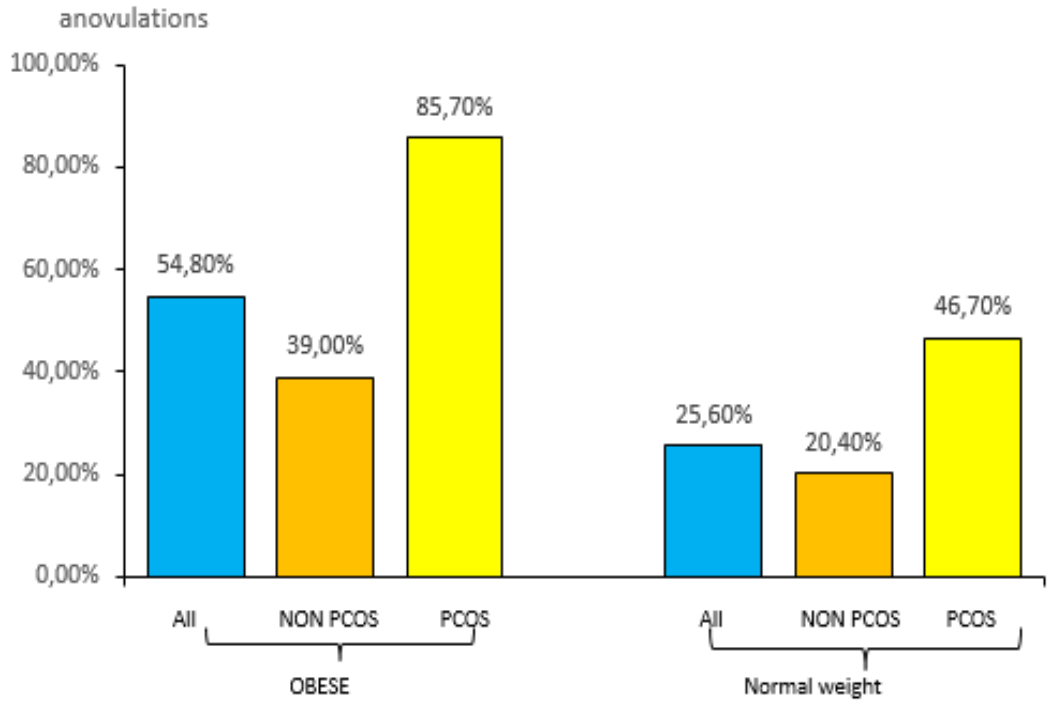


Figure 26.Incidence of anovulatory cycles in obese and normal weight populations

Anovulatory cycles (including amenorrhea) were recorded in 85.7% of obese PCOS patients and in 46.7% of normal weight PCOS women. This prevalence is statistically significantly higher than the prevalence in non-PCOS patients. Anovulation coincided with obesity in 39% of women.

In all BMI groups incidence of anovulatory cycles is statistically significant higher in PCOS patients: (Two sample t-test and CI)

I group	T-value 7,83	p < 0,001
II group	T- value 3,91	p < 0,001
III group	T – value -3,55	p < 0,001
Obese non PCOS patients have significantly higher rates of anovulations than normalweight women- t- value- 28,29 p < 0,001		

Heavy menstrual bleeding (HMB) is predictably frequently associated with obesity and PCOS cycles. The data collected in this study is not based on objective menstrual blood loss measurement, but on subjective impressions and anamnestic data. HMB was reported by 42 patients (23.3%), with the highest incidence, of 9 patients (24.3%), in the overweight and obese PCOS population. This is statistically significantly higher than in the patients with normal body weight.

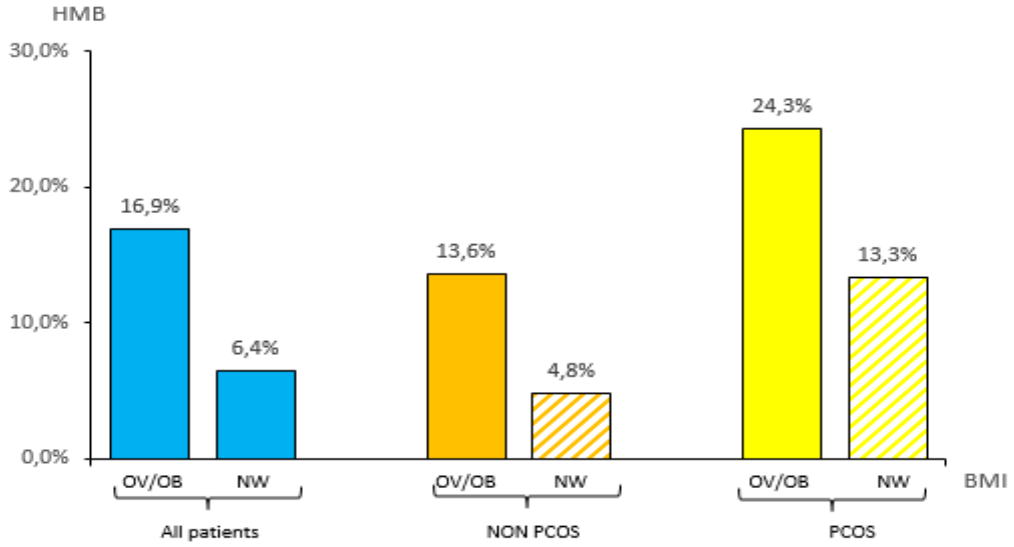


Figure 27. Heavy menstrual bleeding in 182 participants

Menstrual cycle duration changes with obesity, anovulation, hyperandrogenemia and PCOS, and most frequently with anovulatory PCOS.

Table 30. Menstrual cycle length in participating women (mean and SD)

	Menstrual cycle length (days)		
Patients	**All**	**Non PCOS**	**PCOS**
B I Group n=64 SD	**29,13** 4,16	**27,53** 3,51	**33,66** 5,66
M II Group n= 56 SD	**29,75** 4,60	**28,5** 3,17	**34,6** 5,64
I III Group n= 62 SD	**32,35** 4,25	**30,12** 4,30	**39,54** 4,31
TOTAL n= 182 SD	**30,41** 4,11	**28,63** 3,8	**36,42** 5,7

Menstrual cycle was prolonged in all BMI groups (I, II, III) in the study, less prominently in case of obesity only and more prominently with higher BMI and PCOS. Patients with polycystic ovaries have statistically significantly longer cycles than non-PCOS participants (36.4 days vs. 28.6 days).

Obese PCOS women have the longest menstrual cycles (39.54 days), which is statistically significantly longer than the cycles of obese women and normal weight patients ($p < 0,001$).

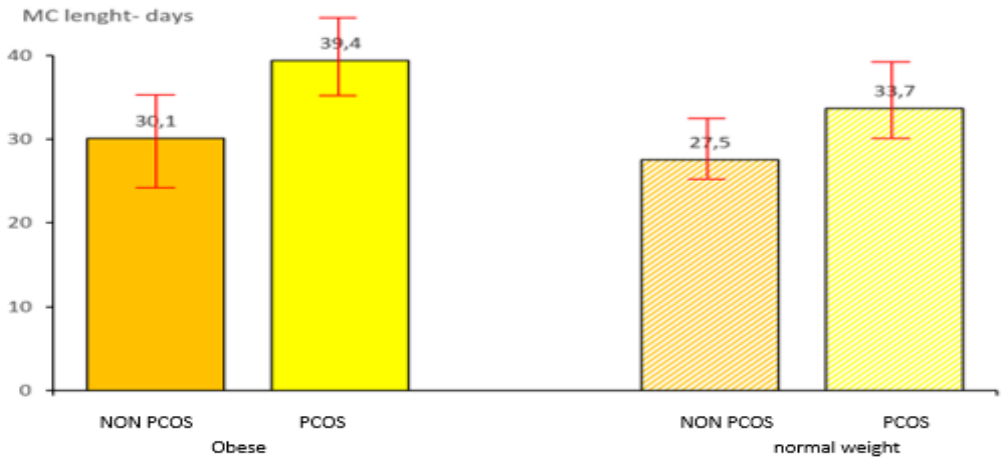


Figure 28. Menstrual cycle length inobese and normal weight patients

5.3 Previous pregnancies

Among 56 secondarily infertile patients (30.76%), there were 91 pregnancies, 41 births (45.05%) and 50 early spontaneous abortions (SAB) (54.94%). Of 50 SABs, 44 (88%) occurred in the overweight and obese population, while 12% occurred in normal weight patients.This study found that the population with long-term infertility has a high prevalence of early spontaneous abortions. Over one half of all early-stage pregnancies were terminated by miscarriage, especially in obese patients.

These 56 subjects provided data in the questionnaire regarding time to pregnancy. In the normal, healthy population time to pregnancy (TTP) is 4.8 months (48,50).

Table 31. Time to pregnancy in two different populations(months)

BMI	Non PCOS **n= 38**	PCOS **n= 18**
18,5- 24,9 SD	**9,5** 3,5	**15,6** 4,2
25- 29,9 SD	**16,8** 4,1	**18,7** 5,2
30- 34,9 SD	**19,9** 5,8	**25,6** 6,1
≥ 40 SD	**27,2** 7,1	**33,4** 7,3

This small sample shows subfecundity and a prolonged TTP with increased BMI up to morbid obesity. This data should be taken with caution due to confounding with other causes of infertility that are independent of obesity and PCOS in our sample as well.

Time to pregnancy is also prolonged in cases of extremely low and high BMI. With an increase in BMI, fecundity decreases in PCOS and non-PCOS populations. Obese women were also subfertile or infertile prior to this study.

Time-to-pregnancy is significantly longer only in normalweight PCOS patients comparing to non PCPS women (Two sample t-test and CI)

I group	T-value - 2,71	p < 0,030
II group	T- value - 0,83	p < 0,427
III group	T – value - 2,01	p < 0,080
Obese non PCOS patients and PCOS patients have significantly longer TTP in comparison to normalweight patients. TTP is significantly the longest in morbid obesity.		
T-value – 5,75	p < 0,001	
T- value – 3,24	p < 0,012	
T – value – 4,75	p < 0,003	

5.4 Hormone analysis

Serum concentrations of folliculostimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were determined and analyzed. In addition to these pituitary hormones, estradiol (E_2), testosterone (T) and sex hormone binding globulin (SHBG) were also analyzed. The aim was to determine how the levels of these hormones were associated with increased body mass index and obesity, and to examine the correlation of hyperandrogenemia (HA) with obesity and polycystic ovary syndrome. In addition to total T, the calculation of free androgen index (FAI) was used. The influence of obesity and PCOS on **gonadotropic hormones (FSH, LH)** is shown in Table 32.

Table 32. Body mass index and PCOS effects on FSH and LH

	FSH IU/L		LH/ IU/L	
Patients	**Non PCOS** n= 130	**PCOS** n= 52	**Non PCOS** n= 130	**PCOS** n= 52
B I Group	**7,53**	**4,97**	**6,77**	**9,98**
SD	2,81	1,16	3,01	5,05
M II Group	**6,87**	**5,41**	**6,82**	**7,53**
SD	2,72	1,26	3,95	3,17
I III Group	**6,14**	**4,67**	**5,72**	**7,31**
SD	1,80	1,47	2,23	3,03
TOTAL	**6,85**	**5,02**	**6,44**	**8,27**
SD	2,30	1,32	3,15	3,86

FSH and LH distribution is shown in histograms, i.e. Figures 30 and 31.High LH is expected in normal weight PCOS patients, while excessive adipose tissue slightly decreases LH levels and activity in groups with a higher BMI.

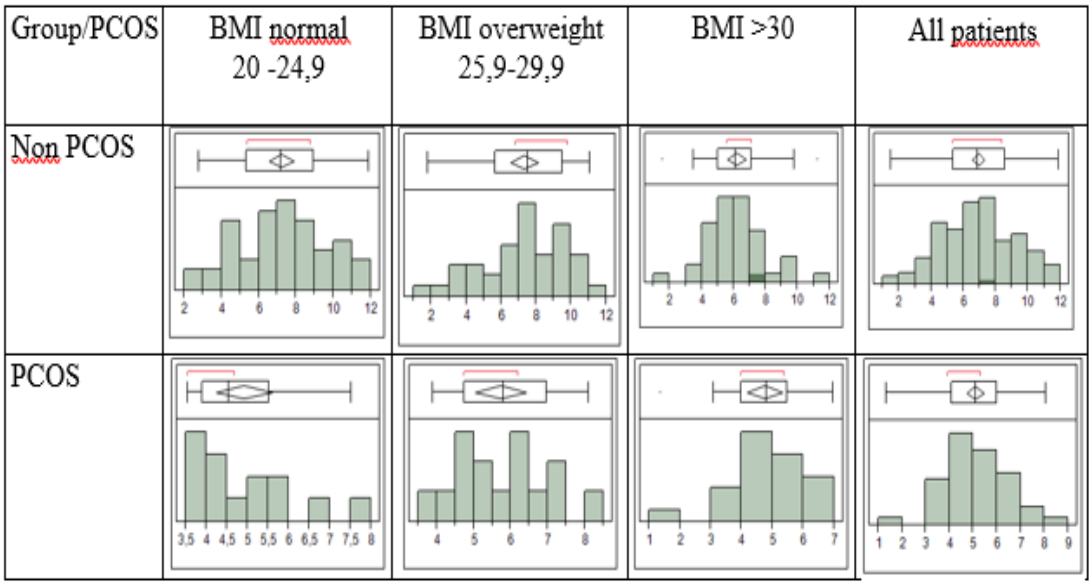


Figure 29. Distribution of FSH in PCOS and non PCOS patients

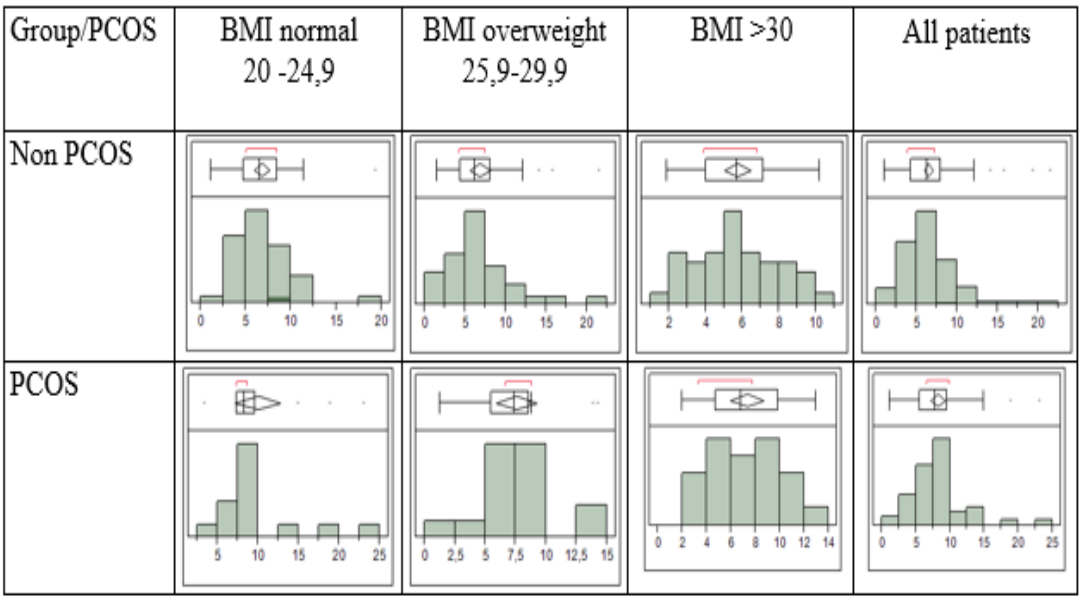


Figure 30. Histogram of LH distribution in two populations

In the group of normal weight patients with PCOS, the LH/FSH ratio is higher than 2.0 due to an elevated LH(LH 9.98/FSH 4.97). Obesity minimally decreases gonadotropin levels, which are the highest with a normal BMI. FSH is lower and LH is higher in PCOS patients compared to non-PCOS patients.

The differences in FSH and LH in obese patients are shown in Figure 32.

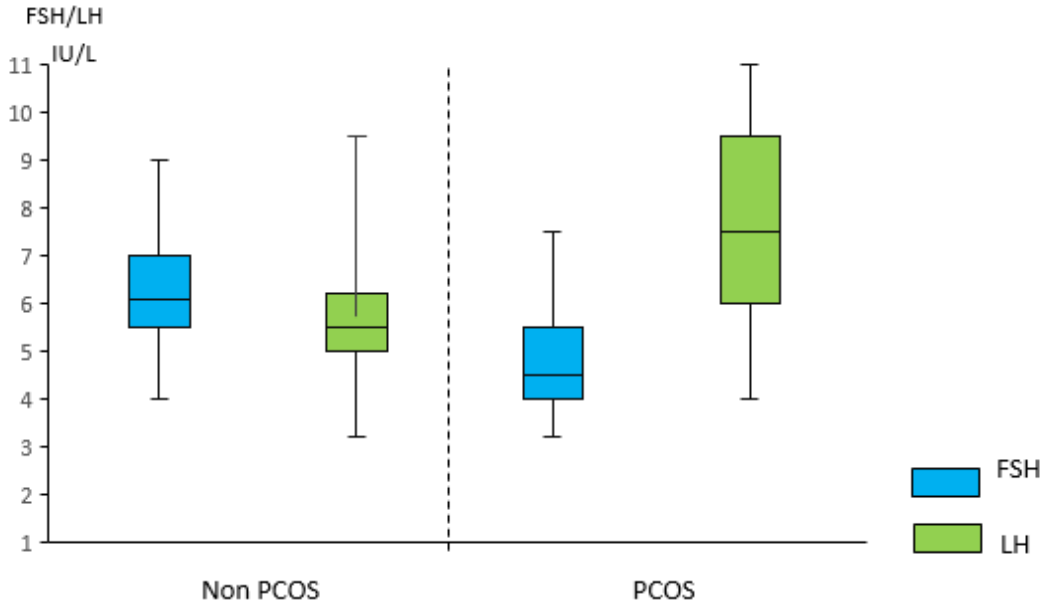


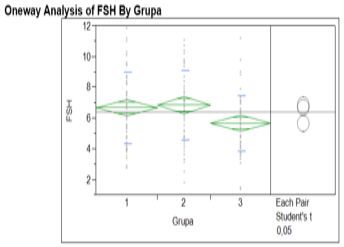
Figure 31. FSH and LH values in obese non PCOS and PCOS patients

In obese patients the difference in serum FSH was statistically significantly higher in non-PCOS patients than in PCOS patients.

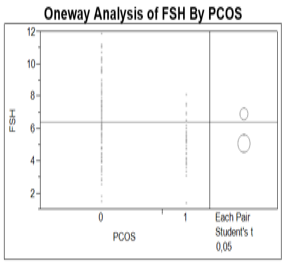
According to Kruskal-Wallis test the LH levels are statistically significantly different in PCOS and non-PCOS patients (P 0.001 - ChiSq).

The expected LH level was statistically significantly different by BMI groups.

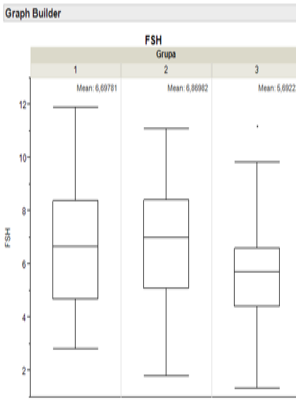
FSH



$H_0: \mu_2 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Valu
$H_1: \mu_2 - \mu_3 \neq 0$		1,177596	0,0033*	
$H_0: \mu_1 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Valu
$H_1: \mu_1 - \mu_3 \neq 0$		1,005590	0,0089*	
$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Valu
$H_1: \mu_2 - \mu_1 \neq 0$		0,172006	0,6626	

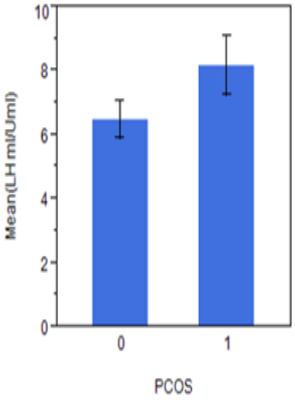


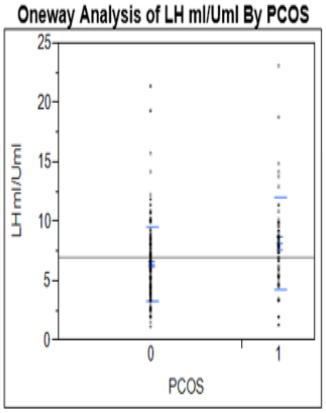
$H_0: \mu_0 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_0 - \mu_1 \neq 0$				
		1,837622	<,0001*	



LH

$H_0: \mu_1 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_1 - \mu_3 \neq 0$		1,260675	0,0403*	
$H_0: \mu_2 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value>α→H0
$H_1: \mu_2 - \mu_3 \neq 0$		0,760541	0,2301	
$H_0: \mu_1 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value<α→H0
$H_1: \mu_1 - \mu_2 \neq 0$		0,500134	0,4260	





$H_0: \mu_1 - \mu_0 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_1 - \mu_0 \neq 0$				
		1,692192	0,0026*	

Prolactin is a stress hormone secreted by the pituitary gland. Apart from its role in lactation, it also affects the endometrium and ovaries. There is no correlation between prolactin and obesity, but prolactin is slightly elevated in 20 to 30% of women with PCOS.

The distribution of prolactin findings is illustrated in the histogram below, clearly showing a wide range of established values:

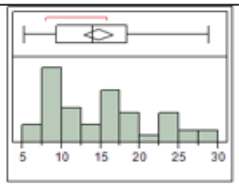
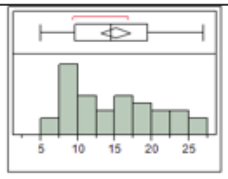
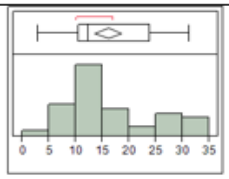
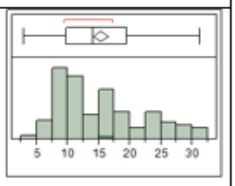
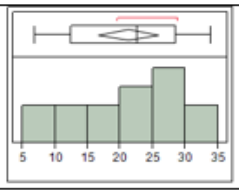
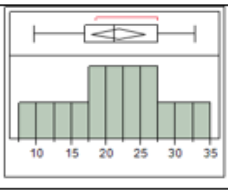
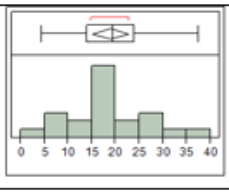
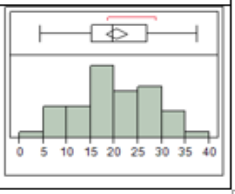
Group/PCOS	BMI normal 20 -24,9	BMI overweight 25,9-29,9	BMI >30	All patients
Non PCOS				
PCOS				

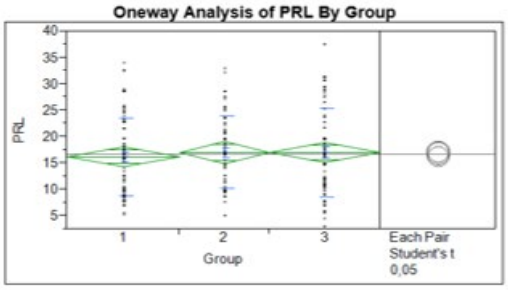
Figure 32. Distribution of serum prolactin in participating patients

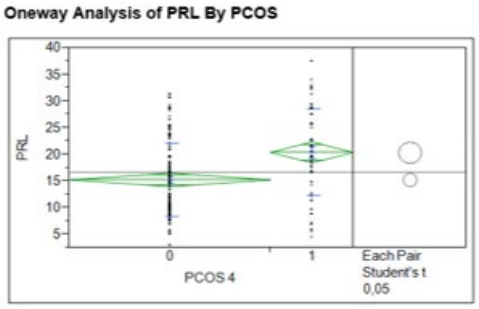
Table 33. Serum prolactin values (mean and SD)

	Prolactin ng/ml	
Patients	**Non PCOS**	**PCOS**
B I Group	**14,67**	**21,22**
SD	6,40	8,42
M II Group	**15,04**	**22,53**
SD	5,89	10,41
I III Group	**16,02**	**18,89**
SD	8,15	8,70
TOTAL	**15,22**	**20,37**
SD	6,86	8,12

There was no difference in PRL concentrationsamong different BMI groups, which means that obesity is not associated with higher PRL production. However, there is a statistically significant difference in mean PRL values between PCOS and non-PCOS patients.

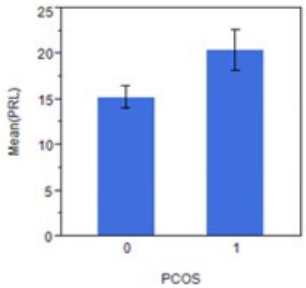
In the participating patients PRL is significantlyhigher (p < 0,001) with PCOS compared to the patients without that syndrome. All mean values are in the normal range.





$H_0: \mu_3 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_3 - \mu_1 \neq 0$		0,8153720	0,5482	
$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_2 - \mu_1 \neq 0$		0,7954841	0,5796	
$H_0: \mu_3 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_3 - \mu_2 \neq 0$		0,0198880	0,9890	

$H_0: \mu_1 - \mu_0 = 0$	α=0,05	Difference	**p-Value**	**p-Value< α→H₁**
$H_1: \mu_1 - \mu_0 \neq 0$		5,159145	<,0001*	



Normal estradiol and TSH levels were also determined, and these were not affected by obesity and PCOS.

Table 34. Serum hormonal concentrations in 3 groups of patients

	BMI		
Parametar	**I Group**	**II Group**	**III Group**
Estradiol (E_2) pg/ml	54,1	56,7	56,2
Prolactin (P_4) µg/L	17,7	19,8	19,2
Thyroid stimulating hormone (TSH) mU/L	2,17	2,76	2,35

Hyperandrogenemia

An increased activity of androgens is characteristic of excessive adipose tissue and PCOS. HA parameters in the study subjects were investigated by comparing the levels of total testosterone (T), sex hormone binding globulin (SHBG) and FAI (normally 1-4 points). It is known that hyperandrogenism depends on the level of androgen production and on the level of androgen transporters in the circulation. Obesity and PCOS play a role in these mechanisms.

Table 35. Total serum testosterone in two populations (mean and SD)

	Testosteron nmol/L	
Patients	**Non PCOS**	**PCOS**
B I Group	**1,38**	**2,53**
SD	0,78	0,76
M II Group	**1,79**	**2,35**
SD	0,85	0,96
I III Group	**1,82**	**2,43**
SD	0,97	0,88
TOTAL	**1,66**	**2,44**
SD	0,79	0,91

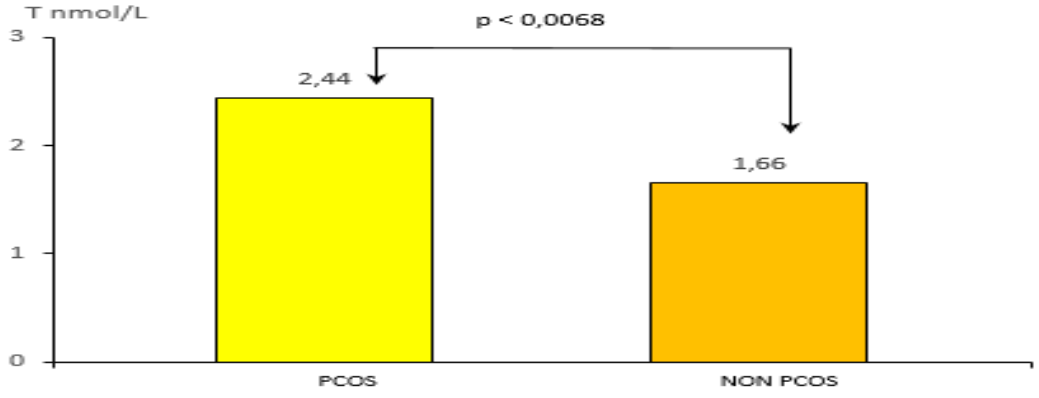
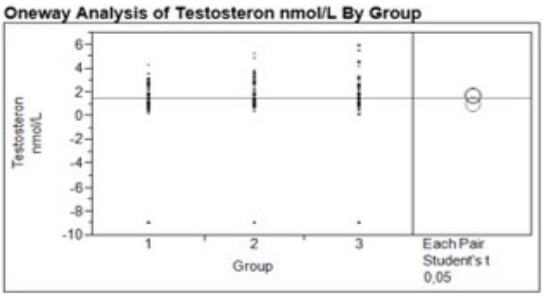
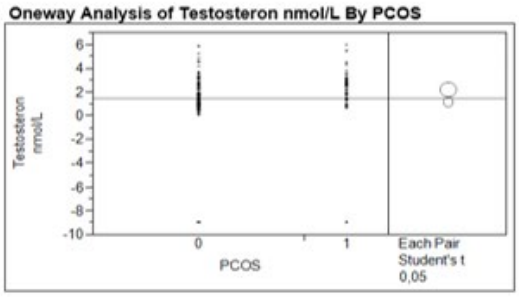


Figure 33. testosterone concentration in all PCOS and non PCOS patients

Testosterone levels do not change with weight gain and increased BMI in PCOS subject group. In this study a statistically significantly higher T was determined in all BMI groups of PCOS patients compared to non-PCOS women. Positive correlation was found between increased adipose tissue and testosterone production in non PCOS participants.





$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_2 - \mu_1 \neq 0$		0,7582599	0,0825	
$H_0: \mu_3 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_3 - \mu_1 \neq 0$		0,6687813	0,1156	
$H_0: \mu_2 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_2 - \mu_3 \neq 0$		0,0894786	0,8382	

$H_0: \mu_1 - \mu_0 = 0$	α=0,05	Difference	p-Value	p-Value<α→H₁
$H_1: \mu_1 - \mu_0 \neq 0$				
		1,053197	0,0068*	

$H_0: \mu_1 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value<α→H₁
$H_1: \mu_1 - \mu_3 \neq 0$		-0,440	0,022	

Testosterone levels are statistically significantly higher in PCOS patients, but there are no statistically significant differences in T levels by BMI in these women. Obese non PCOS women have statistically significant higher T comparing to normal weight participants (T 1,82 vs. 1,38; $p < 0,05$).Obesity does affect serum testosterone levels in patients with and without PCOS.

Sex hormone binding globulin is the main transporter of T and E_2 in the circulation. With a reduced production of SHBG in the liver, the share of these free, unbound hormones increases, and so does their activity. SHBG is a highly sensitive biomarker of HA and PCOS.

Table 36.SHBG values in two groups ofpatients

	SHBG nmol/L	
Patients	**Non PCOS**	**PCOS**
B I Group	**61,10**	**35,39**
SD	30,42	15,3
M II Group	**50,63**	**40,89**
SD	25,8	14,5
I III Group	**55,0**	**32,60**
SD	37,5	19,6
TOTAL	**55,96**	**35,96**
SD	31,6	17,0

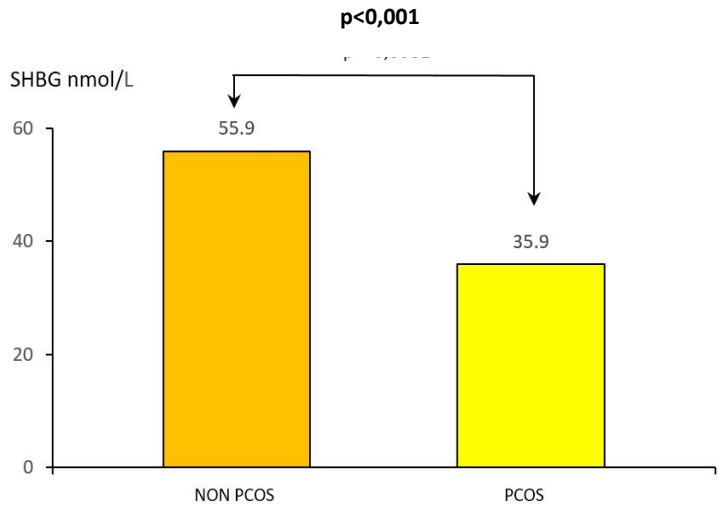


Figure 34. Difference in SHBG values in PCOS/non PCOS patients

The difference in serum SHBG levels between PCOS and non-PCOS patients (in each BMI group and total) is statistically significant. However, there is no significant difference in the expected levels of SHBG in different BMI groups. Obesity decreases the production and levels of SHBG in both patient groups, but the decrease is not statistically significant.

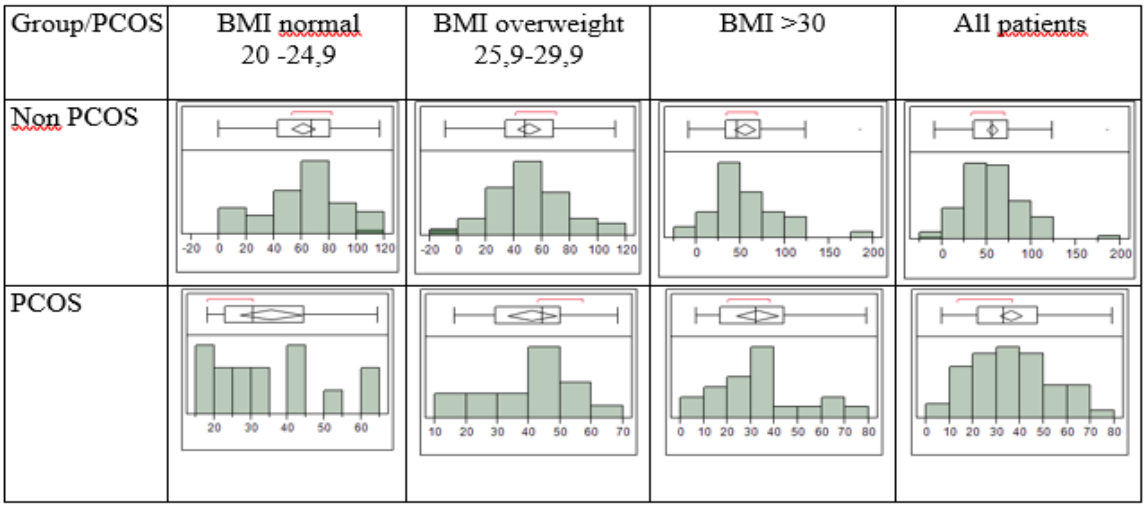
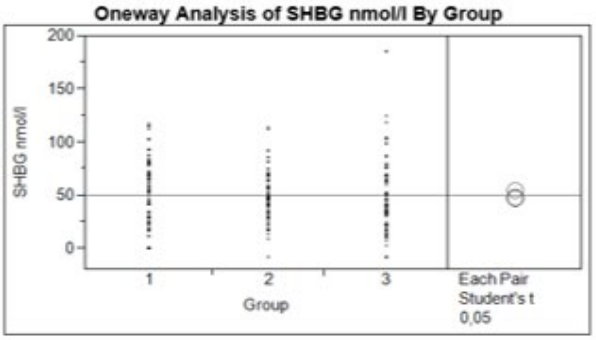
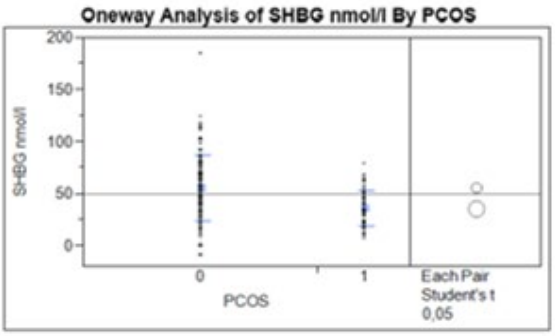


Figure 35. Histogram of SHBG distribution in our patients

Increased androgen activity is best shown by calculating the free androgen index (FAI). Based on T and SHBG results, it was determined that hyperandrogenism is related to PCOS, rather than obesity. In non-PCOS patients FAI was statistically significantly lower in all BMI patient groups. In non-PCOS patients FAI minimally increases with obesity, while FAI is significantly the highest in normal weight PCOS women.





$H_0: \mu_1 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_1 - \mu_3 \neq 0$		7,666905	0,1476	
$H_0: \mu_1 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_1 - \mu_2 \neq 0$		7,232009	0,1833	
$H_0: \mu_2 - \mu_3 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_2 - \mu_3 \neq 0$		0,434896	0,9365	

$H_0: \mu_0 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value<α→H₁
$H_1: \mu_0 - \mu_1 \neq 0$				
		19,99746	<,0001*	

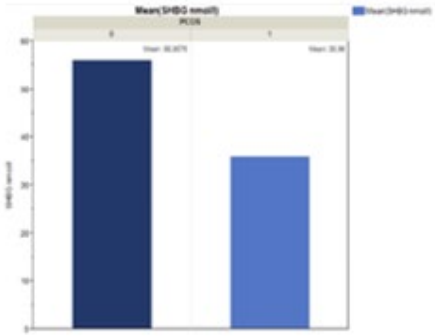


Table 37. Free androgen index in our groups of patients (mean and SD)

	FAI	
Patients	**Non PCOS**	**PCOS**
B I Group	**2,07**	**7,28**
SD	0,9	2,2
M II Group	**3,43**	**5,75**
SD	1,0	1,9
I III Group	**3,12**	**7,45**
SD	1,2	2,1
TOTAL	**2,87**	**6,83**
SD	1,25	2,12

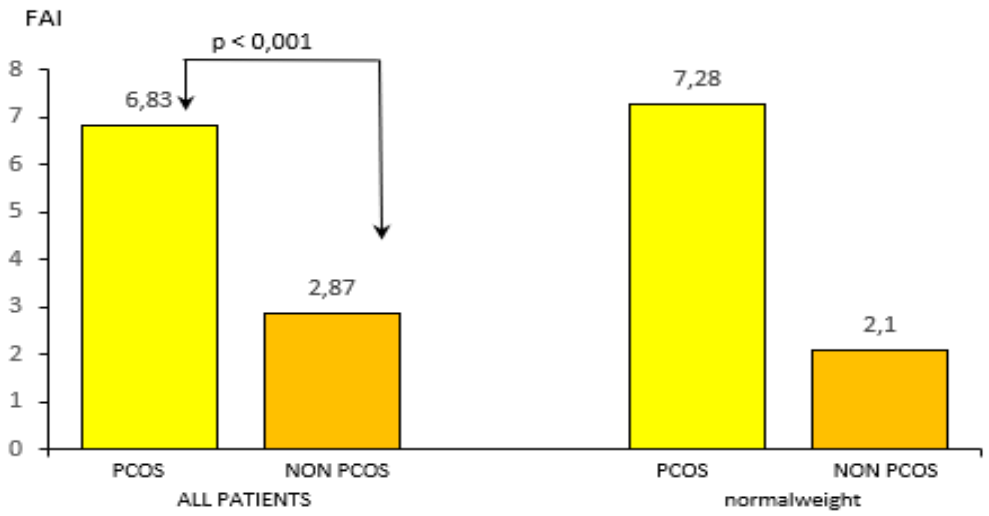


Figure 36.Differences in FAI in all patients and normal weight patients

Statistically significant differences in FAI between PCOS and non PCOS patients and obese PCOS women and control.

BMI increase is associated with unchanged FAI in PCOS subjects, while a minimal FAI increase was found in non-PCOS women.

Table 38 shows all HA markers that were determined in the two patient groups. Overweight and obese patients have higher androgen activity if they are in the non-PCOS group, while in PCOS patients hyperandrogenism is significantly more pronounced in normal weight patients.

Table 38. Markers of hyperandrogenism in PCOS and non PCOS patients according to BMI

Patients	Markers of hyperandrogenism			
	NON PCOS		PCOS	
	OV+OB	Normal W	OV+OB	Normal W
Testosteron nmol/L	1,55	1,27	2,12	2,53
SHBG nmol/L	52,8	61,1	36,7	35,4
FAI	2,93	2,07	5,78	7,15

OV- overweight; **OB-** obese; **NW-** normal weight

In analyzed populations the signs of hyperandrogenism were found mostly in PCOS patients:

- acne 11 patients (21,1%)
- hirsutism 32 patients (61,5%)

According to modified Ferriman- Gallwey score ≥ 7 hirsutism in PCOS women was expressed in 27 patients (51,9%). Hyperandrogenism in non PCOS women has less prevalence- 18 (13,8%), mostly in obese participants.

5.5 Biomarkers of ovarian reserve

The biomarkers of ovarian reserve identified in the patient population are the anti-Müllerian hormone (AMH), antral follicular count (AFC), and ovarian volume (OV).

Table 39. Serum Antimüllerian hormone values in non PCOS and PCOS patients (mean and SD)

Patients	AMH pmol/L		
	All n= 182	Non PCOS n= 130	PCOS n= 52
B I Group	**24,60**	**13,43**	**59,68**
SD	12,8	8,02	23,7
M II Group	**29,52**	**15,73**	**51,49**
SD	11,6	8,38	23,9
I III Group	**28,14**	**15,47**	**57,18**
SD	13,9	6,81	32,6
TOTAL	**28,60**	**14,76**	**56,15**
SD	12,6	7,80	27,4

BMI does not affect serum AMH production and values, as there are no intergroup differences. Therefore, obesity does not affect AMH. AMH also remains unchanged when comparing the overweight and obese patient groups with that with a normal BMI. The wide distribution of AMH results is shown in the histogram below.

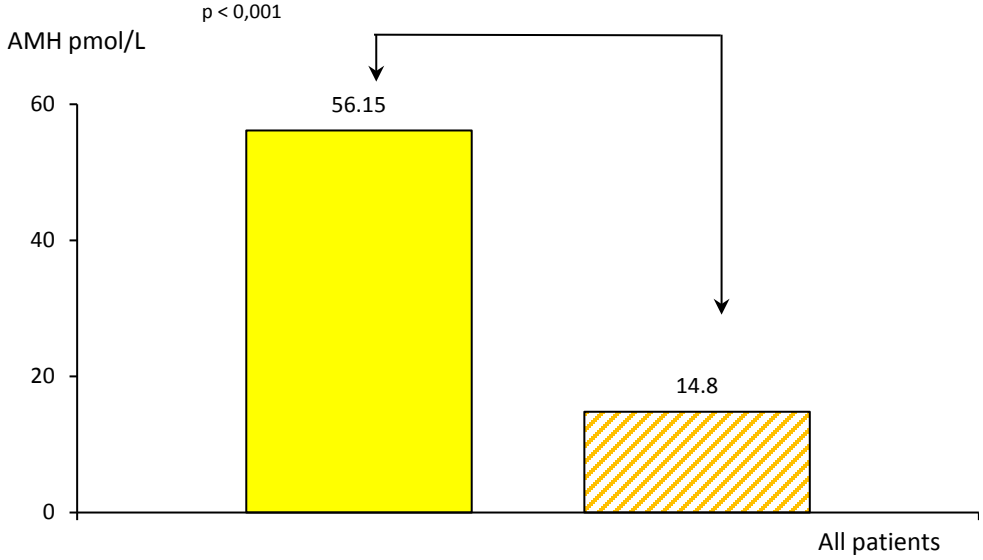


Figure 37.AMH Difference between PCOS

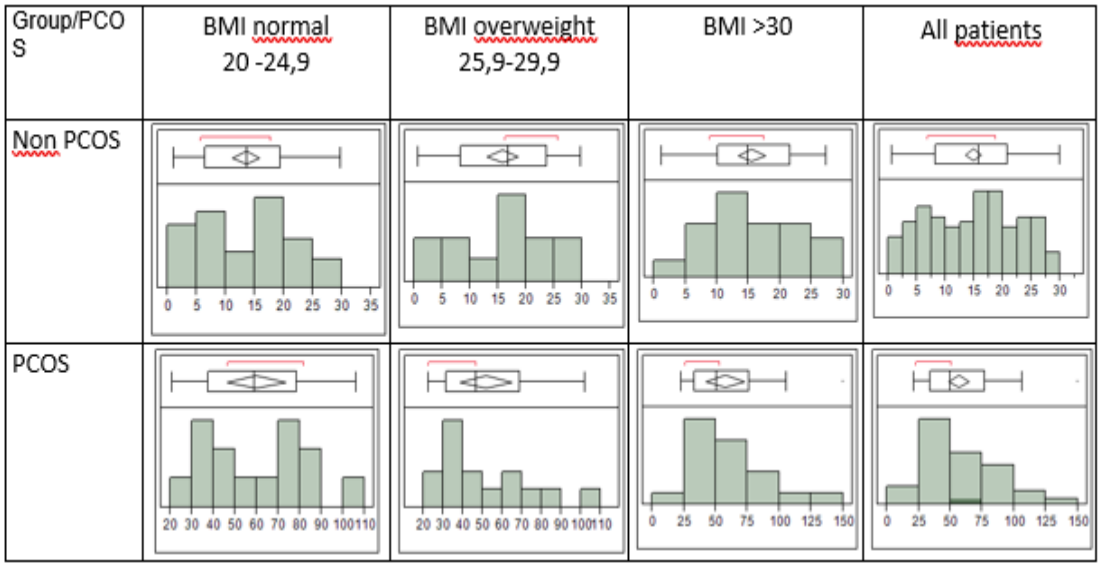


Figure 38. Histogram AMH distribution and non PCOS patients

In all BMI groups AMH in PCOS patients is statistically significantly higher than in women without PCOS (p< 0,001). Despite expectations, there was no negative association of obesity with AMH production.

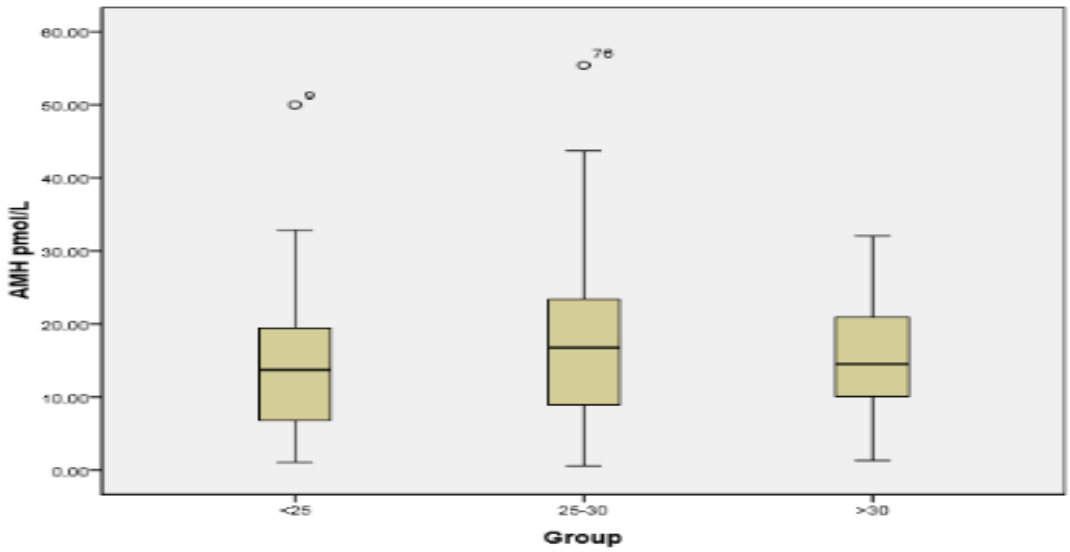


Figure 39. AMH values in non PCOS (mean and range)

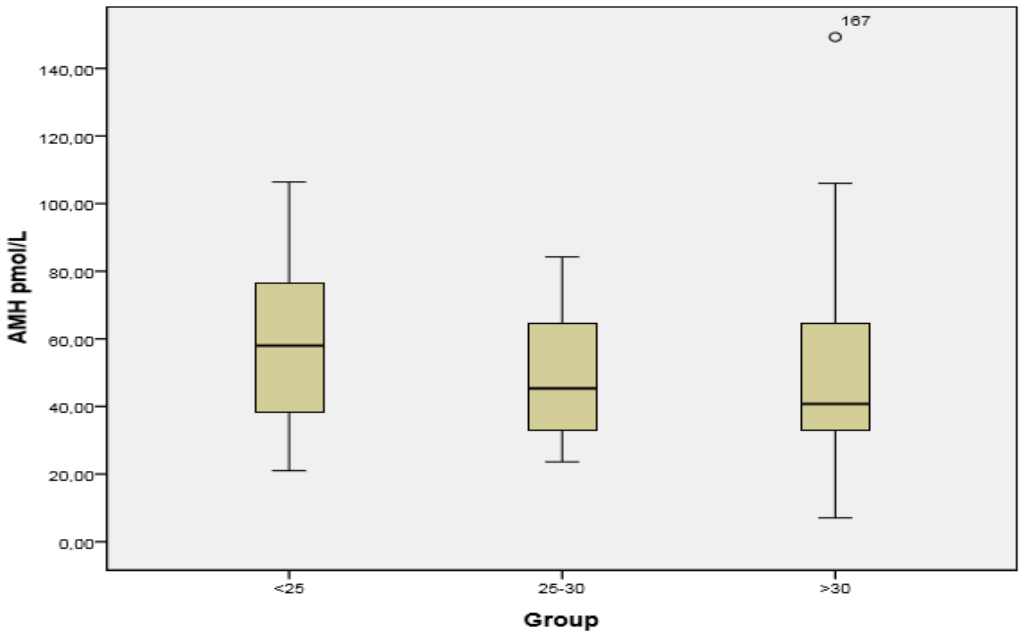
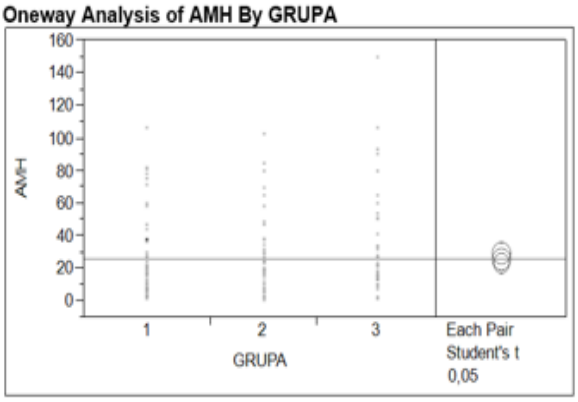
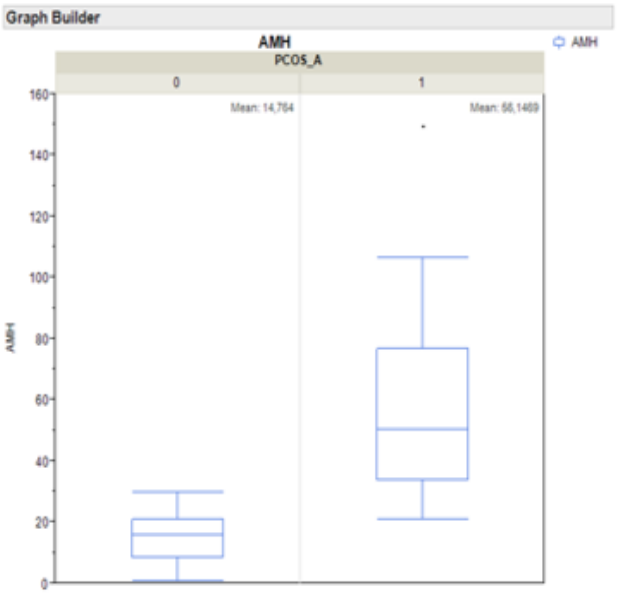


Figure 40. AMH values in PCOS (mean and range)



$H_0: \mu_3 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_3 - \mu_1 \neq 0$		5,911929	0,1832	
$H_0: \mu_3 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_3 - \mu_2 \neq 0$		3,947881	0,3918	
$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H₀
$H_1: \mu_2 - \mu_1 \neq 0$		1,964048	0,6650	



$H_0: \mu_1 - \mu_0 = 0$	α=0,05	Difference	**p-Value**	**p-Value< α→H₁**
$H_1: \mu_1 - \mu_0 \neq 0$		41,3829	<,0001*	

Antral follicular count (AFC) is a measure of the number of 2-10 mm antral follicles in reserve. This ultrasound examination depends on the sensitivity of the vaginal probe, the number of sections through both ovaries and the experience of the reproductive gynecologist performing the examination. Criteria for this examination were set at the beginning of this study with AFC being determined in two sections through each ovary. More recent instructions and more advanced US devices have changed the recommendations and criteria for AFC assessment. For the purpose of uniformity, the earlier criteria were kept for this examination. These are the reasons why AFC levels in all participating patients are uniformly lower.

Table 40. Antral follicular count in our populations (mean and SD)

Patients	AFC n		
	All n= 182	Non PCOS n= 130	PCOS n= 52
B I Group	**12,88**	**8,28**	**24,73**
SD	8,86	4,28	7,17
M II Group	**13,91**	**9,37**	**22,51**
SD	9,39	5,50	10,13
I III Group	**15,15**	**9,41**	**25,76**
SD	10,09	4,16	9,66
TOTAL	**13,98**	**9,02**	**24,33**
SD	9,25	4,20	7,85

Obesity (BMI) does not affect follicular ovarian reserve and there are no intergroup differences. In all the BMI groups AFC is statistically significantly higher in PCOS patients.In individual BMI groups of patients an increase in obesity is not associated with a rise in AFC levels. Obesity does not affect the number of antral follicles.

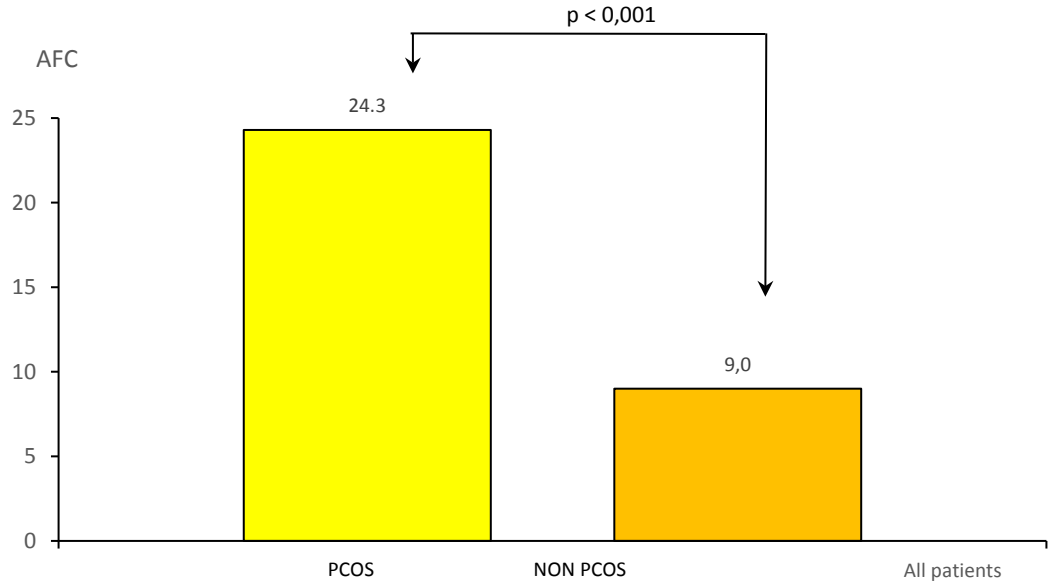


Figure 41. Difference in AFC between PCOS

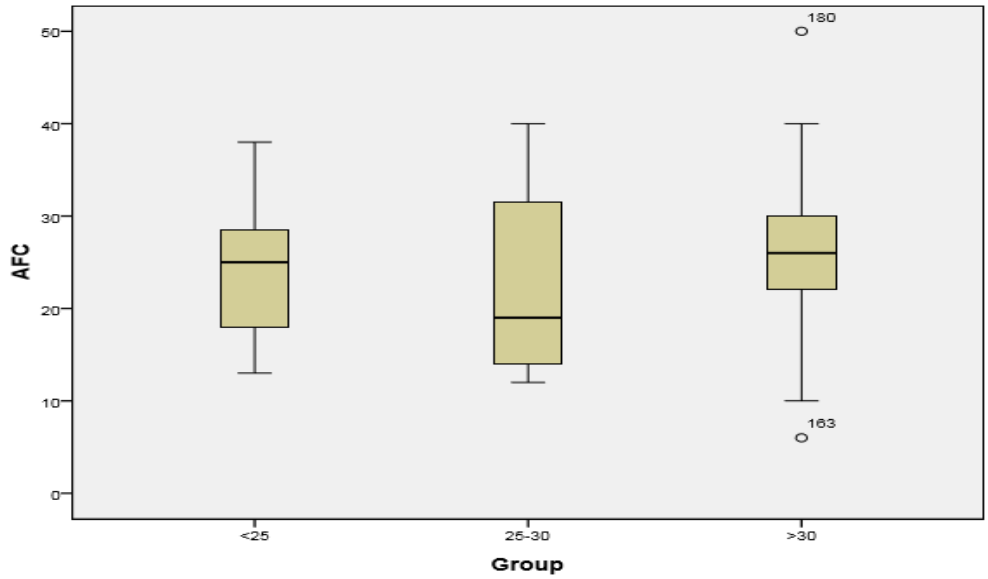
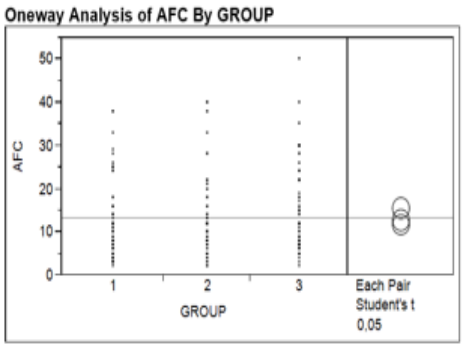
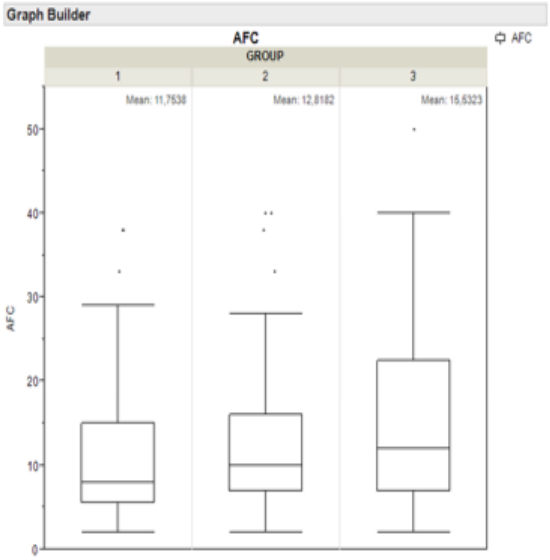
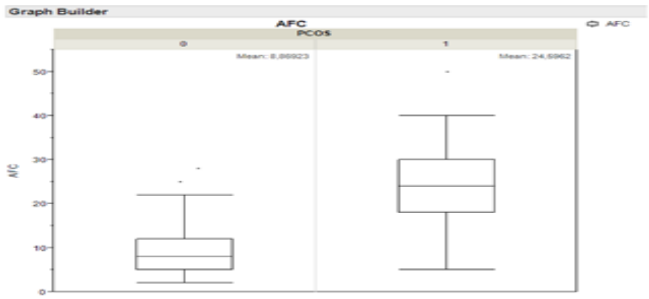


Figure 42. AFC in PCOS patients and non PCOS patients



$H_0: \mu_3 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value<α→H1
$H_1: \mu_3 - \mu_1 \neq 0$		3,778412	0,0264*	
$H_0: \mu_3 - \mu_2 = 0$	α=0,05	Difference	p-Value	p-Value>α→H0
$H_1: \mu_3 - \mu_2 \neq 0$		2,714076	0,1250	
$H_0: \mu_2 - \mu_1 = 0$	α=0,05	Difference	p-Value	p-Value>α→H0
$H_1: \mu_2 - \mu_1 \neq 0$		1,064336	0,5419	





Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
1	0	15,72692	1,056553	13,64210	17,81175	<,0001*

Ovarian volume increases with the total number of reserve follicles (AFC). Ovarian volume (OV) was expressed as the mean value of measured volumes of both ovaries. An increased ovarian volume is one of the US criteria for polycystic ovary morphology (PCOM) or PCOS.

Table 41. Ovarian volume in participating women

	Ovarian volume ml		
Patients	**All** **n= 182**	**Non PCOS** **n= 130**	**PCOS** **n= 52**
B I Group	**7,10**	**6,18**	**10,07**
SD	2,69	1,98	2,59
M II Group	**7,05**	**6,08**	**9,49**
SD	2,28	1,56	1,95
I III Group	**7,79**	**6,69**	**9,87**
SD	2,25	1,60	1,79
TOTAL	**7,31**	**6,32**	**9,81**
SD	2,21	1,76	1,85

As obesity and BMI do not affect the number of antral follicles in the ovaries, there are no differences in OV across BMI patient groups. Obesity does not affect the OV in PCOS patients nor in non-PCOS patients.

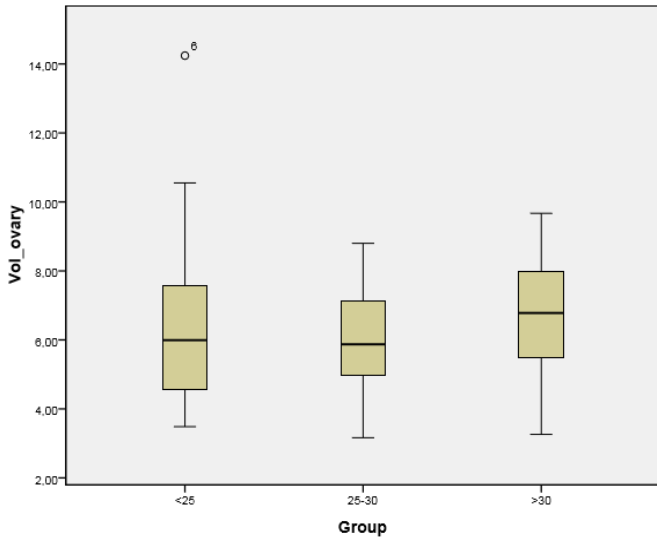


Figure 43. Ovarian volume in non PCOS (mean and range)

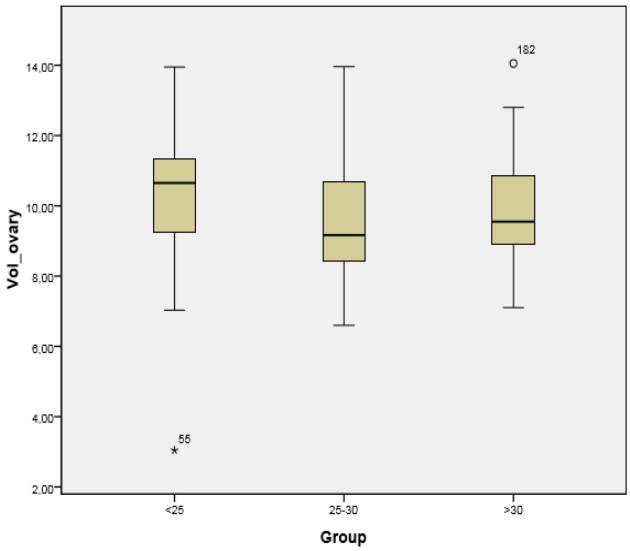


Figure 44. Ovarian volume in PCOS (mean and range)

PCOS patients have a statistically significantly higher ovarian volume than women who do not have this syndrome.

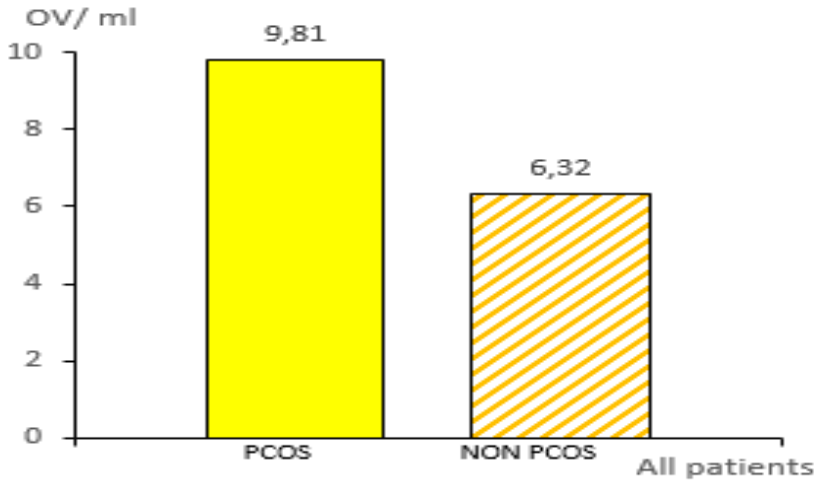


Figure 45. Difference in ovarian volume

Less than 1% of non-PCOS patients and 33 (63.46%) PCOS patients have an ovarian volume greater than 10 ml, which is an expression characteristic of PCOS.
It was investigated whether the duration of the menstrual cycle (≥ 40 days), which is a characteristic more common in PCOS, and anovulation affect AMH levels. Also explored were the levels of the anti-Müllerian hormone in ovulatory and anovulatory cycles of PCOS and non-PCOS patients.

A menstrual cycle longer than 40 days (oligomenorrhoea) is associated with anovulation and an elevated ovarian reserve. Long cycles were identified in 44 patients (24.2%). In all the BMI groups long-lasting oligomenorrheas are associated with elevated AMH levels. In obese patients (group III), long cycles have the highest prevalence of 42%, and these women have a slightly lower AMH than leaner women.

Table 42. Oligomenorrhoea and ovarian reserve

	Long menstrual cycle ≥ 40 days	
Patients	**All** n= 182	**AMH pmol/L**
B I Group n= 64	**9 (14,1%)**	**59,2**
M II Group n= 56	**9 (16,1%)**	**52,1**
I III Group n= 62	**26 (41,9%)**	**45,4**
TOTAL	**44 (24,2%)**	**52,2**

By contrast, anovulation in obese women, and particularly in obese women with PCOS, is associated with statistically significantly the highest AMH. Women with anovulation are more obese (increased BMI) and they have more severe endocrine disorders. Anovulatory PCOS is characterized by statistically significantly the highest AMH.

Table 43. Comparison of ovulatory and anovulatory menstrual cycles

Parametar	**Non PCOS**		**PCOS**	
	Ovulatory n= 94	Anovulatory n= 38	Ovulatory n= 17	Anovulatory n= 35
BMI kg/m^2	26,2	28,4	28,5	32,4
AMH pmol/L	14,1	17,8	45,8	66,4

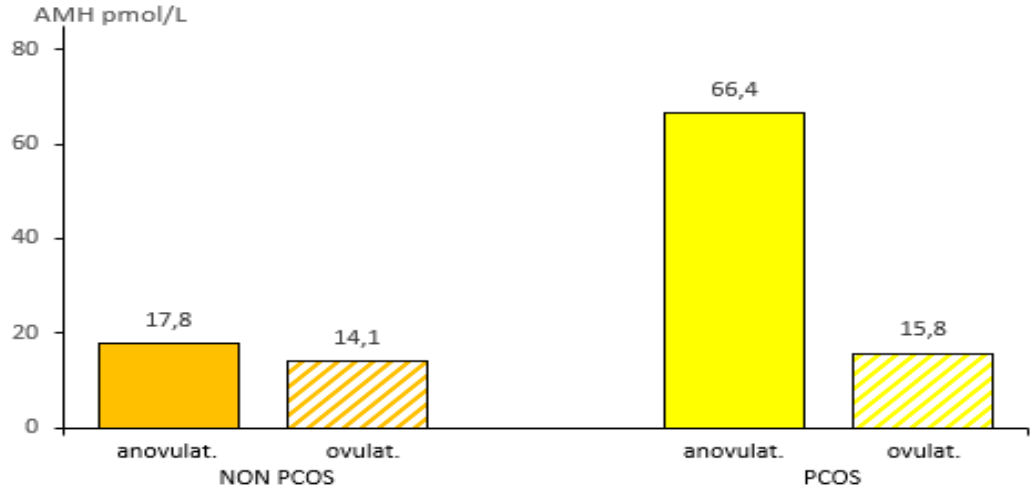


Figure 46. Antimüllerian hormone concentration in ovulatory and anovulatory women

Table 44. Ovarian reserve markers in all patients

	Biomarkers of ovarian reserve		
Parameter	**All n= 182**	**Non PCOS n= 130**	**PCOS n= 52**
AMH pmol/ L	28,60	14,76	56,15
AFC n	13,98	9,02	24,33
Ovarian volume ml	7,31	6,32	9,81
FSH IU/L	6,94	6,85	5,02

With an overall illustration of ovarian reserve markers in all participating patients, we want to confirm the findings presented above. No negative effect of obesity on ovarian reserve was found. Only PCOS patients exhibit a statistically significant elevation of reserve markers. Although the prevalence of morbid obesity in studied populations is low, the findings for this population are also shown.

Table 45. Ovarian reserve markers in morbid obese patients

	Morbid obesity	BMI> 40/kg/m^2
	Non PCOS 4,6%	**PCOS 7,7%**
AMH pmol/ L	**10,56**	**33,47**
AFC n	**7,33**	**17,52**
Ovarian volume ml	**5,52**	**7,72**
FSH IU/L	**4,6**	**5,97**

Although this is not statistically significant, it seems that morbid obesity reduces ovarian reserve markers.Endometrial thickness measured at the beginning of the menstrual cycle was the same in all 3 groups of patients 3.0 to 3.5 mm, and in PCOS patients it was 2.68 to 2.98 mm.

In 133 participating patients we investigated HbA1c which has some advantages over the OGTT because it can be performed at the initial visit with no fasting required.

Obese and PCOS women are at high risk of prediabetes and diabetes (T2D). The OGTT is still regarded primary screening tool for IR and T2D. The American Diabetes Association defines raised HbA1c as > 5,7%, and cut-off value for T2D at >6,4% (101).

In 133 patients we have found HbA1c > 5,7% in 33 (29,3%) women. Association of raised HbA1c with overweight and obesity was in 37,4% and in PCOS patients 11,9%. Incidence of HbA1c> 6,4% was 6 (4,5%). It should be mentioned that this test has not been validated in PCOS so far.

6. DISCUSSION

Adipose tissue (AT) is a highly variable body component both in terms of its total amount and by location. There is a typical gynecoid and android distribution of AT, subcutaneous or central (visceral) fat deposition or distribution in smaller, discrete depots. Adipose tissue is the main energy storage, the controller of its homeostasis and balance. In addition, AT exhibits extensive hormonal activity and controls thermoregulation. Obesity is a multifactorial chronic disease characterized by excessive storage of adipose tissue subcutaneously and viscerally. Obesity is associated with a variety of health disorders, from insulin resistance, metabolic syndrome, glucose intolerance, hormonal disorders to reduced fertility and reproductive risks. The highest risk is caused by central obesity which, along with the above-mentioned risks, is a low-grade inflammation condition (1,7,10,12,11).

The frequency of obesity in the world has doubled over the past 30 years, and in many countries the prevalence of overweight women is 30%, while obese women account for 20-30% (and more) of the population. The prognosis is even worse because the incidence of obesity (with significant geo-epidemiological differences) continues to grow and is considered as a warning epidemic. There is abundant evidence that obesity and especially central obesity reduce fecundity, thus prolonging time to pregnancy, reduce female fertility and increase the risks and complications of pregnancy and childbirth (2,3,4).

Infertility is a disease of the reproductive system that reduces conception and birth. There are numerous female and male causes of infertility, and obesity is becoming an increasingly important epigenetic factor of reduced fertility. Interestingly, in parallel with a rapid increase in obesity, the incidence of infertility in the last 3 decades has increased from 10% to 17% of couples. That is why these two disorders are interrelated. An additional reason for relating obesity and infertility is the frequent association of obesity with polycystic ovary syndrome (PCOS), which also has a high prevalence in the infertile female population (25-35%). It has been shown that obesity and PCOS alone or jointly disturb the menstrual cycle, cause anovulation and cause reproductive

risks through severe hormonal activity disorders and insulin resistance. Each of these diseases can have an important effect on ovarian reserve (OR), which is an important precondition for normal fertility (14,17,20,31,37).

These are the reasons why we investigated the effect of obesity on ovarian reserve. Anthropometric obesity parameters were analysed in order to correlate the independent effect of obesity on ovarian reserve and fertility with the effect of PCOS on these parameters. Obesity alone, and jointly with PCOS, creates an unfavourable hormonal and metabolic milieu, oxidative stress, dyslipidaemia, subclinical inflammation and impaired fibrinolysis. While the effects of PCOS are unquestionable, many effects of obesity, especially on ovarian reserve, remain controversial and no consensus has been achieved (26,40,45,47-59).

Analysis of the ovarian reserve should consider the natural decrease of OR with age, where AMH shows an annual decrease from 0.7 to 1.5 pmol/L after the age of 32 (≈ 5% per annum) (61,67,77).Therefore, this study included young patients of an average age of 32 years.

The study included 182 participants who were divided by BMI values into normalweight (BMI 18.5-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2) and obese (≥ 30 kg/m^2). There were 130 (71.4%) participants affected only by infertility and increased BMI, and 52 (28.5%) PCOS patients. Similarly, the prevalence of PCOS in obese infertile women 21 (33.8%) was identified by several authors (63,73,75). Also, large epidemiological studies (meta-analysis of 35 studies) have found a high incidence of overweight and obesity (61%; OR 2.31: CI 1.67-3.19) in the PCOS population, i.e. 30% of PCOS in the obese population and 75% of obese women in the PCOS population (70,72,77).

In our non-PCOS female population, there were 81 (62.3%) women who were overweight and obese, and the prevalence of such a BMI in the PCOS population was 71.7%. Yildiz and Azziz found the impact of obesity on the prevalence of PCOS to be minimal (73).

Today, the incidence of obesity in the infertile population is 30-35%, and the incidence of anovulatory infertility is 3 times higher than in the normal-weight population. Each increase of BMI by 1 unit (above 25 kg/m^2) reduces fecundity and CPR by 4% and live birth rate (LBR) is lower by 2%. Each further increase of BMI by 1 unit above 30 kg/m^2 reduces IVF success by 7% (14,48,52).

It has been found that an increase in obesity in the PCOS patients significantly increases the incidence of insulin resistance. In normal-weight PCOS women the incidence of IR is 55%, in overweight women 75%, and in obese PCOS patients the incidence of IR is higher than 90% (60,68,74).

Morbid obesity was found in only 5% of the patients, which is less than the numbers found in literature (73).

Participating patients exhibited long-term infertility, primary or secondary, longer than 4 years (ranging from 1 to 17 years), and only 21% had received infertility treatment. This information is indicative of the social environment in which most of the research was carried out. Women marry early and wait long to see a doctor. Obesity is not considered a handicap for fertility, and sometimes infertility lasts for more than 10 years. The average age of our patients was 32 years. The duration of infertility is not prolonged by an increase in obesity (BMI).
In western literature data is different and women report infertility at an average age of 34 years, after 2 years of failure (1,49).

The incidence of male infertility factor today is 40-55% according to most relevant studies (14,40). In our population, male subfertility was identified in 25% to 34% of couples, but it should be noted that women with a completely infertile partner (azoospermia) were excluded from the study. Here we recall that the probability that an obese infertile woman also has an obese partner is around 70% (40).

By analysing the demographic characteristics of our participants, it is understandable that body weight increases significantly with increased BMI. Interestingly, we found

significantly more previous pregnancies, births (secondary infertility) in overweight (21.4%) and obese population (19.3%) than in normal-weight patients (9.4%). Such findings are difficult to interpret because there is less PCOS and anovulation in patients with normal BMI. It is probable that there may be other unidentified causes of infertility in normal-weight women.

Thyroid disorders have roughly the same incidence in all three groups of patients. Therefore, obesity in our patients is not related to subclinical and overt hypothyroidism (TSH > 4.2 mU/L). However, if we observe BMI groups II and III and morbid obesity, elevated TSH levels above 3.5 mU/L are found in 26 patients (22.3%). This is in accordance with more frequent claims in the literature about a **positive correlation between TSH** and progressive weight gain (BMI).It is also stated that hypothyroidism has a higher prevalence of obesity and that obesity occurs with thyroid dysfunction (1,7). Such findings are expected because the thyroid is involved in the control of energy homeostasis, thermoregulation and leptin activity.

Waist circumference is the basic measure of central (abdominal) obesity, leading to the most severe hormonal and metabolic disorders. No WC differences were found within individual BMI groups. Obese patients have a statistically significantly higher WC than overweight and normal-weight women. Obese PCOS patients have a high prevalence of visceral obesity according to all anthropometric parameters, WC is greater than 88 cm in 76% of these patients, WHR > 0.85 in 66.7% of women. Combined indicators of central obesity are positive in as many as 90% of obese PCOS patients. Obesity alone (without PCOS) is associated with central obesity in 68% of participants. Normal-weight participants have a low incidence of central obesity.

Waist-to-hip ratio (WHR) is a reliable indicator of upper body obesity (and visceral obesity) and points to the predominance of android obesity over gynecoid obesity. Borderline WHR was found in obese non-PCOS patients (0.84), while WHR was pathological (0.88) in obese PCOS women. WHR is statistically significantly higher in obese participants compared to lean women. Half of our obese patients had central obesity according to WHR. In our anthropometric analyses, the most powerful indicator

of central obesity was the ratio of WC to patient height – **waist to stature ratio (WSR).** Pathological values of that ratio (>0.50) were recorded in almost all obese patients. According to all indicators, the prevalence of central obesity in our patients was as follows:

Patients	**Non-PCOS**	**PCOS**
Normal-weight	10%	20%
Overweight	15%	31.2%
Obese	68.3%	90.4%

Similar incidence of central obesity in women with increased BMI was also found in other studies, where the prevalence ranges between 65% and 85% (5,9,10,11). There is sample evidence of a strong effect of central obesity on subfertility, due to frequent occurrence of oligomenorrhea and anovulation (OR 0.60, 0.4-0.89) and the association with insulin resistance (IR). Palomba found statistically significantly lower fecundity in women with: BMI > 40 kg/m^2, WHR > 0.85 or WC > 90 cm (70). Conversely, Dumešić (141) claims that obesity leads to reproductive disorders only with IR, and increased insulin levels are found in more than 90% of women with BMI > 30 kg/m^2 and central obesity. Central obesity phenotype induces higher risk PCOS phenotypes (A and B), thereby contributing to higher reproductive risks. Obesity alone decreases ovarian reserve by 40-60% (56,59,70). By contrast, Escobar-Morreale finds that while obesity alone does not diminish the ovarian reserve, that effect is exerted by obesity and PCOS. It was also found that any increase in WHR by 0.1 reduces fecundity by 30% – OR 0.70 (CI 0.56-0.88) (110). It is emphasized that WHR > 80 reduces in vitro fertilization (IVF) success rates by more than 50% – clinical pregnancy rate (CPR) OR 0.42 (CI 0.2-0.90). In Carmina et al., the prevalence of central obesity was reported to be 30% for lean PCOS patients, and 71% for overweight and obese PCOS women (89,104).

Several (direct and indirect) associations have been highlighted where central obesity with polycystic ovary syndrome affects the anti-Müllerian hormone levels and ovarian

reserve. These include increased LH and androgen activity, the effect on AMH receptors (AMH-R2), recruitment of small antral follicles and IR (1,46,47,110).

Hip circumference (HC) is a measure of subcutaneous gynecoid obesity phenotype, which does not increase metabolic and reproductive risks. In this study, no HC differences were determined within BMI patient groups, while increased BMI led to statistically significant differences between obese and lean patients. The more pronounced the gynecoid obesity type, there is less likelihood of central obesity. Our patients were characterized by expressed upper body fat and a high WC contributing to central obesity.

Menstrual cycle -Obesity and PCOS are powerful factors that disturb the menstrual cycle because both syndromes are involved in the control of the hypothalamic-pituitary axis. The adipokine leptin is an important central biosignal that informs the brain of energy stored in adipose tissue. It participates in modulating the function of the appetite centre and the neuroendocrine control of GnRH and its pulsatile neurosecretion. Adipokines also affect the function of follicles in the ovaries. The necessary amount of adipose tissue (energy reserve) influences menarche and the ovulation cycle, while excess AT, or obesity, can disturb the menstrual cycle, cause earlier onset of menarche and induce anovulation (14,20,24,34). Central obesity and PCOS lead to chronic oligo/anovulation, hyperandrogenism and IR (48,49).

In this study, earlier onset of menarche was recorded in obese patients with PCOS (11.6 years), which is statistically significantly earlier than late menarche in polycystic ovary patients (13.9 years). PCOS sometimes postponed menarche until the age of 16. Irregular cycles, oligo/anovulation, were recorded in 21% of all non PCOS women and in 25% of obese patients in that group. In contrast, 69.2% of PCOS patients had irregular menstrual cycles and anovulation. The highest prevalence of chronic anovulations was found when obesity was combined with PCOS (85.7%), and likewise irregular cycles in 80.9% of patients. This frequency is significantly higher than that in lower BMI patients and non-PCOS patients. With obesity alone, amenorrhea was rare (4-7%), depending on BMI, while 17.3% of PCOS patients reported amenorrhoea.

Obese women had longer and heavier menstrual bleeding than normal-weight participants. In obese PCOS patients, the average duration of menstruation was 5.82 days (3-8 days), and HMB was reported by 24.3% of obese and overweight PCOS women. Heavy menstruation is less common in women of normal body weight.

Menstrual cycle is prolonged by obesity. In our study, the average cycle in obese women was 3 days longer (30.12 vs. 27.53 days) in non PCOS patients, and PCOS women had 6 days longer cycle (39.5 vs. 33.7 days). Oligomenorrhea is characteristic of overweight and obese PCOS patients. In all BMI groups, patients had shorter cycles if there was no polycystic ovary syndrome. Obese PCOS patients have a statistically significantly longer cycle (39.5 days) compared to all other patient groups. Menstrual cycle characteristics in participating populations were the following:

Patients	**Non-PCOS**	**PCOS**
Menarche Age/Y	11.9	13.5
Menstrual cycle length Days	28.6	36.4
Irregular cycles %	20.8%	69.2%
Anovulation %	29.2%	67.3%
Heavy menstrual bleeding OV/OB %	13.6%	24.3%

OV - overweight; OB - obese

There is a lot of ongoing discussion on the impact of obesity or PCOS on the menstrual cycle. Several studies have found that increased BMI prolongs the cycle and leads to oligomenorrhea and irregular cycles (RR 3.1 CI 2.2-4.4). Obese adolescents have a higher incidence of irregular cycles than the lean control (54% vs. 19%), and 40% have cycle disturbances with abnormal uterine bleeding (AUB). These patients are at a significant risk of infertility later in life (OR 2.84; CI 1.59-5.10) (14,50,52).

Elevated BMI and obesity significantly reduce ovulation (OR 0.92; CI 0.88-0.96) (14,139,141). The incidence of infertility with obesity is 30-35% (52,75), mainly due to anovulation.The prevalence of anovulation in occult PCOS is 14.5%, while in real PCOS anovulation incidence is 58% (141).

Women with BMI ≥ 30 kg/m^2 were also found to have statistically significantly more endometrial polyps (52% vs. 15.6%) and AUB (131).

Women with oligomenorrhea or amenorrhea have a 90% probability for PCOS, but it is stated that 20-30% of PCOS cycles may be ovulatory (80). The same consensus points out that according to the Rotterdam criteria, 20-30% of PCOS patients have regular cycles, 40-50% have oligomenorrhea, 21% have amenorrhea and 7% have polymenorrhea. According to NIH criteria for the diagnosis of PCOS, all patients have hyperandrogenism and irregular cycles (70,72,77,80,101).

In a study of 26,000 women it was found that cyclic irregularity and anovulation are correlated with overweight and obesity. Obese women have 3.1 times more menstrual disorders than lean women (B and B). Elia et al. emphasize that oligomenorrhea is associated with central obesity (48).

In 1741 British women with PCOS, 70% have menstrual cycle irregularities, while obese PCOS women have a higher prevalence (78%) of cycle irregularities (14,110). Similarly, a smaller study with 263 PCOS women found that obese women have an 88% chance of cycle irregularities, while non-obese women have an irregular cycle in 72% of cases (110). Central obesity with or without PCOS has a strong effect on chronic anovulation, cycle irregularities and reduced fertility in women.

The disorders described in the conclusions of these studies are associated with central and peripheral hormonal and metabolic effects of adipokines and PCOS. The pathophysiology of anovulation is based on the disturbed control of kisspeptin and neurosecretion of GnRH, and on the effects of hyperandrogenemia and hyperinsulinemia (70,74).

The mechanism of prolonged and heavy menstrual bleeding (HMB) involves a disbalance in the production and activity of estrogen and progesterone, and focal and abnormal endometrial transformation. Also interesting are meta-analysis findings about

weight loss in morbidly obese patients. A significant drop in BMI decreases the prevalence of PCOS and hirsutism, leading to a reduction in menstrual cycle irregularity (from 56.2% to 7.7%) and infertility (from 18.2% to 4.3%) (14,79,135,138,139).

Previous pregnancies- Unlike normal-weight women who achieve an 81.4% probability of pregnancy in one-year, obese women have a 66.4% chance of getting pregnant (14,45). This means that obese women are subfertile, while obese PCOS women are 10 times more likely to be infertile. While for normal couples it takes 4.8 months on average to achieve pregnancy (TTP – time-to-pregnancy), with obesity and PCOS this time is extended 3-5 times (1,12). If both partners are obese, it takes 60% more time to achieve pregnancy (1,7,124), i.e. OR for subfecundity is 2.74 (CI 2.27-3.30).Although our research does not have the strength (because of the number of patients) or design to investigate fertility parameters, some findings are unexpected. This primarily concerns the number of earlier pregnancies and developed secondary infertility. In 56 (30.8%) secondary infertile patients in the investigated population, there were as many as 91 pregnancies. In that number, there were 50 early spontaneous abortions (59.9%), significantly more in overweight and obese populations (88%). In the normal population TTP reported by the patients was 9.5 months. Obesity alone without PCOS prolongs the time to earlier pregnancy two times. In obese PCOS patients, pregnancy (birth or miscarriage) occurred after two years of exposure (25.6 months). Morbid obesity further prolongs TTP by 26% or 23%, depending on whether PCOS is involved. These results, although obtained on a small sample, are consistent with larger studies (136,139).

Ramlau-Hansen et al. analysed 47,835 couples from the Danish registry and found that female obesity prolonged TTP by 30% (OR 1.32: CI 1.26-1.37) (50). According to Broughton and Moley, obesity significantly increases TTP and reduces fecundability, obese women are subfertile even without anovulation (124). This fact is explained by IR, hyperandrogenism and adipokine dysfunction, as well as the effects on oocyte development and endometrial receptivity. Obesity is associated with subfecundity (OR 0.72: CI 0.63-0.83), as shown in a study conducted on 7,327 women (14).

A high incidence of early spontaneous abortions (SAB) with elevated BMI and (or) PCOS has been demonstrated by numerous studies, although findings are not fully consistent. (14,106,110,111,117,120,126,127,139).

In a meta-analysis of 33 studies and 47,967 IVF cycles, Rittenberg et al. (120) have demonstrated that an elevated SAB risk is associated with patient obesity – OR 1.43 (CI 1.22-1.67). This finding is confirmed by other studies and meta-analyses in which an analysis in natural and IVF pregnancies increases RR risk for SAB by 30-60% (14). Tian et al. (122) link the high risk of SAB with insulin resistance, which creates a big difference compared to the normal population – 47.8% vs. 9.5% (OR with IR = 8.32: CI 2.65-26.17). This finding is even more significant if we take into consideration that 90% of obese PCOS patients are IR (31,45,49,58,139,140).

In a large study including 9,068 women with PCOS who were obese in 54% of the cases (14). The increased incidence of spontaneous abortions in this population was statistically significant - OR 1.70 (CI 1.56-1.84).
Important research has also been published that does not confirm an association between obesity and SAB (80,133,134). In contrast to this controversy, findings about the association between spontaneous abortions and PCOS are consistent. On a sample of 1,962 cycles it was found that with elevated levels of testosterone and AMH (> 50 pmol/L), which are characteristic of a riskier phenotype of PCOS, there is a significant increase of SAB incidence (30% vs 19%), i.e. OR 1.39 (CI 1.22-2.86) (103). There are findings about SAB incidence of 30-50% in obese PCOS patients (117,122). Goldman did not find more aneuploidies in miscarriages, which has been confirmed by other authors (123,130).In 2,349 pregnancies of obese and morbidly obese women Wang et al. found a high risk for SAB (OR 1.71 and 2.19 respectively) (130).

Effects of obesity and PCOS on regulatory hormones- Normal folliculogenesis, ovulation and endometrial maturation require a complex interaction and balance of hormones. Obesity and PCOS disturb the production and activity of regulatory hormones by direct and indirect mechanisms. Hormone disorders also include adipokine imbalance and hyperinsulinemia, and the effect of both syndromes on

neurophysiological events in the hypothalamus and the pituitary gland. Central and peripheral hormonal disorders are even more complex when obesity and PCOS are combined. In accordance with our possibilities, we have investigated the changes in some pituitary hormones and the response of ovarian hormones in patients with infertility due to obesity and PCOS. It is known that obesity reduces FSH and LH and decreases LH amplitudes and increases hyperandrogenemia. PCOS reduces FSH, increases LH and its pulsatility, mildly increases PRL, and more strongly increases the androgens. Elevated LH in lean women with PCOS opens a path to hyperandrogenemia. Defective insulin signalling leads to IR, and receptor phosphorylation and abdominal AT phenotype increase de novo androgen production in adipocytes. Both syndromes reduce the production of SHBG and thus increase the activity of androgens. Gonadotropins and androgens are co-factors in the control of ovarian reserve and anti-Müllerian hormone (1,7,34,40,47).

In this study, we have found that weight gain and increasing BMI lead to a reduction of FSH, which is significantly lowest in the obese PCOS population (4.67 IU/L). Obesity also reduces LH, which is highest in the lean non-PCOS and PCOS patient group. It is expected that LH levels (and activity) are the highest in the normal-weight PCOS population (9.98 IU/L), in which only the LH/FSH ratio recorded the inversion characteristic for PCOS (> 2.0). In all BMI groups of participating women with PCOS, LH levels were higher than FSH levels. These results are in accordance with the data about the pathophysiological changes in obesity and PCOS, especially with the interpretations of oligo/anovulation and cycle prolongation due to disturbed gonadotropins, as described above. All hormonal analyses were performed under basal conditions. The mean prolactin level (PRL) in our infertile patients was within the normal range. In all BMI groups PRL is statistically significantly higher in PCOS patients. Overall, PCOS patients had 25% higher PRL (20.37 ng/mL vs. 15.22 ng/mL) and 6 (11.5%) had slightly abnormal PRL levels. Only 4 (3.1%) women without PCOS had abnormal PRL levels.

The levels of estradiol are similar in all three investigated BMI groups. No difference in E_2 was found between obese only and PCOS patients. This hormonal analysis is partially in line with the findings in the literature, with more mildly expressed

hormonal changes than described. It should be noted that the basal E_2 is an indirect ovarian reserve marker.

Hypersecretion of LH and elevated LH/FSH ratio have been shown to be unfavourable controllers of folliculogenesis in obese infertile women (1,34,35). These disorders are even more strongly expressed in polycystic ovarian syndrome (58,59,61). High LH and increased pulsatility stimulate AMH hypersecretion and are the main pathophysiological mechanism of hyperandrogenemia. In obese PCOS patients, the path to elevated androgens is also achieved with IR. Insulin is an active factor (cogonadotropin) in the stimulation of steroidogenesis in theca cells. An imbalance of FSH and LH in the follicle is transmitted to autocrine and paracrine secretion disorders (1,40,141,142). The hormonal ovarian disorders in obesity also include leptin, which disrupts steroidogenesis and folliculogenesis. Leptin participates (with insulin) in controlling the secretion of gonadotropin (41,49). Weight loss leads to a reduction in LH secretion but does not affect pulsatility.The central effects of hormones in PCOS disturb dopamine secretion and may result in mild hyperprolactinemia (1,7,57).

Androgen activity- Androgens play an important role in pathophysiological disorders associated with adiposity, visceral obesity and polycystic ovary syndrome. Hyperandrogenemia regularly occurs with more severe obesity and PCOS phenotypes. Since both obesity and PCOS are combined in 60-80% of patients, the mechanism of increased androgen activity in these syndromes is complex. Increased androgen activity depends on the production in theca cells, adipocytes and the adrenal gland. In adipose tissue, androgens are created de novo from cholesterol, or by the conversion of androstedione to testosterone. Serine phosphorylation of the enzyme P450c17 additionally stimulates hyperandrogenism (26,28,29,70,72,94). SHBG level is an important regulator of androgen and estrogen activity. As insulin and TNF-α inhibit SHBG, its low levels lead to more free androgens in the circulation. This means that IR and elevated androgens are interdependent. In addition, elevated LH levels and pronounced pulsatility in PCOS upregulate LH receptors in theca cells, which are the main source of ovarian androgens. Hyperandrogenemia stimulates lipolysis and induces central obesity adipocyte hypertrophy. It stimulates IR and disturbs the cycle centrally

and peripherally, leading to anovulation. Androgens accumulate in adipose tissue. Increased androgen activity positively correlates with ovarian reserve (81,85,87).

In this study we investigated the relationship of obesity and PCOS with testosterone levels, SHBG and free androgen index (FAI). In patients without PCOS, BMI increase is associated with mildly elevated testosterone levels (T), and T is significantly higher in overweight and obese patients compared to normal-weight women (1.82 vs. 1.38). T levels in PCOS patients are significantly higher than those of non PCOS women (2.44 vs. 1.66 nmol/L), but there are no differences in T levels in PCOS women of different weights. The insignificantly highest T levels appear in lean PCOS patients. SHBG levels in our patients show a wide distribution. In line with expectations, the production of SHBG and its levels in the circulation are reduced with obesity. SHBG was statistically significantly lower in PCOS patients compared to patients with obesity alone (35.96 vs. 55.96 nmol/L). Obesity alone slightly reduces SHBG, and when combined with PCOS, SHBG decrease is more pronounced (32.60 nmol/L).

Androgen activity is expressed by an average FAI index which was within normal limits in all groups of patients without PCOS. No association was found between increased androgen activity and weight gain. All BMI groups of PCOS patients have elevated FAI (average 6.83). FAI was statistically significantly higher in lean and obese PCOS patients compared to non-PCOS patients of the same weight:

Patients	**Non-PCOS**	**PCOS**
Testosterone nmol/L	1.66	2.44
SHBG nmol/L	55.96	35.96
FAI	2.87	6.83

In our normal patient's androgen activity measurements were the following: testosterone 1.38 nmol/L, SHBG 61.1 nmol/L, and FAI 2.07. These androgen results correlate well with the data about oligo/anovulation and general and central obesity measurements.

Obesity has a smaller independent effect on androgens than the expected findings from the literature. Therefore, even a mild increase in androgen levels can influence cycle disorders, most likely through changes in androgenic receptors.

Crujeras et al. (52) explain that obesity affects the reduction of reproductive capacity by supressing LH amplitudes and SHBG, and the reduction of free androgens through insulin stimulation. The same findings on the effects of obesity are highlighted by the Practice Committee ASRM (14). The significance of hyperinsulinemia for HA in PCOS patients is also frequently highlighted. When BMI is normalized, the significance of HA (66,67) may be reduced 50 percent. Broughton et al. (124) have found subfertility in obese patients due to lipotoxicity and oxidative stress even when there is no ovulation dysfunction.

Norman et al. (77) state that 75% of PCOS patients have gonadotropin dysregulation. Also, the concentration of the KISS neuropeptide is negatively correlated with BMI, FAI and IR. There is ample evidence that central obesity increases T levels and inhibits SHBG, thereby increasing FAI. Also described is the notion of asymptomatic hyperandrogenemia (functional HA), which is based on intracrine activity and variable androgenic synthesis (1,7). Androgens increase LH activity and inhibit FSH.
Dumešić et al. (141) emphasize that SHBG is the most sensitive measure of hyperandrogenism due to elevated free T. The risk of complications in pregnancy is 4 times higher with hyperandrogenism (78,139). Insulin resistance is the pathophysiological link between obesity and PCOS in the stimulation of hyperandrogenemia. Compensatory hyperinsulinemia affects ovaries, which remain responsive to insulin, stimulating androgenesis in theca cells (139,140). Elevated androgens in obese women and PCOS patients thus lead to oligoovulation/anovulation and subfertility. In 20 to 40% of PCOS patient's androgen levels are not elevated (1,7), but there may be a stronger effect on androgen receptors. Increased androgen levels increase the ovarian reserve of preantral and small antral follicles, which is important in the pathogenesis of PCOS (66).

Ovarian reserve- Ovarian reserve (OR) is the total number of follicles in early stages of development that can be recruited and further developed by folliculogenesis. Ovarian reserve is a fundamental factor in female reproductive potential and is estimated by the number of small antral follicles measuring 2 to 10 mm in diameter, AMH levels and ovarian volume (OV). Antral follicle count (AFC) is determined using vaginal ultrasound by scanning multiple sections of the ovary. The results of this test depend on the sensitivity of the US machine, the number of scanned sections, and on the experience of the expert. Many reserve follicles directly increase ovarian volume. In an increased number of follicles and in more active granulosis, a larger amount of AMH is produced. The ovarian reserve, i.e. the results of its biomarkers, depends on a number of factors such as age, genetic and epigenetic effects. Also, some diseases such as endometriosis, tumours, malignant and autoimmune diseases can significantly reduce OR. Any ovarian surgery or oncological treatment can significantly reduce the reproductive potential. Age, or aging, is the most constant factor of OR reduction. It has been found that with every year after the age of 32 AMH levels are naturally reduced by 0.7-1.4 pmol/L, or 2.5-5% per year. In contrast, excision of endometrioma can reduce AMH by as much as 35% (32,81-93).

Several studies have warned that obesity may reduce ovarian reserve by as much as 40-50% (56,81). In contrast, PCOS is characterised by high ovarian reserve, dominated by abnormal AMH and AFC. An indirect measure for normal ovarian reserve and fertility is a favourable level of basal FSH and E_2(100,141,142).

Ovarian reserve markers are not only a powerful predictor of fertility and sensitivity of the ovaries to gonadotropins, but also of infertility treatment success. High OR may also point to the risk of ovarian hyperstimulation, while low ovarian reserve is an indicator of poor ovarian response and resistance to gonadotropins. The findings about OR as a measure of oocyte quality are inconsistent, as there are claims that reserve biomarkers are only an indicator of quantity (95-104).

In investigating this population, we wanted to determine the ovarian reserve in obese and PCOS patients. We also sought to explore other fertility indicators and their correlation with OR biomarkers.

It was found that increased BMI did not lead to changes in AMH levels in the circulation, i.e. obesity in neither non-PCOS or PCOS patients reduced the production of that hormone. As expected, AMH is statistically significantly higher in PCOS patients in all body weight categories (56.15 pmol/L vs. 14.76 pmol/L).

Antral follicle count (AFC) is also independent of the amount of adipose tissue, i.e. obesity, because there are no intergroup differences. PCOS patients in all BMI groups have statistically significantly higher AFC (24.3 vs. 9.02). These findings are consistent with the diagnostic criteria (Rotterdam) for PCOS/PCOM (80,88,89).

The highest ovarian volume was found in normal-weight PCOS patients, which is statistically significantly higher than in non-PCOS patients (9.84 vs. 6.33 ml). No correlation was found between OV and the amount of adipose tissue. Patients without PCOS do not exhibit a change of ovarian volume even when they are obese. Only PCOS patients (63.5%) have OV greater than 10 ml.

Oligomenorrhoea or menstrual cycle longer than 40 days and anovulation have an elevated ovarian reserve compared to AMH levels. Such cycles are characteristic of PCOS patients. Anovulation cycles always have higher AMH levels in the circulation compared to the normal cycle (66.4 pmol/L vs. 45.8 pmol/L in PCOS population).

In our morbidly obese patients, ovarian reserve markers are reduced, but these differences are not significant due to a small number of extremely obese patients. The effect of obesity on ovarian reserve biomarkers is shown in Table 38.

Table 46. Ovarian reserve markers in obese patients

Patients **BMI ≥ 30 kg/m^2**	**Non-PCOS**	**PCOS**
AMH **pmol/L**	15.47	57.18
AFC	9.41	25.76
Ovarian volume **Ml**	6.69	9.87
FSH **IU/L**	6.14	4.67

Obesity is not a factor affecting the ovarian reserve in our patients, and FSH levels are not a reliable marker for OR evaluation. According to these findings, OR markers are a good indicator for infertility treatment and individualization of gonadotropin doses in ovarian stimulation. These findings can be a reliable predictor of treatment success. Since obesity does not reduce OR markers, it also does not diminish the risk of ovarian hyperstimulation. No association was found between obesity and weak ovarian sensitivity to fertility drugs, but this claim does not have a solid foundation in our analyses. Obese patients in our study have the same ovarian reserve as the lean (normal) population. All patients with PCOS and amenorrhea (9 women) had AMH levels above 90 pmol/L.

Numerous studies have investigated the relationship of obesity and PCOS with ovarian reserve markers and female fertility. AMH levels have become a criterion for the diagnosis of PCOS, and a positive correlation of AMH with LH, AFC (especially with 2-5mm follicles), hyperandrogenism and anovulation (98,100,141) has been confirmed repeatedly. Also, the association of AMH with insulin resistance and ovarian hyperstimulation syndrome (OHSS) has been demonstrated, as well as a negative correlation of AMH with BMI, FSH and ovulation cycles (100,101,142). High levels of AMH (> 50 pmol/L) are combined with high ovarian sensitivity to FSH (ovarian sensitivity index) and an elevated risk for OHSS (OR 6.8: CI 4.9-9.6) (40,80,81). Elevated AMH levels and IR are associated with a significantly higher risk of spontaneous abortions (139,142).

The Practice Committee of ASRM (81) has confirmed that obesity alone does not affect AMH, but that AMH levels are reduced in obese PCOS patients. Responses to that question remain controversial.

Bhide et al. have established criteria for PCOS according to ovarian reserve findings: AMH 55 pmol/L, AFC 30, AMH/AFC ratio > 1.90 (86). An AMH/AFC ratio level lower than 1.20 was the criterion for PCOM diagnosis.

No correlation was found between high ovarian reserve in PCOS women and longer "window of fertility", because after the age of 40 these patients experience a faster decrease of ovarian reserve (84,90). AMH has central and peripheral effects on ovulation control and folliculogenesis, it inhibits FSH activity and is a diagnostic marker for hyperandrogenism (66,95). Dumešić claims that high AMH and androgen levels, and

oligomenorrhea are clear signs of insulin resistance (141). This is also confirmed by Crujeiras, who finds that 94% of obese PCOS patients have IR (52).

Jungheim found that women who developed OHSS were lean with high AMH levels, while all women with poor ovarian response (POR) had BMI > 30 kg/m ^{2} and low AMH levels (136).

Although the number and quality of oocytes decline with age, fertility is highly variable within the same age group. Ovarian reserve tests are neither an absolute measure of fertility, nor of the onset of menopause. OR testing contributes to the diagnosis of women with diminished ovarian reserve (DOR) and poor responders to therapy (81,141,142). The same study and many others point out that ovarian reserve tests should not be the only criterion for withdrawal from treatment because they do not mean (even when they are very low) a complete impossibility of getting pregnant. With aging and decreased ovarian reserve, and with DOR, there is a rise in basal FSH (≥ 12 IU/L) and estradiol (> 70 pg/ml) levels with a shortening of the follicular phase and the menstrual cycle as a whole (40). These indicators together with ovarian reserve tests have an important prognostic role in the treatment of infertility and IVF success. A high ovarian reserve points to a risk of OHSS and to high responders, while a low reserve is linked to a prognosis of POR and poor success. AMH levels lower than 5 nmol/L, AFC < 4 and ovarian volume < 3 ml point to very low OR (40,139,142). In that case the prognosis for treatment and success (most frequently IVF) is very poor.

In contrast, very high ovarian reserve complicates treatment due to high response and decreased endometrial receptivity, which is avoided using "freeze all" technology. It has been shown that ovulation stimulation (OS) and laparoscopic ovarian drilling (LOD) are more successful if AMH levels are below 50 pmol/L (101,133).

In our young (32) but long-term infertile patients, we have not found that elevated BMI was associated with a higher frequency of low ovarian reserve. DOR or poor ovarian reserve was equally represented in all three patient groups. The POR criterion was one or more of the following findings: AMH ≤ 5 nmol/L, AFC ≤ 4 ili FSH > 10 IU/L.

Table 47. Markers of poor ovarian reserve in non-PCOS patients

BMI	Group I n = 64	Group II n = 56	Group III n = 62
PORn (%)	9 (14.1%)	10 (17.8%)	5 (8.1%)
Estradiol> 70 pg/mL n (%)	7 (10.9%)	3 (5.3%)	5 (8.1%)

Similar findings are also shown by elevated basal estradiol levels. Patients with polycystic ovary syndrome did not have POR results, and only 5 overweight and obese PCOS women had elevated E_2 (5/32 – 14.3%).

Increased FSH, and high or low AMH levels are not associated with the risk of aneuploidy in pregnancies following IVF (141,142,143). More frequent spontaneous abortions in these populations don't seem to be related to OR, but to obesity (or PCOS) and insulin resistance. Single FSH value has limited value due to inter-cycle variability (40,81). Ovarian reserve screening tests are valuable for the clinician if they are predictive. Using only FSH is therefore less sensitive for the prediction of success or failure of infertility treatment. There is a similar consensus on the diagnostic and predictive value of basal estradiol (1,40).

For a long time, research has been attempting to determine the cutoff AMH values for predicting reduced success, POR and high response in IVF procedures. As mentioned in the introduction, these AMH values are 7.5 for poor and 25 pmol/L for high response (81,91,92). AFC cutpoints are 3-4 follicles for POR and 20 follicles for HR. Ovarian volume in low ovarian reserve patients is lower than 4 ml and for PCOM/PCOS patients greater than 10 ml (81). Ovarian volume is a weak predictor for IVF pregnancies. It is increasingly recommended to use a combination of ovarian reserve tests to achieve a better prediction of success, failure and risks of treatment. Today's consensus is the recommendation to evaluate OR through a combination of AMH, AFC and ovarian volume (81,139,141,142,143).

Opinions on AMH were presented as a good or unreliable predictor of IVF success differ (121). Longer cycles, elevated AMH levels and high AFC are predictors

of high LBR (88,112). It should be noted that gonadotropins and AMH have different isoforms and different types of receptors, which can significantly alter their activity.

Also important is the transfer of pulsatility from growth hormone to leptin and FFA, and the fact that only changes in pulsatility may alter the activity of the hormones.

According to the results of our study, obesity, central obesity and PCOS, either independently or jointly, are a significant factor of reduced fertility and infertility. Long-term infertility (mean 4 years) in this population, where we excluded important potential causes such as age, tubal factor, infertile partner and severe endometriosis, has a greater potential of analyzing the effects of obesity and PCOS. In addition to these two main syndromes, the remaining possible causes of infertility in our sample are oligoovulation/anovulation, subfertile partner, mild endometriosis and idiopathic sterility. Proven or unproven hormonal disorders and likely insulin resistance associated with obesity and PCOS (50-90% of patients) should be linked with the negative effects on the oocyte and endometrium. We were not able to prove the positive or negative effect of obesity on the ovarian reserve. Therefore, with these mechanisms' obesity does not alter the fertility of the investigated women. Since in this analysis obesity does not even alter the ovarian reserve of PCOS patients, it is useful to know that the risk for OHSS in these patients remains unchanged. This is also confirmed in our clinical practice. Some contrary findings have been reported in the literature about this risk (81,91,139).

It is a consensus in world literature that obesity and PCOS alone, and especially when they are combined, significantly contribute to infertility and reproductive risks. The negative effects of these two syndromes result in low fecundity and reduced treatment success, especially in in vitro fertilization (IVF) procedures. Also, obesity and distribution of AT do not cause but may exacerbate the PCOS manifestations and phenotype. Women with PCOS have central obesity in 66 percent of the cases (70,75). Obese women are insulin resistant in 70 percent of the cases and more than 90 percent of obese PCOS patients have IR (31,78). Lean PCOS patients also develop IR in 30-50 percent of the cases. There is thus a consensus that all obese PCOS women are in a state of hyperinsulinemia (70,74). An equally significant disorder for the reproductive axis is hyperandrogenemia or hyperandrogenism (HA). The effects of elevated androgens exist in 30-40% of obese patients and in 80% of women with PCOS

(28,60,61). According to findings in literature, the effects of obesity and PCOS on reproduction are shown in Table 48. (48,49,52,58,61,68)

Table 48 Obesity, central obesity and PCOS effects on reproduction

Fertility impairment	Risks
• Menstrual cycle irregularity • Anovulation • Low fecundity • ↑ Time to pregnancy • Infertility • Low oocyte quality • Gonadotropin resistance • ↑ Cycle cancellation • ↓ Ovulation rate • ↓ Endometrial receptivity • ↓ Implantation • Lower IVF success	• Venous thromboembolism • Multiple pregnancies • OHSS • Spontaneous abortions (SAB) • Perinatal risks - gestational DM - hypertension - prematurity - mortality • Congenital abnormalities • Neonatal • Maternal (SC, bleeding)

SC - Caesarean section; OHSS- ovarian hyperstimulation syndrome

The negative effects of obesity and PCOS on reproduction have been recorded in all forms of conception–natural, ovulation stimulation, IVF/ICSI, oocyte donation (139,141,142,143).

In a meta-analysis of 13 studies with 2326 cycles, Mulders et al. a significantly more frequent cycle cancellation (OR 1.86: CI 1.13- 3.06), and a significant decrease of clinical pregnancy rate (CPR) and increase of SAB (OR 3.05: CI1.45-6.49) as a result of obesity and IR. Numerous studies have confirmed the reduction of CPR, live birth rate (LBR) and a significant increase of spontaneous abortions with obesity and PCOS (125). The highest rate of SAB has been shown by Tian et al. (122), who claim that IR is associated with a high risk of spontaneous abortion – OR 8.32 (CI 2.65-26.17), where the incidence of spontaneous abortions compared to control subjects is 47.8% vs. 9.5%. The risk for SAB is 2-3 times higher due to hyperandrogenism, elevated LH, IR and high PAI-1 (116,117). High T and AMH levels increase the risk for spontaneous abortion

(103). Obesity decreases LBR by 30-60%, and success is significantly reduced with the progressive increase of BMI (126). With morbid obesity, IVF success is lower by 70% (116,117,118,132). Bellver and Provost (115,116) associate the reduction of LBR in obesity with reduced endometrial receptivity. In the same model of study conducted on obese donor recipients, Luke and Jungheim (132,136) did not find endometrial receptivity to be significant for IVF success. The highest rate of IVF success is achieved in lean PCOS patients with a larger number of available oocytes. It has been proven that these women have the highest OHSS risk (19.6%), while this risk is reduced with obesity to 3.2% (141,142,143).

Findings about the adverse effects of obesity on reproduction are not consistent and there is no consensus on the causes of reduced fertility. There is an ongoing debate about reduced count and quality of oocytes, fertilization rate, embryo quality and implantation disorders (139,142). There is a uniformly confirmed consensus on the effect of obesity and PCOS on the cycle, decreased fecundity, anovulation, and more frequent withdrawal from treatment (45,50,139). Obese patients are less sensitive to gonadotropins and it has been proven that 10-20% more drugs are needed to stimulate ovulation. Metabolic disorders in these patients appear to affect LBR and SAB. These are primarily IR, low grade of chronic inflammation and the consequences of high androgen levels. A crucial role is played by the molecular activity of hormones and sophisticated central and peripheral (paracrine) control of folliculogenesis. LH pulsatility or leptin disorders, which are frequent in obesity, may alone affect neurosecretion and folliculogenesis. A complex control of the density and function of the receptors is involved in the expression of hormonal activity. Also affecting fertility are a number of non-provable epigenetic effects and endocrine disruptors.

The silent effects of adipokines, vitamin D, oxysterol, oxidative stress and intra-follicular signals can significantly disturb fertility without clear biosignal disturbances in the circulation. It is an undisputable fact that obesity increases the incidence of infertility three times, and obesity and PCOS together 10-15 times. Spontaneous abortions are more common, but they are euploid (123,130).

Obesity is associated with low responders, which are 70% more frequent in IVF (OR 1.70: CI 1.27-2.26) (132,139).

Due to a low number of quality oocytes and embryos, the possibility of cryostorage is reduced to 10% (128). It is interesting that weight loss does not lead to a significant increase of LBR (135,138), but regular exercise increases LBR even without weight loss (139). Brewer and Balen have reported that infertility (OR 2.7) significantly increases with obesity, anovulatory infertility even more so (OR 3.1) (110). In 1721 first-time procedures, Shah et al. (45) found that the treated patients were 20% overweight, 18% obese and 5% morbidly obese. Success of CPR was 50% lower in obese women, but BMI had no influence on SAB. In PCOS and obesity, there is a high level of intrafollicular leptin, leading to local disorders in the communication with the oocyte (48). There is an opinion that obesity causes reproductive disorders only with insulin resistance (14,31,70). Hospitalization due to infertility is more frequent with obesity (40.9% vs. 4.6%) and IVF treatment is 3-5 times more frequent (77). Elevated high sensitivity CRP levels as a sign of silent inflammation with obesity have been proven to lead to decreased fecundity and higher SAB rates (134). Broughton warns that obese women are subfertile due to a reduction of LH amplitudes and lipotoxicity of FFA, and ectopic lipid accumulation. Chronic inflammation leads to diminished fertility and euploid spontaneous abortions (124). The embryo rapidly progresses to blastocyst, and trophoectoderm exhibits lower cellularity. There are incoherent findings on the effect of obesity on lower fertilization rates and the reduction of embryo quality (110,132).

In our study, a high prevalence of obesity and PCOS was determined in younger infertile patients. PCOS incidence was different in 3 BMI patient groups – 23.4%, 28.6% and in obese women 33.9%. Such prevalence was expected about known geo-epidemiological data and exclusion criteria. About one-third of the patients in each BMI group had normal body weight. In 56 (30.8%) secondary infertile women there were 91 pregnancies, of which 50 were SAB (59.9% of all pregnancies). Significantly more abortions were recorded in the overweight and obese population, which is consistent with the findings in the literature. In secondary infertile women obesity and PCOS prolonged time-to-pregnancy to 2 years. The minimum prolonged waiting time to previous pregnancy was 9.5 months for lean women. According to the criteria WC > 88 cm and WHR > 0.85, central (abdominal) obesity was determined in 42% of PCOS patients and 28% of non PCOS patients. Obese women in these subgroups had visceral obesity in 76% and 63% of cases respectively. Central obesity was statistically

significantly more frequent in PCOS patients. Considering all parameters of central obesity (and WSR), its prevalence is 50% in women with a high BMI (≥ 30 kg/m^2). Oligo/anovulation was determined in 30% of non-PCOS patients and 67% of PCOS patients, and as many as 86% of obese PCOS patients.

Increasing BMI leads to a significant increase of central obesity and anovulation rates. Cycles are often irregular, especially in PCOS patients. Weight gain prolongs the cycle by 2.6 days in normal patients and by 6 days in PCOS patient. PCOS patients on average have 9 days longer cycles than control patients. FSH levels are higher in patients without PCOS, while LH is significantly higher in women with PCOS. Weight gain increases hyperandrogenemia (increase in T) and androgenic activity due to a significant decrease in SHBG levels. T, SHBG and FAI levels are significantly different between PCOS and non-PCOS patients. Androgen activity is not dependent on BMI in PCOS patients. Ovarian reserve markers are normal in non-PCOS patients and high in PCOS women. The determined values of AMH, AFC, OV do not show intergroup differences. No negative effect of obesity on ovarian reserve was found. Obesity in our patients does not diminish or increase the ovarian reserve. This finding is important for the programming and individualizing of infertility treatments. Specifically, 60 to 80% of infertile patients should be treated with ovarian stimulation (with or without IVF). According to our findings, opinions that obesity protects women from OHSS, frequently encountered in literature, are incorrect and may mislead the clinician. Also, no relationship was established between obesity (BMI) and prevalence of POR. Therefore, the findings from this study population show that young women with high BMI can expect the same treatment results and the same risks. It can be assumed that the reasons for infertility in this population include obesity, HA, anovulation, and several previously described pathophysiological events associated with adipokine and insulin disorders.

We believe this research can have multiple benefits for clinicians and reproductive subspecialists. In analysing and treating infertility, a close association between obesity and reduced fertility should be considered. Our findings may contribute to clinical decision-making about the individualization of treatment and care for the safety of this vulnerable population.

7. CONCLUSIONS

✓ In the investigated sample of infertile patients, 35% were normal-weight women and 65% were overweight and obese. Of that number, 28.6% exhibited clear results supporting polycystic ovary syndrome. The highest prevalence of abnormal BMI, 71%, was in the PCOS group. Only 5.5% of patients were morbidly obese (BMI > 40 kg/m^2).

✓ The participating women were of approximately the same age (32 years) and height, and based on the study design, they were assigned to 3 groups according to increasing BMI, i.e. weight. Primary or secondary infertility lasted on average for 4.2 years.

✓ Secondary infertility was found in 56 patients (30.8%), least frequently among normal-weight participants. Out of a total of 91 previous pregnancies, there were 41 births and 50 early spontaneous abortions (54.9% of all pregnancies). Most SABs (88%) occurred in the overweight and obese populations, more than one half in PCOS women with an increased BMI. These results are consistent with some findings in the literature which link SAB to obesity and PCOS, but mainly to IR. Around 20% of overweight and obese women had previously given birth.

✓ Around 80% of all participants had not previously intensively investigated infertility or received infertility treatments. This percentage reflects the perception and the attitude towards infertility in the investigated population. Passive behaviour in some participating women was justified because of secondary infertility.

✓ According to all anthropometric studies, statistically significant differences of WC and HC were found among patients with different BMI characteristics. On average obese women had abnormal WC and HC measurements, with the highest values recorded in obese PCOS patients. Following the recommended criteria, central (visceral) obesity (WC > 88 cm, WHR> 0.85, WSR> 0.50) was diagnosed in 68% of non-PCOS women and 90% of obese PCOS patients. Abdominal obesity was a reliable surrogate marker for insulin resistance because there is uniform evidence of a high correlation between central obesity and IR.

✓ In our study obese women also have menstrual cycle disorders. Menarche occurs early in obese patients (11.6 years), while in PCOS patients it was postponed until 13.9 years on average. Obese PCOS women have longer and heavier menstrual

bleeding (5.8 days vs. 4.3 days) than normal-weight patients in the same group. According to subjective impressions and anamnesis, heavy menstrual bleeding was reported by 23% of patients, most frequently by overweight and obese PCOS women.

- ✓ Obesity alone prolongs the menstrual cycle by 2.6 days on average. When obesity is accompanied by PCOS, the duration of the cycle in most overweight patients is almost 40 days on average (statistically significantly the longest). Even in normal-weight participants PCOS significantly prolongs the cycle. In overweight and obese PCOS women, oligomenorrhea dominates (mean cycle duration of 37.1 days) and more than 80% of participants in that group have a cycle > 35 days. Of all patients, 42% of obese patients have a menstrual cycle with the duration ≥ 40 days. Anovulatory cycles were recorded in 85,7% obese PCOS patients, and 39% obese woman.
- ✓ Obesity doubles the required time-to-pregnancy rate in patients with secondary infertility (19.9 vs. 9.5 months). It took obese PCOS patients 25.6 months to achieve their first pregnancy. In this small sample, the established subfecundity should be taken with caution because it can be confused with other causes of infertility (other than obesity and PCOS).
- ✓ FSH levels are the highest (7.53 IU/L) in normal-weight patients from the control group (non-PCOS), while obese PCOS patients have slightly lower FSH levels (4.67 IU/L). LH is the highest in PCOS patients with a normal BMI (9.98 IU/L). Further, obesity slightly decreases gonadotropin levels. In addition, FSH/LH inversion (LH > 2 times) was found in normal-weight PCOS women. These findings are consistent with the established pathophysiological mechanisms through which obesity and PCOS affect the regulation of the ovarian function and folliculogenesis. The negative correlation of AMH with FSH and the positive correlation of AMH with LH should be borne in mind. In PCOS patients, prolactin levels were significantly higher than in the control group (20.4 ng/ml vs. 15.2 ng/ml), but they are still in the normal range.
- ✓ Elevated basal FSH (> 10 IU/L) and E_2 (> 70 pg/ml) as surrogate markers of a low ovarian reserve and DOR were found in 15% of non-PCOS patients.
- ✓ In the control group (non-PCOS), it was established that AT produces more testosterone with weight gain (1.82 vs. 1.38 nmol/L). All PCOS patients, regardless of BMI, have statistically significantly higher T levels than non-PCOS patients (2.44 nmol/L vs. 1.66 nmol/L). Adipose tissue and obesity in PCOS patients

significantly reduce SHBG. Thus, significantly increased androgen activity was recorded in both study groups. HA is the fundamental characteristic of PCOS patients. FAI was used as a surrogate marker for free T. It was significantly higher and pathological in all PCOS patients, and it was the highest in the normal-weight group.

- ✓ Increased androgen activity and HA in the participating patients are associated with a high incidence of oligoanovulation in the whole sample. Among causes of infertility in our study population, HA and insulin resistance are highlighted as the main disorders accompanying obesity and PCOS.
- ✓ Biomarkers of ovarian reserve remain normal and unchanged with higher BMI in non-PCOS patients. AMH, AFC and ovarian volume are significantly higher in PCOS women. Positive or negative effects of BMI on ovarian reserve were not determined even in that study group. Since obesity does not lower AMH levels, a lower risk for OHSS cannot be expected, as claimed by some studies. Ovarian volume > 10 ml was found in 63% of PCOS patients. Anovulatory PCOS cycles are associated with the highest ovarian reserve (AMH 66.4 pmol/L) and the highest average BMI (32.4 kg/m^2).
- ✓ The characteristics of increasing obesity are accompanied by prolonged cycles, elevated androgen levels (probably IR as well) and anovulation. It seems that only morbid obesity slightly lowers OR markers.
- ✓ The analysis of the prevalence of low ovarian reserve in non-PCOS patients, i.e. the effect of obesity alone, has identified a similar incidence of poor ovarian response (POR) in women with normal and abnormal body weight. The prevalence of POR (low AMH, AFC, OV) was similar (14.1% vs. 13.2%). An elevated basal estradiol as a possible sign of POR was found in 10.4% of participants, equally in lean and obese patients.
- ✓ Obesity does not significantly affect ovarian reserve biomarkers, but it is undoubtedly associated with irregular cycles, anovulation and hyperandrogenism, especially if accompanied by PCOS, in which case a high incidence of insulin resistance should also be expected.
- ✓ These findings contribute to an understanding of reproductive risks associated with obesity and PCOS. We believe that they will contribute to clinical decision-making about ovulation stimulation and IVF treatment and support an individualized approach to long-term infertile female patients suffering from these two syndromes.

✓ According to above findings the minimal workup for obese infertile patients could be: BMI, WC, serum concentration of FSH, T, AMH and US analysis of uterus and ovaries. With these basic findings we can get enough information's needed for the personal approach.

8. SAŽETAK

Debljina je najčešća kronična bolest. U zapadnom svijetu i ovim prostorima učestalost prekomjerne tjelesne težine i debljine je 50-60%. Paralelno s porastom debljine posljednjih 30 godina raste i učestalost neplodnosti. Debljina je često udružena s sindromom policističnih jajnika (engl. Polycystic ovary syndrome- PCOS), pa udružena patofiziologija može biti razlog za niz metaboličkih i reprodukcijskih poremećaja. Debljina je povezana sa subfertilitetom i infertilitetom u žena i muškaraca. Uz debljinu je produljen time-to-pregnancy, tri puta je viši rizik neplodnosti i povišen je rizik za rane spontane pobačaje. Sa navedenim reprodukcijskim poremećajima posebno se povezuje centralna (abdominalna) debljina. Te povezanosti se temelje na disbalansu hormona, adipokina, hiperandrogenizmu i inzulinskoj rezistenciji. Također se često opisuje negativni učinak debljine na ovarijsku rezervu.

Cilj istraživanja:

U ovom istraživanju željeli smo utvrditi učinke debljine i centralne debljine na ovarijsku rezervu. Također smo nastojali utvrditi povezanosti debljine s promjenama reprodukcijskih hormona koje mogu biti razlog za subfertilitet. Nastojali smo odvojeno istražiti utjecaje debljine i PCOS-a na reprodukcijski kapacitet. Markere ovarijske rezerve (antimüllerov hormon AMH,antral follicularcount- AFC i volumen jajnika) istražili smo prema indeksu tjelesne mase (engl. body mass indeks- BMI) populacije na kojoj smo proveli istraživanje. Istražili smo kakav utjecaj imaju reprodukcijski hormoni i debljina na osobine menstrualnog ciklusa. Promjene ovarijske rezerve mogu bitno utjecati na odabir načina i uspjeha liječenja.

Pacijentice i metode:

U ovoj prospektnoj studiji na 182 žena grupiranih u 3 kategorije prema indeksu tjelesne mase (normalan, prekomjeran, debljina),a od toga je bilo 52 žene s PCOS. U tim BMI grupama bio je podjednak broj pacijentica- 64, 56, 62. Istražili smo i usporedili nalaze hormona i ovarijske rezerve u svakoj BMI grupi pacijentica. Također smo istražili utjecaj debljine i centralne debljine, te PCOS-a na karakteristike menstrualnog ciklusa i ovulacije. Istraživane pacijentice su bile primarno ili sekundarno neplodne prosječno 4 godine, i prosječne dobi 32 godine. Iz studije su isključene pacijentice s uterinim anomalijama, tubarnom neplodnošću, teškom endometriozom i tumorima uterusa ili

jajnika, endokrinim bolestima ili azoospermijom partnera. U istraživanim skupinama odredili smo i usporedili biomarkere ovarijske rezerve- antimüllerov hormon (AMH) antral follicular count (AFC) i volumen jajnika (engl. ovarian volume - OV). Također smo usporedili nalaze hormona- folikulostimulirajući (FSH), luteinizirajući (LH), prolaktin (PRL) i androgena.

Statistička analiza:

Statističke analize koristile su paket SPSS 17.0., a nalazi su prikazani kao srednja vrijednost i standardna devijacija. Parametričke podatke smo usporedili student-ovim *t* testom /ANOVA, a neparametrijske KrusckalWallis- ovim testom. Vrijednost statističke značajnosti bila je na razini 5% ($p \leq 0.05$).

Rezultati:

U istraživanom uzorku neplodnih pacijentica bilo je 35% normalnog BMI-a, a 65% žena bile su prekomjerne tjelesne težine ili debele (BMI ≥ 25 kg/m^2).

Od toga su 28,6% imale PCOS, a u toj grupi pacijentica prevalencija abnormalnog BMI-a bila je 71%. Od sekundarno neplodnih pacijentica (56 žena-30,8%), ukupno smo zabilježili 91 trudnoću. Od toga je bilo 54,9% spontanih pobačaja. Većina pobačaja (88%) bila je u populacijama abnormalnog BMI-a. Centralnu debljinu utvrdili smo u 68% debelih ispitanica, i u 90% debelih PCOS pacijentica. Abdominalnu debljinu povezali smo s inzulinskom rezistencijom jer je takva udruženost opće prihvaćena. Centralna debljina je značajno češća u debelih non PCOS pacijentica (68,3% vs. 15%) nego onih nižeg BMI-a.

U debelih žena ranije je nastupila menarha (11,6 godina), dok PCOS odgađa menarhu na 13,9 godina. Menstrualni ciklus produljuje debljina za 2,6 dana u prosjeku, a PCOS i debljina za više od 11 dana. Oligomenoreje (> 35 dana), iregularne cikluse, ili anovulacije imalo je više od 80% PCOS pacijentica i 40% pacijentica s debljinom.

Debljina i PCOS razlozi su za subfertilitet uz produljenje vremena za postizanje trudnoće- time-to-pregnancy (TTP) za 10 do 15 mjeseci. U debelih PCOS pacijentica TTP je bio statistički značajno dulji od onog u non PCOS grupi (25,6 mj vs. 19,9 mj).

Porastom BMI blago raste razina serumskog testosterona (1,82 vs. 1,38 nmol/L), koji je statistički značajno najviši u PCOS pacijentica (2,44 nmol/L vs. 1,66 nmol/L). Hiperandrogenizam je izražen u obje istraživane skupine pacijentica, značajno najviše u PCOS pacijentica (free androgen indeks – FAI 6,83 vs. 2,87).

U našem istraživanju utvrdili smo da debljina inhibira razinu cirkulacijskog sex hormone binding globulina (SHBG), a on je značajno najniži u PCOS pacijentica. To je dodatni doprinos androgenoj aktivnosti u obje skupine istraživanih žena, što pokazuju abnormalne, vrijednosti FAI. Razine FSH su niže u PCOS pacijentica (5,02 IU/L vs. 6,85 IU/L),a LH je značajno viši u PCOS-u u odnosu na non PCOS pacijentice (8,27 IU/L vs. 6,44 IU/L), Najizraženiju inverziju FSH/LH utvrdili smo u PCOS pacijentica normalne tjelesne težine. Serumski prolaktin (PRL) je viši u PCOS pacijentica (20,4 ng/ml vs. 15,22 ng/ml), a u 11,5% tih pacijentica je imalo blago nenormalnu razinu PRL.

Porastom debljine (BMI) u obje skupine istraživanih pacijentica nije zabilježena razlika u ovarijskoj rezervi unutar svake težinske skupine žena. Debljina ne snižava AMH, AFC i OV, dok PCOS statistički značajno povisuje ovarijsku rezervu (AMH 55,15 pmol/L vs. 14,76 pmol/L).

Antral follicular count i OV ne ovise o količini masnog tkiva i BMI-u pa se ne može ni očekivati da bi u ovoj populaciji debljina bila povezana s rezistencijom na gonadotropine u stimulaciji ovulacije. PCOS pacijentice imale su značajno viši AFC (24,3 vs. 9,02) i volumen jajnika (9,87 vs. 6,69 ml). Također nismo utvrdili povezanost povišenog BMI s niskom ovarijskom rezervom (engl. poor ovarian response/reserve- POR). Učestalost POR bila je podjednaka u sve 3 grupe ispitanica (8,1- 17,8%).

Anovulacijski ciklusi u PCOS pacijentica bili su povezani s najvišom ovarijskom rezervom (AMH 66,4 pmol/L) i najvišim prosječnim BMI (32,4 kg/m^2). Prevalencija poor ovarian reserve (POR) podjednaka je u pacijentica normalne tjelesne težine i debelih..

Zaključci:

Porastom debljine i u PCOS pacijentica produljuje se menstrualni ciklus i značajno su češće oligomenoreje i anovulacije. Utvrđeno je da su ti poremećaji, kao i hiperandrogenizam najizraženiji uz centralnu debljinu. Kako je već ranije dokazana povezanost navedenih pokazatelja s inzulinskom rezistencijom.Na temelju naših rezultata možemo istaknuti visoku povezanost debljine, centralne debljine i PCOS-a sa subfertilitetom i dugotrajnim infertilitetom. Nismo utvrdili pozitivan ili negativan efekt suvišnih masnog tkiva na markere ovarijske rezerve.

U ovom istraživanju našli smo visoku povezanost debljine i PCOS-a s produljenim TTP (19 do 25 mjeseci) i s trajanjem neplodnosti (primarne ili sekundarne) prosječno 4

godine. Iako su poznati učinci ta dva sindroma na plodnost, obrada i liječenje neplodnosti dakle kasni. Na subfertilitet istraživane populacije ukazuje i visoka učestalost ranih spontanih pobačaja u sekundarno neplodnih ispitanica (54,9% svih trudnoća).

Za dijagnozu centralne debljine našli smo da su najpouzdanije mjere WC > 88 cm i WSR > 0,50. Na WHR je snažan utjecaj imala izraženost visokog hip circumference u našoj populaciji debelih pacijentica. Centralna debljina je snažno povezana s anovulacijama, hiperandrogenizmom (HA) i oligomenorejom. Te smo poremećaje shvatili kao glavne razloge za neplodnost, a ne udruženost debljine s poremećenom ovarijskom rezervom.

Debljina minimalno, a PCOS snažno utječu na disbalans reprodukcijskih hormona (FSH, LH, E_2, i povišenu androgenu aktivnost (testosteron, SHBG, FAI). Ispitanice s anovulacijama, HA i PCOS-om imale su značajno više pokazatelje ovarijske rezerve.

Ovo istraživanje može unaprijediti postupke i obradu pacijentica koje su prekomjerne tjelesne težine ili debele. Minimum pretraga i racionalan pristup bio bi odrediti BMI, WC, testosteron, FSH i AMH, uz UZV analizu jajnika.

Ovi nalazi doprinos su poimanju reprodukcijskih rizika uz debljinu i PCOS, a svakako mogu pomoći u kliničkom odlučivanju za opseg obrade i individualizirani načina liječenja dugotrajno neplodnih pacijentica

Ključne riječi: Debljina, centralna debljina, sindrom policističnih jajnika, neplodnost, rezerva jajnika

9. SUMMARY- Abstract in English

“Impact of obesity in vvariar reserve”, Albert Lila, 2019

Obesity is the most frequent chronic disease. In the Western world and in these areas the incidence of overweight and obesity is 50–60%. In parallel with an increase in obesity over the past 30 years, there is also an increase in infertility incidence. Obesity is often associated with PCOS and the combined pathophysiology can cause a variety of metabolic and reproductive disorders. Obesity is also associated with subfertility and infertility in men and women..

Aim of the study:

The aim of this study was to identify the effects of obesity and central obesity on ovarian reserve. We also tried to identify the correlation of obesity with changes in reproductive hormones, which could be a cause of subfertility. We separately investigated the effects of obesity and PCOS on reproductive capacity. Ovarian reserve markers (AMH, AFC and ovarian volume) were investigated according to the body mass index of participating patients.

Methodology and methods:

In this prospective cross-sectional cohort study on 182 women grouped in 3 categories according to body mass index (normal weight, overweight and obese), 52 had PCOS. We determined and compared hormone and ovarian reserve results in each BMI patient group.

Statistical analysis:

Statistical analysis was performed by using the statistical package SPSS 17.0. Data is reported as mean ± standard deviation**.** Student’s *t* test/ANOVA was used to compare the means of parametric data and the Man-Whitney test / the Krusckal Wallis test to compare non-parametric data. The Chi square test were used to analyze categorical outcomes. For statistical significance the value of the factor alpha is ≤ 0.05.

Results:

In the investigated sample of infertile patients, 35% were normal-weight women, and 65% were overweight or obese. Of that number, 28.6% had PCOS, while the prevalence of abnormal BMI in that patient group was 71%. Central obesity was identified in 68% of obese patients and in 90% of obese PCOS patients.

Prolongs of the menstrual cycle, oligomenorrheae, anovulation, hyperandrogenism are more presente in obesity and PCOS patients than in obese respectivly control group.

Obesity does not lower AMH, AFC and OV levels.

Conclusion

Since the relationship between the indicators and insulin resistance has already been proven, our results emphasize a high correlation of obesity, central obesity and PCOS with subfertility and long-term infertility. Positive or negative effects of excessive adipose tissue on ovarian reserve markers were not identified.

Key words: Obesity, central obesity, polycystic ovary syndrome (PCOS), infertility, ovarian reserve

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11. CURRICULUM VITAE

Albert Lila born on July 22, 1967 in Gjakova Kosovo, where he completed primary school and gymnasium.

Education and professional training (graduation year):
MD: School of Medicine, University of Prishtina, 1993
Specialization in Gynecology and Obstetrics: Kosovo University Clinical Centre (KUCC), 2004

Postgraduate Training:
CME postgraduate course on Gynecology and Obstetrics, Infertility in Salzburg, Austria, 2010

Publications: 12 papers in peer reviewed journals, including International Journal of Gynecology, FIGO, Gynecology Endocrinology, Acta medica-BIH, Acta Clinica Croatica and 1 original paper in Acta Clinica Croatica

Author and co-author of comparative study of humane reproduction in Kosovo, Perinatal situation by years in Kosovo and manual for screening of best and cervical cancer.
Oral Presenations and poster presentation in national conferecnes and congresses, also in Europian-EBCOG and FIGO congress in 2009, 2012, 2015.

Languages: Albanian, English and Croatian.

More than 20 years working in Kosovo Occupational Health Institute as Chief of Gynecology Cabinet and leacturer at Faculty of Midwife and Nursing –University Fehmi Agani in Gjakova.

Married and have two sons.