ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

THE EFFECT OF ESTROUS CYCLE ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN RATS

MASTER OF SCIENCE

TALAT OĞULCAN ÖZARSLAN

BOLU, SEPTEMBER 2016

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APPROVAL OF THE THESIS

The Effect of Estrous Cycle on Ischemia Reperfusion Induced Arrhythmias in Rats submitted by **Talat Oğulcan ÖZARSLAN** in partial fulfillment of the requirements for the degree of Master of Science in **Department of Biology, Abant Izzet Baysal University** by,

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September 01, 2016

Prof. Dr. Duran KARAKAS ……

Director, **Graduate School of Natural and Applied Sciences**

To my family

Nihal ARAPTARLI, Özcan ÖZARSLAN
and Burakhan ÖZARSLAN

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Talat Oğulcan ÖZARSLAN

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ABSTRACT

THE EFFECT OF ESTROUS CYCLE ON ISCHEMIA REPERFUSION INDUCED ARRHYTHMIAS IN RATS
MSC THESIS
TALAT OĞULCAN ÖZARSLAN
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY
(SUPERVISOR: PROF. DR. ÖMER BOZDOĞAN)

BOLU, SEPTEMBER 2016

Arrhythmia caused by coronary artery occlusion is a major cause of sudden death both in women and men. Ventricular tachycardia and fibrillation are lethal arrhythmias that may cause sudden death due to ischemia-reperfusion injury. The incidences of these arrhythmias show differences in women and men. There is a cardioprotective difference between women and men. Women are more resistant to heart attack than men. However the cardioprotective effect disappears after menopause in women. Since estrogen diminishes after menopause in women, and it is well known that the administration of estrogen have a cardioprotective effect against ischemia-reperfusion induced arrhythmias, it is thought that the cardioprotective effect in pre-menopausal women is caused by estrogen levels. However, the estrogen levels are fluctuating during menstrual cycle in women and during the estrous cycle in rats. It was shown that there is a difference between phases of estrous cycle in the maintaining of cardiac function after injury. That is why in the present study the effect of estrous cycle on ischemia-reperfusion induced arrhythmias and the role of endogenous estrogen were researched. In the present study 6-7 months old 28 female Sprague Dawley rats were used. The phases of estrous cycle were determined by the observation of the cells present in the vaginal smears. Four groups were created according to the phases of estrous cycle; proestrus, estrus, metestrus, diestrus. Left anterior descending coronary artery was occluded to create ischemia for 6 minutes, and the artery was released for reperfusion for 6 minutes. Electrocardiogram and blood pressure are recorded

during ischemia and reperfusion. Blood samples were collected after reperfusion, and serum 17ß-Estradiol levels were determined. After occlusion, blood pressures decreased and ST segment elevation or depression were observed in all animals. There were no differences in arrhythmia score, the incidences of arrhythmias, and arrhythmic periods among the groups. Higher blood pressures were observed in proestrus group, however the difference was not statistically significant. Lower heart rates were observed in estrus group during 6 minutes of ischemia and 6 minutes of reperfusion ($P<0.05$). 17ß-Estradiol level of proestrus was found higher than the other groups ($P<0.05$). In conclusion, in the present study it was found that the estrous cycle and endogenous estrogen do not have an effect on ischemia-reperfusion induced arrhythmias, however they may have an effect on the heart rate and blood pressure during ischemia-reperfusion.

KEYWORDS: Ischemia, Reperfusion, Arrhythmia, Estrous Cycle, 17ß-Estradiol

ÖZET

SIÇANLARDA ÖSTRUS DÖNEMİNİN İSKEMİ REPERFÜZYON İLE UYARILAN ARİTMİLER ÜZERİNE ETKİSİ
YÜKSEK LİSANS TEZİ
TALAT OĞULCAN ÖZARSLAN
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI
(TEZ DANIŞMANI: PROF. DR. ÖMER BOZDOĞAN)

BOLU, EYLÜL - 2016

Koroner arter tıkanıklığından kaynaklanan aritmiler hem kadınlarda hem de erkeklerde başlıca ani ölüm sebeplerinden birisidir. İskemi-reperfüzyon ile uyarılan ventriküler taşikardi ve fibrilasyon ani ölüme sebep olan aritmilerdir. Bu aritmilerin görülme oranları kadınlar ve erkekler arasında farklılıklar göstermektedir. Kadınlar kalp krizine karşı erkeklerden daha dayanıklıdır. Ancak kadınlarda görülen kardiyak koruyucu etki menopozdan sonra ortadan kalkmaktadır. Menopoz sonrası östrojen seviyesindeki azalma ve östrojenin iskemi-reperfüzyon aritmilerine karşı koruyucu etkisinin olduğunun bilinmesi yüzünden, kadınlardaki menopoz öncesi dönemde görülen kardiyak koruyucu etkinin östrojen seviyesinden kaynaklandığını düşünülmektedir. Ancak kadınlarda menstrüel siklus ve hayvanlarda östrus siklusunda östrojen seviyesinde dalgalanmalar olmaktadır. Östrus siklusunun fazları arasında, hasar sonrası kardiyak fonksiyonlarda farklılıklar olduğu gösterilmiştir. Bu nedenle bu çalışmada östrus siklusunun iskemi-reperfüzyon ile uyarılan aritmiler üzerine etkisi ve endojen östrojenin rolü araştırılmıştır. Bu çalışmada 6-7 aylık 28 adet Sprague Dawley cinsi sıçan kullanılmıştır. Östrus siklusunun fazları vajinal smeardaki hücreler gözlemlenerek belirlenmiştir. Hayvanların östrus fazlarına göre 4 grup oluşturuldu; proöstrus, östrus, metöstrus ve diöstrus. Sol koroner arterin ön inen dalı (LAD) tıkanarak 6 dakikalık iskemi ve arterin açılmasıyla 6 dakikalık reperfüzyon oluşturuldu. Elektrokardiyogram ve kan basıncı iskemi-

reperfüzyon süresince kaydedildi. Reperfüzyonun ardından kan örnekleri alındı ve serum 17ß-Estradiol seviyeleri belirlendi. Tıkanmanın ardından tüm hayvanlarda kan basınçları azaldı ve ST segmentinde yükselme ya da çökme görüldü. Aritmi skorunda, aritmi oranlarında ve aritmi periyodunda gruplar arasında bir farklılık gözlemlenmedi. Proöstrus grubunda yüksek kan basıncı gözlemlendi ancak bu farklılık istatistiksel olarak anlamlı bulunmadı. 6 dakikalık iskemi ve 6 dakikalık reperfüzyon süresince, östrus grubunun dakika kalp atım sayısının diğer gruplardan daha düşük olduğu gözlemlendi ($P<0.05$). 17ß-Estradiol seviyesi, proöstrus grubunda diğer gruplara göre daha yüksek bulundu ($P<0.05$). Sonuç olarak, bu çalışmada östrus siklusunun ve endojen östrojenin iskemi-reperfüzyon ile uyarılan aritmiler üzerine bir etkisi olmadığı gösterilmiştir, ancak östrus siklusu ve endojen östrojen iskemi-reperfüzyon süresince dakika kalp atım sayısı ve kan basıncını etkileyebilir.

ANAHTAR KELİMELER: İskemi, Reperfüzyon, Aritmi, Östrus Siklusu, 17ß-Estradiol

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LIST OF ABBREVIATIONS AND SYMBOLS

eNOS	**:** Endothelial nitric oxide synthase
NO	**:** Nitric oxide
ANS	**:** Autonomic nervous system
SA	**:** Sinoatrial
AV	**:** Atrioventricular
Na^+	**:** Sodium
K^+	**:** Potassium
Ca^{2+}	**:** Calcium
g Ca^{2+}	**:** Calcium conductance
g K^+	**:** Potasssium conductance
ECG	**:** Eloectrocardiogram
AF	**:** Atrial fibrillation
VPC	**:** Ventricular premature complex
VT	**:** Ventricular tachycardia
VF	**:** Ventricular fibrillation
DAD	**:** Delayed afterdepolarization
EAD	**:** Early afterdepolarization
ATP	**:** Adenosine triphosphate
Na^+-K^+- ATPase	**:** Sodium-potassium adenosine triphosphatase
Ca^{2+} ATPase	**:** Calcium adenosine triphosphatase
K_{ATP}	**:** ATP dependent potassium channel
TNFα	**:** Tumor necrosis factor alpha
ICAM-1	**:** Intercellular adhesion molecule-1
PRO	**:** Proestrus
EST	**:** Estrus
MET	**:** Metestrus
DIE	**:** Diestrus
FSH	**:** Follicle stimulationg hormone
LH	**:** Luteinizing hormone
GnRH	**:** Gonadotropin releasing hormone
HR	**:** Heart rate
BP	**:** Systolic blood pressure

LPS	**:** Lipopolysaccharide
iNOS	**:** Inducible nitric oxide synthase
COX-2	**:** Cyclooxygenase-2
NaCl	**:** Sodium cloride
LAD	**:** Left anterior descending coronary artery
AAR	**:** Area at risk of infarction
RPM	**:** Revolutions per minute
Isc1	**:** 1 minutes after ischemia
Isc3	**:** 3 minutes after ischemia
Isc5	**:** 5 minutes after ischemia
Rep1	**:** 1 minutes after reperfusion
Rep3	**:** 3 minutes after reperfusion
Rep5	**:** 5 minutes after reperfusion
Rep_Total	**:** Total durations of arrhythmia during reperfusion
Isc_Total	**:** Total durations of arrhtyhmia during ischemia
TOTAL	**:** Total durations of arrhythmia during ischemia and reperfusion

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1. INTRODUCTION

Cardiovascular diseases followed by irreversible and lethal arrhythmias are known as the major cause of death in both women and men. Recently, the 30% of death worldwide is caused by cardiovascular diseases (Gaziano, 2005). Ventricular arrhythmias caused by myocardial ischemia, infarction or reperfusion causes many of these deaths. A study shows that the 84% percent of sudden death in patients with cardiovascular disease is caused by ventricular tachyarrhythmias and 16% is caused by bradyarrhythmias (Luna et al., 1989). As a result of myocardial ischemia, ionic and metabolic alterations occur in the myocardium. These alterations affect resting membrane and action potentials, which cause the arrhythmias.

Previous studies have shown that there is a difference in cardioprotective response to ischemia between women and man. Men are more sensitive to heart diseases then women are. There are both clinical and animal studies that support this aspect (Connor, 1997; Gonca et al., 2004). This cardioprotective effect in women diminishes after menopause, however it can be re-obtained with hormone replacement therapy (Nabulsi et al., 1993). 17ß-Estradiol is known to have a cardioprotective effect against cardiac injury (Hale et al., 1996; Boot et al., 2003; Terrell et al., 2007). Besides the 17ß-Estradiol, raloxifene (a selective estrogen receptor modulator) also have a cardioprotective effect against ischemia-reperfusion induced myocardial injury (Ogita et al., 2002). Whereas, estrogen receptor selective antagonists decrease the cardioprotective effect of estrogen (Booth et al., 2005). Since, the administration of estrogen is known to have a cardioprotective effect and the level of estrogen decreases after menopause, it is believed that the cardioprotective effect in women is caused by estrogen production and its metabolism. Contrarily, a study was done with 384,878 patients have shown that the rates of death during hospitalization is higher in younger women, but not older women, then men of the same age (Vaccarino et al., 1999).

During the estrous cycle, plasma levels of gonadotropins and ovarian steroids fluctuate. Plasma levels of 17ß-Estradiol, luteinizing hormone, follicle stimulating hormone, prolactin and progesterone reach a peak level in proestrus and decrease during estrus, metestrus and diestrus. During proestrus levels of eNOS and NO in

median eminence increases. It has been suggested that this increase may be caused by the increase in the Estradiol level. The cardioprotective feature of nitric oxide has been shown (Jones and Bolli, 2006) (Heusch et al., 2008). The increase in plasma Estradiol level in proestrus may increase plasma NO levels and trigger a cardioprotective effect in proestrus animals.

Earlier studies have shown that during estrous (Lewis and Newman, 1984) and menstrual cycle (Moran et al., 2000) hemodynamic parameters may change. The change in the hemodynamic parameters and the endogenous estrogen may affect the arrhythmias during the estrous cycle. A study was done in rats to show the effect of estrous cycle on ischemia-reperfusion induced arrhythmias (Fraiser et al., 2013). In the study it was shown that there are no differences in basal heart rate and incidences of ventricular fibrillation among the phases of estrous cycle. However, there are no information about pre-ischemic and post-ischemic arterial blood pressure, heart rate, onset and ending times of arrhythmias, and incidences of arrhythmias except ventricular fibrillation.

It is known that there are differences in cardioprotective response between men and women, however it is not clear whether if there is a difference in the cardioprotective response between the phases of estrous or not. Since the female animals are used in the ischemia-reperfusion studies without determining the estrous phase, it is important to know the difference between the phases. This study was planned to clear this issue.

1.1 Physiology and Anatomy of the Heart

1.1.1 Anatomy of the Heart

The heart is an organ that functions as a pump. It is located in thorax, between lung leaflets and localized just above the diaphragm. There is a covering membrane around the heart, the pericardium (Iaizzo, 2005). There are 2 main functions of heart; first one is collecting blood from peripheral tissues and pumping it to the lungs, and second one is collecting blood from lungs and pumping it to peripheral tissues (Iaizzo, 2005).

The heart is composed of cardiac muscle cells. Cardiac muscle, myocardium, is composed of cells that are branched and having one nucleus. At the end of each cell there are intercalated discs that allow action potential to pass the adjacent cell quickly (Scanlon and Sanders, 2007). There are 3 kinds of cardiac muscle cells found in the different parts of heart, these cardiac muscle cells or fibers are; atrial muscle, ventricular muscle, and specialized excitatory and conductive muscle fibers (Guyton and Hall, 2006). Atrial and ventricular muscle cells are found in the atria and ventricles. Main function of these type of muscle cells are to contract and pump the blood into the next chamber or the aorta (Guyton and Hall, 2006). The main function of specialized excitatory and conductive muscle cells is to generate action potential or to conduct the action potential (Guyton and Hall, 2006).

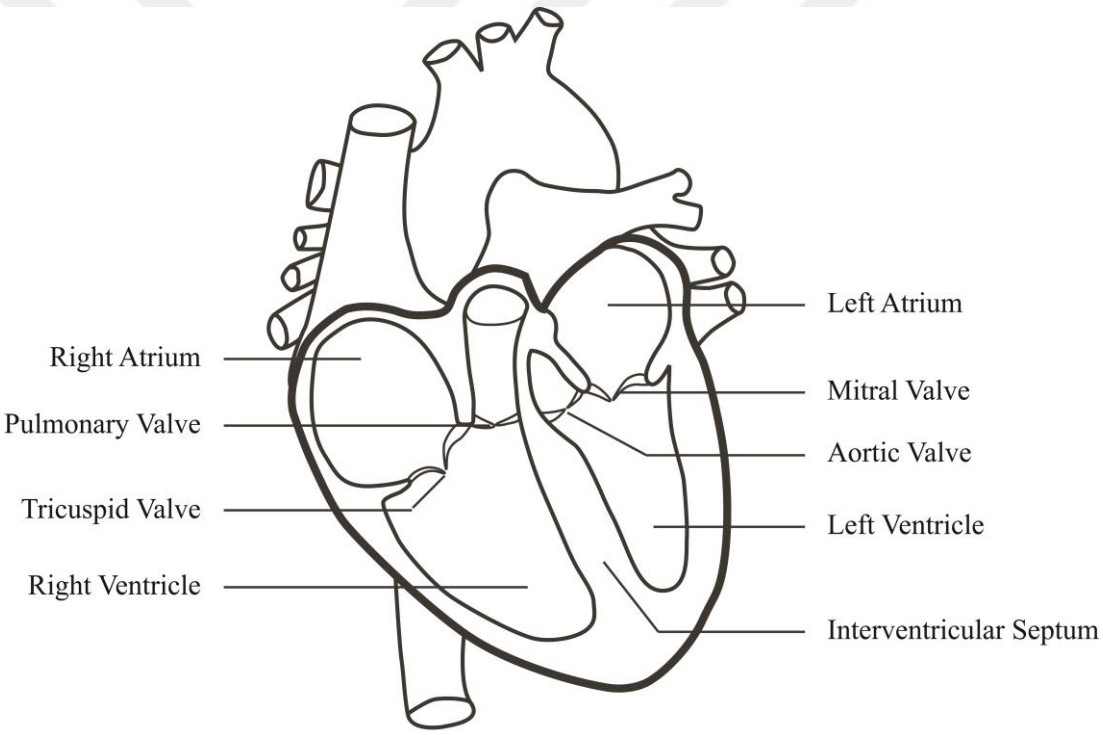

Figure 1.1. Chambers of heart.

The heart is divided by septum into 2 halves; left and right halves. Each half is divided into 2 chambers; the upper chambers are called as atria and the lower ones are called as ventricles (Figure 1.1). Between atria and ventricles, there are atrioventricular valves; the tricuspid valve is found between right atrium and ventricle, and the mitral valve is found between left atrium and ventricle. These valves allow the blood to pass through itself and enter the ventricle from the atrium.

There are 2 more valves that are located between ventricles and vessel; pulmonary valve, and aortic valve. Pulmonary valve connects right ventricle and pulmonary artery to each other, whereas aortic valve connects left ventricle and aorta.

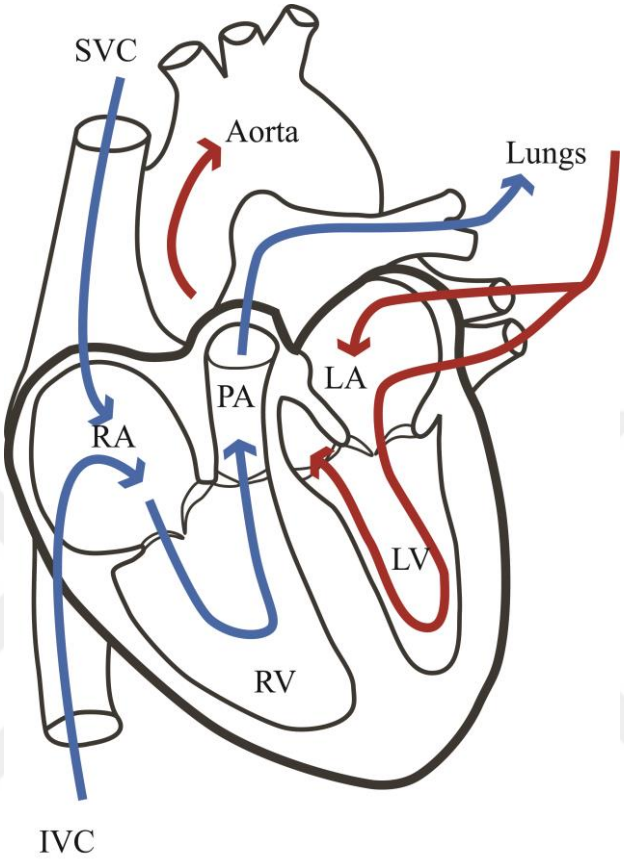

Figure 1.2. Blood flow within the heart. RA: right atrium, SVC: superior vena cava, IVC: inferior vena cava, RV: right ventricle, PA: pulmonary artery, LA: left atrium, LV: left ventricle.

Right atrium is larger in volume than the left atrium, however the walls of left atrium are thicker than the walls of right atrium (Standring et al., 2008). It collects deoxygenated blood from the systemic circulation via superior and inferior vena cava, then pump the blood into the right ventricle through the tricuspid valve. Right ventricle pumps the blood that was filled inside by right atrium into pulmonary artery through pulmonary valve. The blood that oxygenated in the lungs return to the left atrium via pulmonary veins and left atria pump the blood into the left ventricle through mitral valve (Figure 1.2). The left ventricle is longer and narrower than the right ventricle, also its wall is thicker than right ventricle's (Standring et al., 2008). The left ventricle is a powerful pump that provides blood flow to the arteries which

have a high blood pressure. After the left ventricle is filled with blood, it contracts powerfully and pump the blood through aortic valve into the aorta and then to the systemic circulation.

1.1.2 Electrophysiology of the Heart

The heart is an autorhythmic organ. The nerves which innervates the heart control the function of the heart. They may decrease or increase the rate and contractility of heart. Even though the heart is highly innervated with autonomic nerves, it produces its own rhythmic action potentials without the help of nervous system. It continues to generate rhythmic action potentials even if all the nerves going into the heart are blocked or cut. The autonomic cells of the heart generates action potentials which travel along the heart and generate regular contractions. Any disruption in the generation of the action potential or in the conduction system causes arrhythmias.

1.1.2.1 Neural Innervation of Heart

The heart itself is innervated by both sympathetic and parasympathetic (vagal) branches of autonomic nervous system (ANS). Sympathetic nerves are distributed to the all parts of the heart, and they have a great influence on ventricular muscle. On the other hand, parasympathetic nerves are distributed mainly to sinoatrial (SA) and atrioventricular (AV) node, and they have a lesser influence on ventricular and atrial muscles of the heart. Stimulation of sympathetic nerves causes the release of norepinephrine from the sympathetic nerve endings, whereas stimulation of parasympathetic nerves causes the release of acetylcholine from the parasympathetic nerve endings. Sympathetic stimulation increases both heart rate and force of contraction, whereas parasympathetic stimulation decreases both heart rate and contraction. However, since parasympathetic nerves innervates atrial muscles more than ventricular muscles, parasympathetic stimulation causes a great decrease in heart rate rather than muscle contraction.

Both of the sympathetic and parasympathetic stimulation of heart are controlled by medulla oblongata which is located in the brain stem.

1.1.2.2 Impulse Generation and Conduction

Under normal conditions the primary pacemaker site of the heart is the SA node which has the highest firing rate in the heart. Secondary pacemakers may take control of the heart rate if the SA node is unable to control the heart rate. Secondary pacemakers are AV node, bundle of His and the Purkinje fibers. Since the firing rates of secondary pacemakers are lower than SA node, heart rate decreases. These kind of rhythm disturbances in heart are caused by ectopic focus or abnormal conduction.

1.1.2.2.1 Autonomic Activity

There are 3 intrinsic rhythmical excitation center in the heart; SA node, AV node and Purkinje fibers (Figure 1.3). When there is no external stimuli, AV node spontaneously discharges at a rate of 40-60 times per minute, and Purkinje fibers discharges at a rate of 15-40 times per minute. However, SA node can discharge at a rate of 70 to 80 times per minute. The discharge rate of SA node is faster than other regions, which allows it to control the heart rate. When SA node discharges, the impulses are conducted to both AV node and Purkinje fibers, creating a depolarization wave throughout the heart. When the discharging of SA node is complete, it discharges again before AV node and Purkinje fibers reach their own threshold level. Thus SA node controls the impulse generation of heart and it is the natural pacemaker of the heart, since its rate of rhythmical discharge is faster than AV node and Purkinje fibers.

In order to create an impulse that stimulates the heart, an action potential must be produced. When the membrane potential reach to threshold level, action potential is generated. There are 2 kinds of action potentials present in the heart; nonpacemaker action potential, and pacemaker action potential.

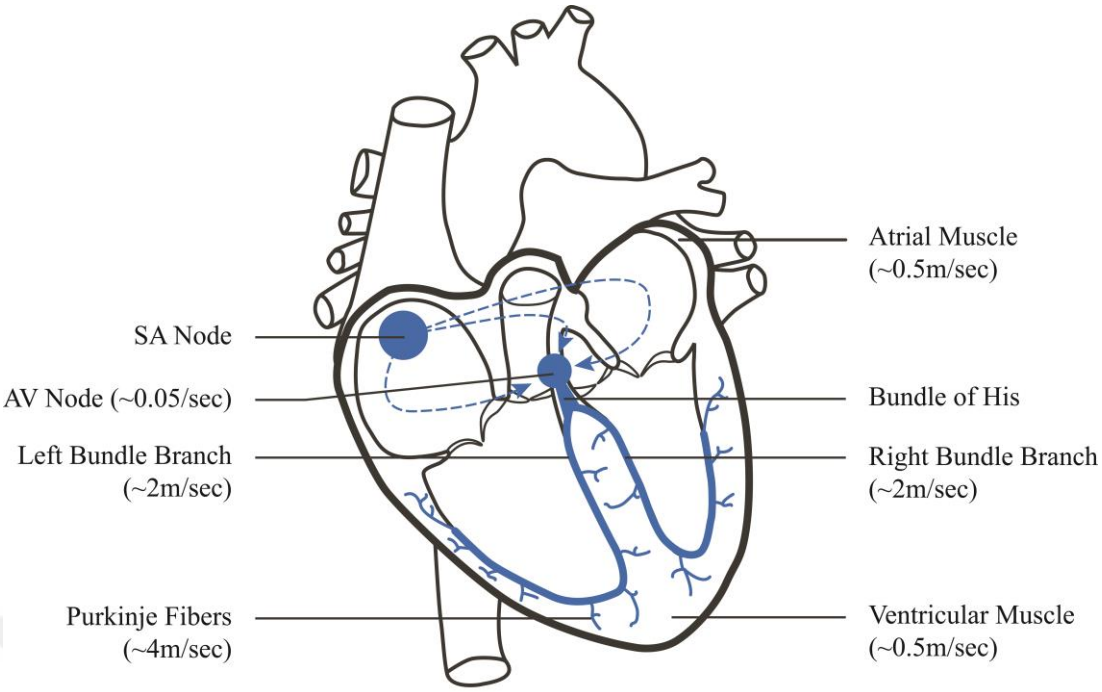

Figure 1.3. Conduction pathway within the heart. SA: sinoatrial, AV: atrioventricular. Conduction velocities are beneath the parts, and in parentheses. Purkinje fibers have the highest conduction velocity, whereas AV node has the lowest.

1.1.2.2.2 Nonpacemaker Action Potential

The cells which do not possess the pacemaker activity are named as nonpacemaker cells. Atrial and ventricular myocardial cells are nonpacemaker cells. Nonpacemaker cells have true resting membrane potential.

At resting state, nonpacemaker myocardial cell's membrane potential is about -90mV. When a nonpacemaker cell depolarizes from -90mV to threshold voltage (about -70mV) action potential is produced and it is conducted to adjacent cells.

There are 5 phases in nonpacemaker action potential. Phase 0, depolarization, is based on the rapid increase in Na^+ conductance. Phase 1, initial repolarization, is based on the opening of the special K^+ channels. Phase 2, plateau, is based on an increase in slow inward Ca^{2+} channels. Phase 3, repolarization, is based on an increase in K^+ conductance and a decrease in Ca^{2+} conductance. Phase 4, resting phase, at this phase the cell has a resting membrane potential, and this potential is

caused by a high amount of K^+ inside of the cell and low amount outside (Figure 1.4).

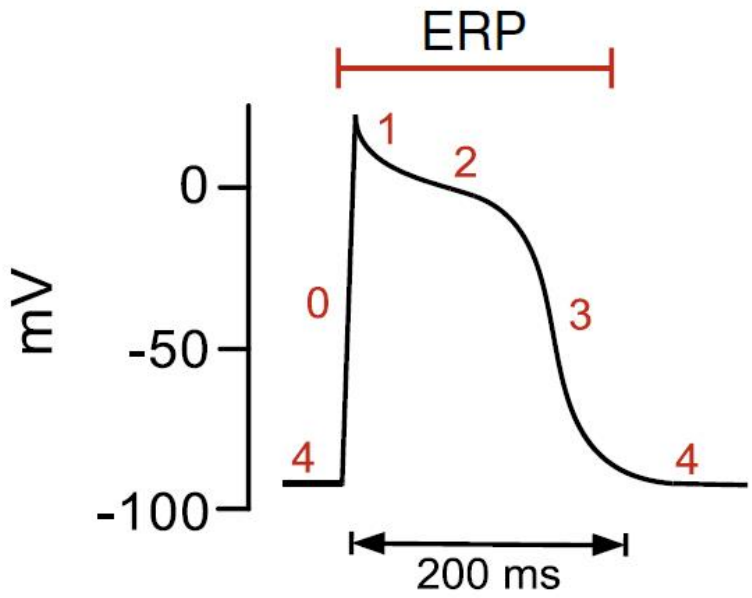

Figure 1.4. Non-pacemaker action potential. 0: depolarization, 1: initial repolarization, 2: plateau phase, 3: repolarization, 4: resting phase. ERP: effective refractory period. (Klabunde, 2011).

The cell is in refractory period during phase 0, 1, 2, and a part of phase 3. At this refractory period the cell is unexcitable, and cannot produce a new action potential. Thus, this period is called as effective refractory period.

1.1.2.2.3 Pacemaker Action Potential

SA node is the primary pacemaker region in the heart (Bozdoğan, 2004). There are true pacemaker fibers, subsidiary pacemaker cells and cells with little or no prepotential in the SA node (Lu et al., 1965). The cells which are located in the lower parts of SA node have a slower and more irregular firing rate than the cells in the upper parts of the SA node (Lange, 1965). The myocytes within the SA node do not have a true resting potential. The generated action potentials are regular and spontaneous. Unlike other myocytes in the heart (e.g. myocytes in Purkinje fibers) which use fast Na^+ channels, the myocytes in the SA node use relatively slow L-type

Ca^{2+} channels for depolarizing the cell. The usage of slow Ca^{2+} currents causes decreased rate of depolarization. Since the depolarization phase is slow, the action potentials which are generated via the usage of Ca^{2+} currents are called as slow response action potentials.

There are 3 phases of the SA nodal action potential; phase 0 is the depolarization phase, phase 3 is the repolarization phase, and phase 4 is the spontaneous depolarization phase (Figure 1.5).

Phase 0 occurs due to the opening of the voltage-operated L-type Ca^{2+} channels when the membrane is depolarized to threshold voltage (-40 mV). As the L-type Ca^{2+} channels open, Ca^{2+} conductance (gCa^{2+}) increases and Ca^{2+} ions begin to flow inward the cell. Since the flow of Ca^{2+} ions is slow, the slope of depolarization phase (phase 0) is not as steep as it is in non-pacemaker cell action potential.

At the end of depolarization, voltage-operated K^+ channels open, and the L-type Ca^{2+} channels close (phase 3). K^+ conductance (gK^+) increases while gCa^{2+} decreases. K^+ ions start to flow outwards the cell, thereby decreasing the membrane potential to about -65 mV and repolarize the cell.

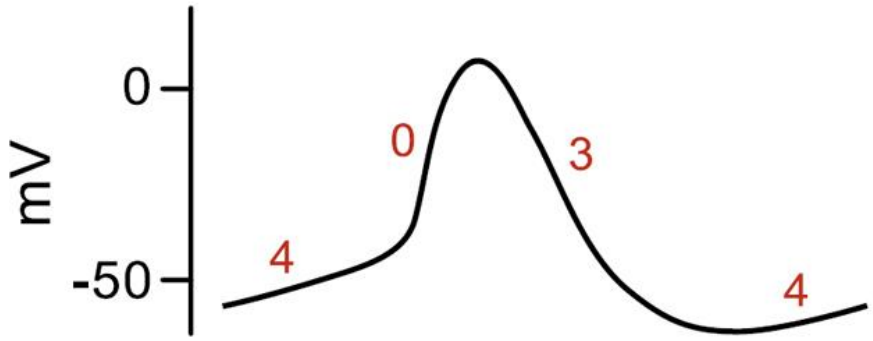

Figure 1.5. SA nodal action potential. 0: depolarization, 3: repolarization, 4: spontaneous depolarization (Klabunde, 2011).

There are 3 ions; K^+, Ca^{2+}, Na^+ that contributes to the spontaneous depolarization (phase 4) of the pacemaker potential. At the end of repolarization and the beginning of phase 4, the K+ channels begin to close thereby decreasing gK^+. Decreased gK^+ initiates slow inward flow of Na^+, and increased gCa^{2+} caused by T-

type Ca^{2+} channels contributes to the depolarization. Unlike L-type Ca^{2+} channels, T-type Ca^{2+} channels open when the membrane potential is about -50 mV. When the membrane potential begin to reach the threshold level L-type Ca^{2+} channels open and initiate the phase 0 (Klabunde, 2011).

1.1.2.3 The Electrocardiogram and Sinus Rhythm

The electrocardiogram (ECG) is a recording which is used to diagnose rhythm disturbances, changes in electrical conduction and myocardial ischemia and infarction. To record an ECG trace, electrodes are placed at specific locations on the body. The electrodes measure electrical currents which are generated as a result of depolarization and repolarization of the cardiac cells. In the ECG there are repeating waves which represent depolarization and repolarization of the atria and ventricles.

As the SA node initiate the first depolarization wave, the wave spreads throughout the atria. This depolarization wave is measured by the electrodes and it is called the P wave. Therefore the P wave becomes the first wave of the ECG. The P wave, in a normal heart, lasts 0.08 to 0.1 seconds. Since the atrial repolarization and the ventricular depolarization occur at the same time, there is no wave seen in the ECG representing the repolarization of atria. Because repolarization wave of atria is masked by the ventricular depolarization wave. There is an isoelectric period between P wave and QRS complex. After the atria depolarize, the atrial cells stay depolarized for a time, and the generated impulse travels through the AV node, in which the conduction velocity is decreased greatly. The isoelectric period represents the time needed for the impulse to travel within the AV node. The period from the beginning of P wave to the beginning of the QRS complex is called P-R interval, which normally lasts 0.12 to 0.2 seconds in human (Figure 1.6).

After P wave, the QRS complex is seen in the ECG. The QRS complex represents the ventricular depolarization. Ventricular depolarization is a rapid process and the duration of it ranges from 0.06 to 0.1 seconds in human heart.

There is another isoelectric period after the QRS complex. This period represents the time in which the cells stay depolarized, and it is called ST segment.

Elevated or depressed ST segment indicates nonuniform membrane potentials in the ventricular cells (Klabunde, 2011), and they are used to diagnose ventricular ischemia.

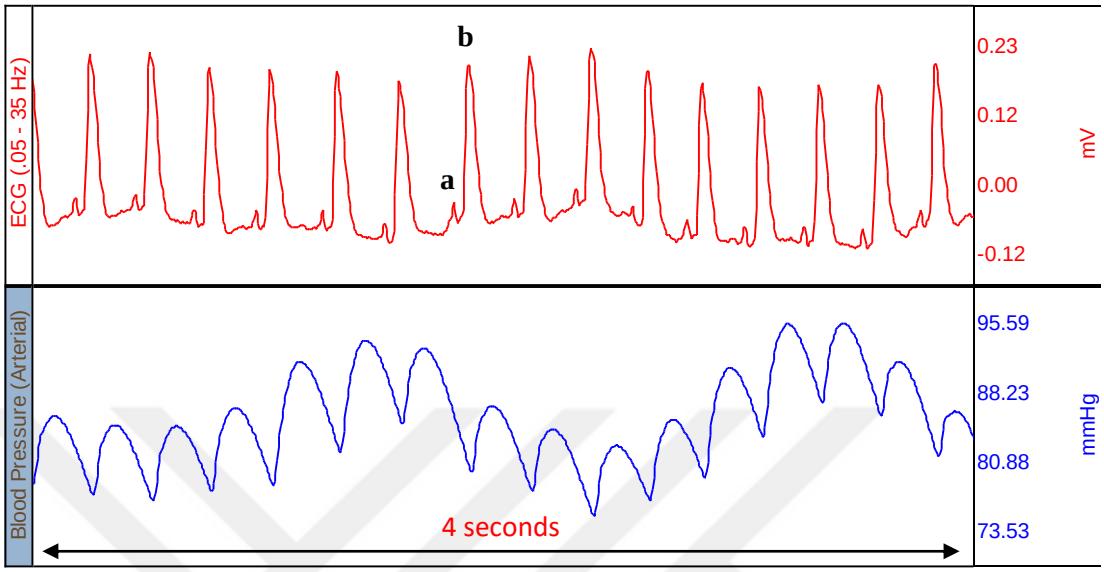

Figure 1.6. Sinus rhythm, heart rate: 255 beats/min, arterial blood pressure: 95,63 mmHg. **a.** P wave**. b.** QRS complex. T wave is hardly visible or hidden in rat ECG.

After ventricular depolarization, ventricular repolarization occurs and it is represented by the T wave in the ECG. T wave lasts longer than the QRS complex, indicating the depolarization is faster than repolarization.

The time period from the beginning of the QRS complex to the end of T wave is called as Q-T interval that represents the duration of ventricular action potential. Q-T interval can range from 0.2 to 0.4 seconds, and it depends on heart rate. Q-T interval is shorter at high heart rates and longer at lower heart rates.

In a normal ECG, each P wave is followed by a QRS complex, indicating that the ventricular depolarization is triggered by atrial depolarization. Under these conditions the SA node is controlling the cardiac rhythm, therefore the heart is in sinus rhythm. In sinus rhythm heart rate is between 60 and 100 beats in human.

1.1.3 Cardiac Circulation

There are four chambers of heart that are filled with blood. Heart muscle itself cannot be supplied with the blood in the chambers. Since the walls of ventricles and atria are too thick, heart cannot supply itself by diffusion (Iaizzo, 2005). To supply myocytes with nutrients and oxygen, a special circulation (cardiac circulation) is needed. Cardiac circulation is achieved by three main components of the coronary system; arteries, capillaries, and veins. There are two main coronary arteries; left and right coronary arteries. Both of these arteries exit the ascending aorta just above the aortic valve. These arteries branch into arterioles, then the arterioles branch into capillaries. Capillaries join together and form venules, which carry the deoxygenated blood to the right atrium.

1.1.3.1 Coronary Circulation

Oxygenated blood in the left ventricle is pumped out into the aorta. Blood only enters to the coronary arteries during the diastole, since the arteries collapse during the systole because of the high extravascular pressure produced by the contracting myocytes. After entering the arteries, the blood is distributed through the heart by the capillaries, and supplies all of the myocytes in the four chambers of heart. In the capillaries oxygenated blood becomes deoxygenated after supplying the myocytes with oxygen and nutrients. Deoxygenated blood then enter the venules and return back to the right atrium. The blood is mixed with the systemic deoxygenated blood in the right atrium and enter to the right ventricle, in which it will be pumped to the lungs to be oxygenated.

The right coronary artery travels along the right anterior atrioventricular groove and reaches to the posterior surface of the heart. When it reaches here, it becomes the posterior descending artery which carries blood to the apex of the left ventricle. The right coronary artery have many braches that supply the sinus node and atrioventricular node.

The left main coronary artery, after exiting the ascending aorta, splits in to left circumflex artery and left anterior descending artery (Figure 1.7). The left

circumflex artery supplies blood the posterior surface of the left ventricle and left atrium. In some cases, a branch of the left circumflex artery runs toward the superior vena cava, so that it can supply the sinus node. The left anterior descending artery supplies anterior and apical portions of the left ventricle, and most portions of the ventricular septum.

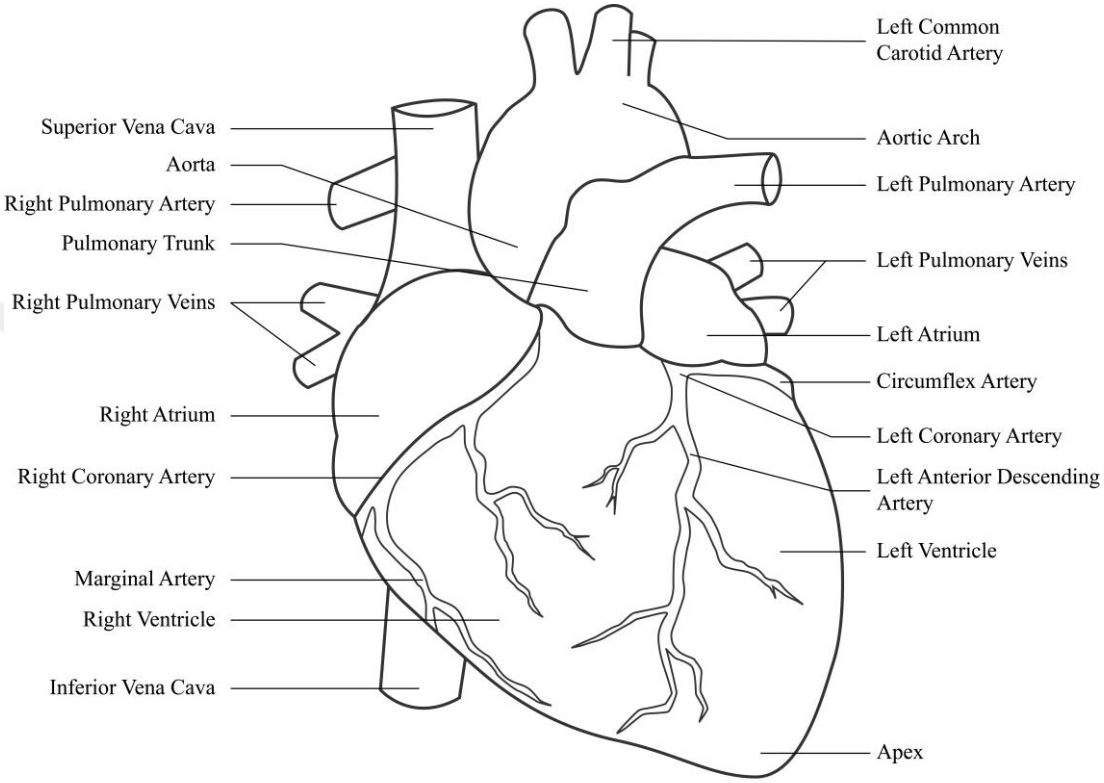

Figure 1.7. Coronary arteries in human heart.

Coronary arterioles branches into numerous cardiac capillaries which provides the transportation of the nutrients and metabolic end products between vessels and the myocytes. During the diastole the coronary artery pressure is higher than the venous pressure, thus the blood flow through the capillaries occurs passively.

Coronary veins are localized very close to the coronary arteries, and they form a network between the ends of capillaries and right atrium. The largest vessel in the coronary venous system is the coronary sinus which carries deoxygenated blood from left ventricle to the right atrium. There are several veins that empties into the coronary sinus; the great cardiac vein, the oblique vein of the Marshall, the lateral

and posterior veins of the left ventricle, the middle cardiac vein and the small cardiac vein. Besides the coronary sinus, there are many tiny veins which drains deoxygenated blood from right ventricular and atrial myocardium, and empties directly into these chambers. These veins are called as Thebesian veins.

1.1.3.2 Coronary Circulation of Rat Heart

In rat heart the right coronary artery originates from the aorta and it supplies the right ventricle, most of the interventricular septum, and a small part of the surface of left ventricle. The left artery originates from the aorta and it gives off branches; left descending anterior coronary artery and circumflex artery (Figure 1.8). The circumflex artery supplies the left margin and the posterior surface of the left ventricle. Left descending anterior coronary artery supplies a portion of the anterior region of the interventricular septum. In rats, right coronary artery contributes to the blood supply to the interventricular septum more than the right coronary artery in human. The rat's coronary circulation is similar to the coronary circulation of human with a small difference (Ahmed et al., 1978).

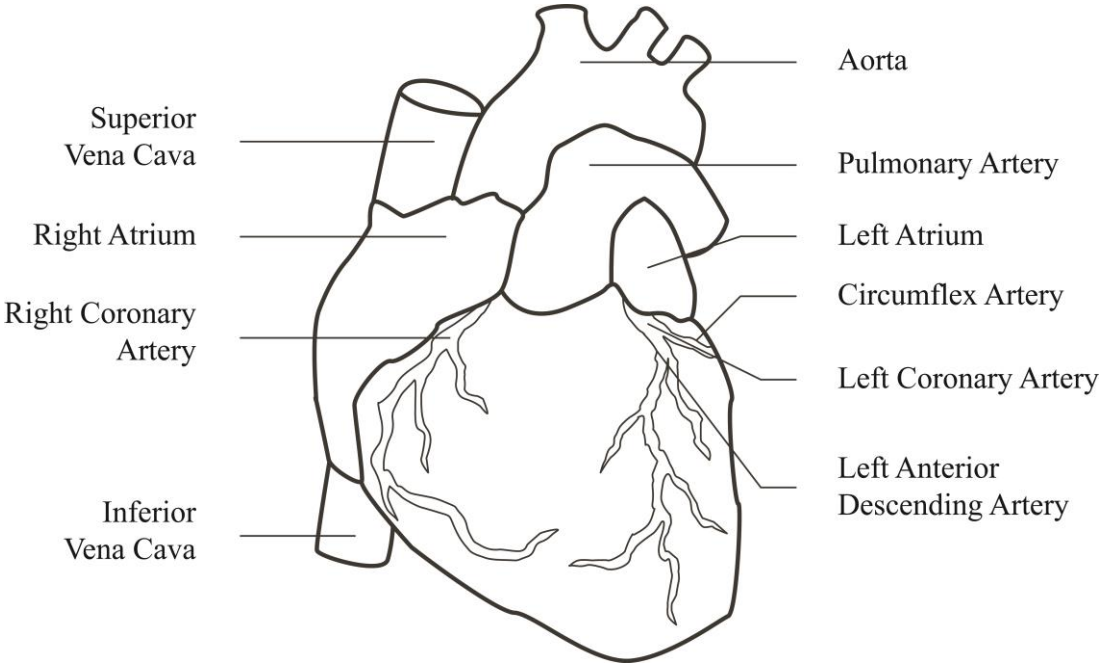

Figure 1.8. Coronary arteries in rat heart.

1.2 Cardiac Arrhythmias

Spontaneous impulse generation from SA node initiates and maintains the normal autonomic rhythm of the heart. As SA node initiates the rhythm, contractions are synchronic and the intervals between the beats are equal. This type of synchronic contraction is termed as sinus rhythm. Arrhythmias are abnormal rhythms or heart rates, and they override the sinus rhythm. There are mainly three mechanism of the generation of arrhythmias; abnormal automaticity, triggered automaticity and re-entrant circuits.

1.2.1 Types of Arrhythmias

1.2.1.1 Sinus Tachycardia and Bradycardia

Autonomic nerve activity modulates the intrinsic firing rate of SA node. Both sympathetic and vagal activations affect the firing rate. In the presence of sinus rhythm, sympathetic and vagal tones are in an equilibrium. Increased sympathetic activity increases sympathetic tone, therefore causing increased heart rates. An abnormal increase in sympathetic tone causes sinus tachycardia, in which each P wave is still being followed by 1 QRS complex, and intervals between beats are equal, however heart rate exceeds normal limits, 100 beats/min in human (Klabunde, 2011) and more than 450 beats/minute in rats (Figure 1.9).

Increased parasympathetic activity increases vagal tone, therefore causing decreased heart rate. Abnormally increased vagal tone causes sinus bradycardia, in which, likewise sinus tachycardia, each P wave is still being followed by 1 QRS complex, and intervals between beats are equal, however, this time, heart rate decreases below normal limits, 60 beats/min in human, (Klabunde, 2011) and less than 200 beats/minute in rats (Figure 1.10) (Gonca, 2015).

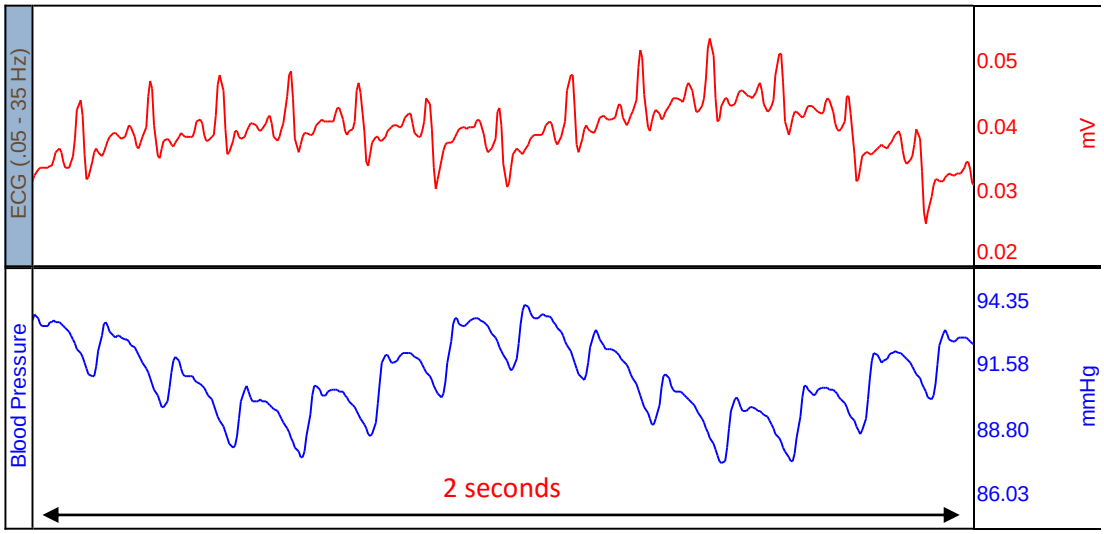

Figure 1.9. Sinus tachycardia. Heart rate: 413,79 beats/min, arterial blood pressure (systolic): 93,37 mmHg.

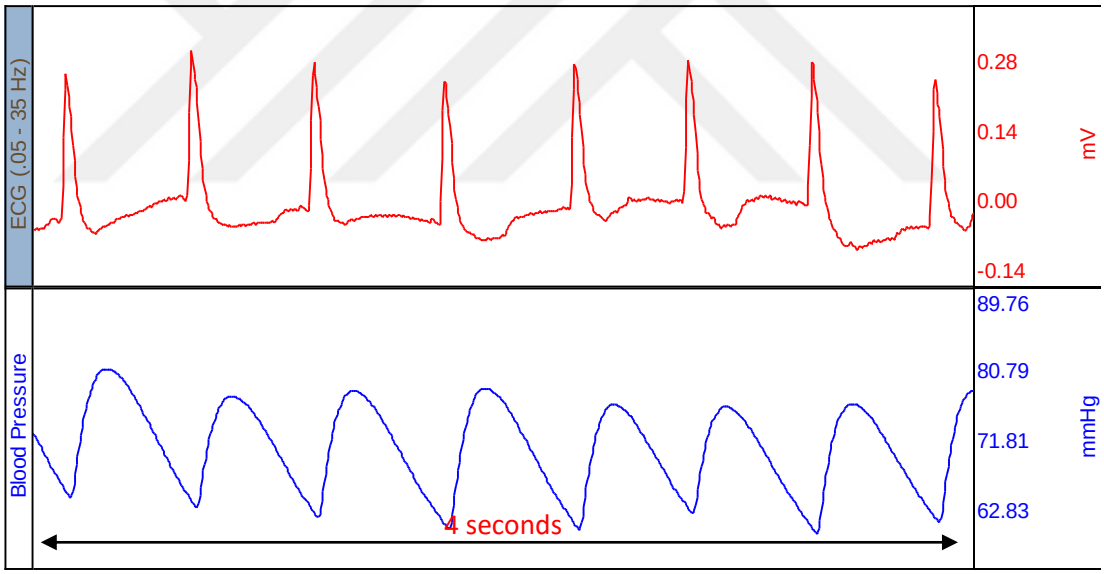

Figure 1.10. Sinus bradycardia - heart rate: 109,09 beats/min, arterial blood pressure: 78,72 mmHg.

1.2.1.2 Atrial Arrhythmias

1.2.1.2.1 Atrial Flutter

Atrial flutter is atrial tachycardia, in which there is little or no isoelectric interval between flutter waves (Yang, 2005). Unlike the sinus rhythm, the frequency of P waves and QRS complexes show differences in atrial flutter. Atrial rate increases, and not all of the impulses are conducted to ventricles. Therefore more than one P wave occurs before QRS complex.

1.2.1.2.2 Atrial Fibrillation

Atrial fibrillation (AF) is related to dilatation and fibrosis of the atria (Hauer, 2005). In atrial fibrillation, there are many weak P waves which have opposite polarity. Therefore P waves either cannot be seen or many waves with low voltages are seen in the ECG. In atrial fibrillation, many impulses are generated in the atria, however the atria and the ventricles are separated from each other by fibrous tissue, therefore impulses cannot enter and induce ventricles. In AF many impulses arrive to the A-V node, however the A-V node does not let impulses to pass before 0.35 seconds. The impulses which passed the A-V, induce the ventricles, however the intervals between each QRS complexes are not equal. In AF heart rate is usually between 125 and 150 beats/min (Guyton and Hall, 2006).

1.2.1.3 Ventricular Arrhythmias

1.2.1.3.1 Ventricular Premature Contractions and Bigeminy

Premature contractions are contractions that occur before they were expected, they are also called as extrasystole. Premature contractions caused mostly by ectopic foci, which can be caused by ischemia. Ventricular premature contractions (VPC) are caused by ectopic foci in the ventricles and they usually occur immediately after

QRS complexes in the ECG (Figure 1.11). Since VPCs occur before the blood fills in the ventricle, blood pressure decreases during the VPCs. Bigeminy is a type of VPC, in which there is a VPC after every normal sinus beat. Bigeminal VPCs have the same shape and timing (Figure 1.12).

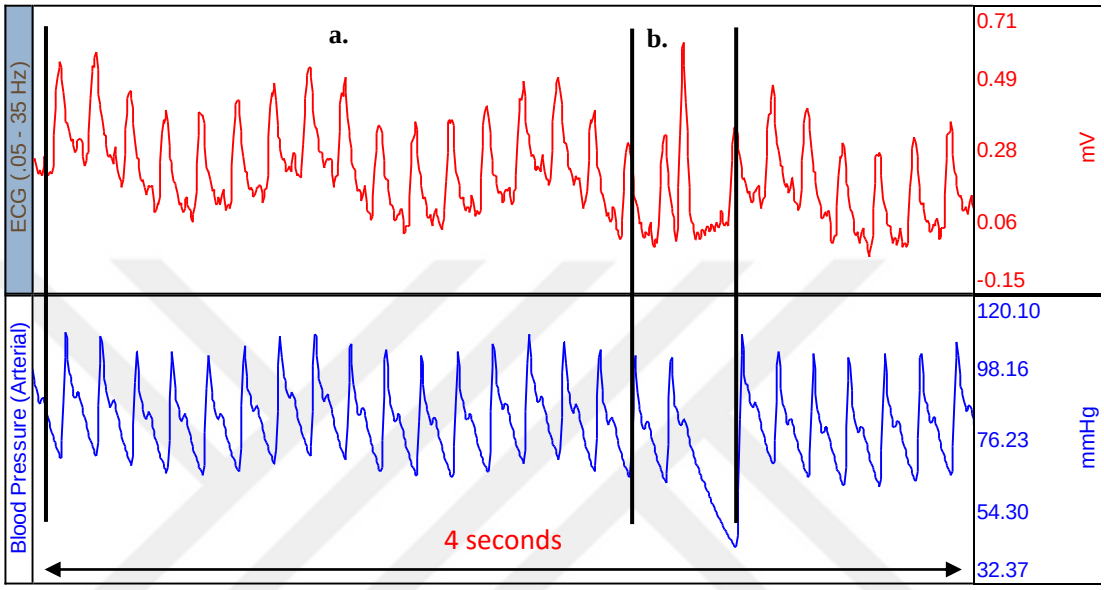

Figure 1.11. ECG of ventricular premature contraction. **a.** sinus rhythm – heart rate: 387,10 beats/min, arterial blood pressure (systolic): 99,12 mmHg. **b.** VPC – heart rate: 800 beats/min, arterial blood pressure (systolic): 81,79 mmHg.

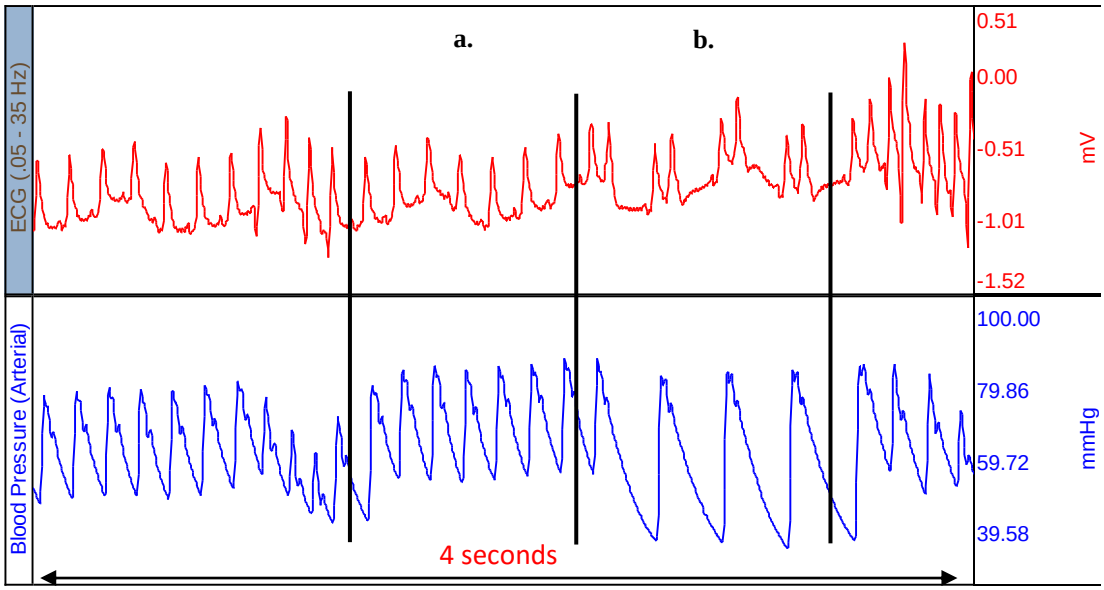

Figure 1.12. ECG of bigeminal ventricular premature contractions. **a.** sinus rhythm – heart rate: 285 beats/min, arterial blood pressure (systolic): 86,67 mmHg. **b.** bigeminal VPC – heart rate: 545 beats/min, arterial blood pressure (systolic): 84,35 mmHg.

1.2.1.3.2 Ventricular Tachycardia

Ventricular tachycardia (VT) is a term which entitles the condition, in which the frequency of QRS complexes is greater than the frequency of P waves (Klabunde, 2011). In VT heart rate is more than 100 beats/min in human and more than 450 beats in rats (Figure 1.13). The blood pressure decreases greatly during VT. VT are caused by abnormal impulse conduction or ectopic foci in the ventricles. VT can be lethal if not intervened, since it can lead to ventricular fibrillation.

"VT is a sequence of a minimum 4 consecutive ventricular complexes" as defined in The Lambeth Conventions (Curtis, 2013).

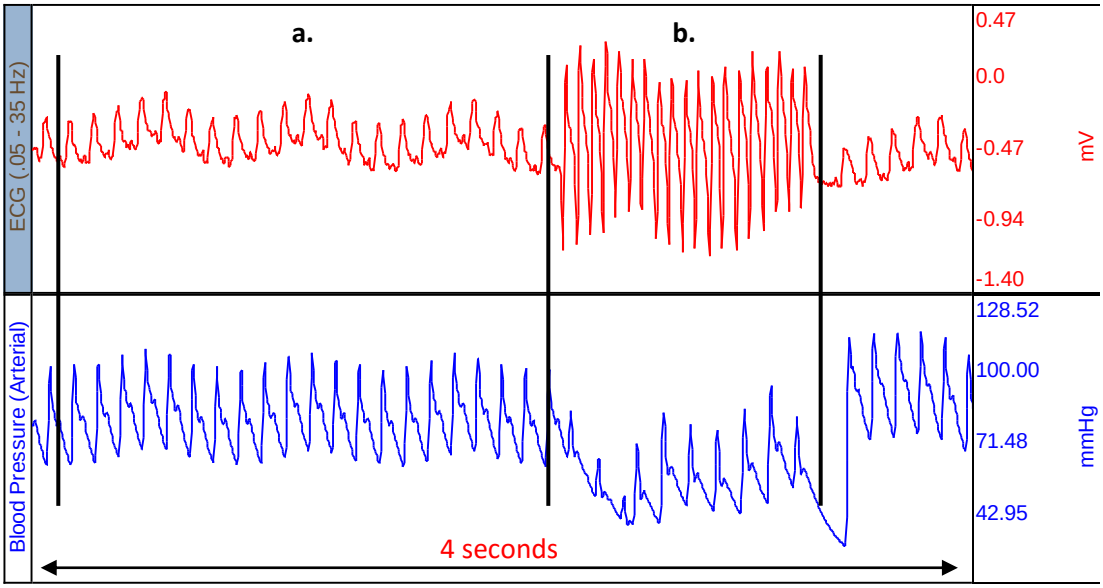

Figure 1.13. Ventricular tachycardia. **a.** sinus rhythm – heart rate: 375 beats/min, arterial blood pressure (systolic): 100,34 mmHg. **b.** ventricular tachycardia – heart rate: 600 beats/min, arterial blood pressure (systolic): 81,79 mmHg.

1.2.1.3.3 Ventricular Fibrillation

Ventricular fibrillation (VF) is rapid, irregular, asynchronous contraction of the ventricle (Figure 1.14) (Fuster, 2011). VF is mainly caused by ischemia or electrical shock to the heart. Since there is no coordinated contraction of the muscles, blood cannot be pumped out of the heart and blood circulation is ceased, therefore, if not intervened, it leads patient to death.

"VF is a sequence of a minimum 4 consecutive ventricular complexes without intervening diastolic pauses" as defined in The Lambeth Conventions (Curtis, 2013).

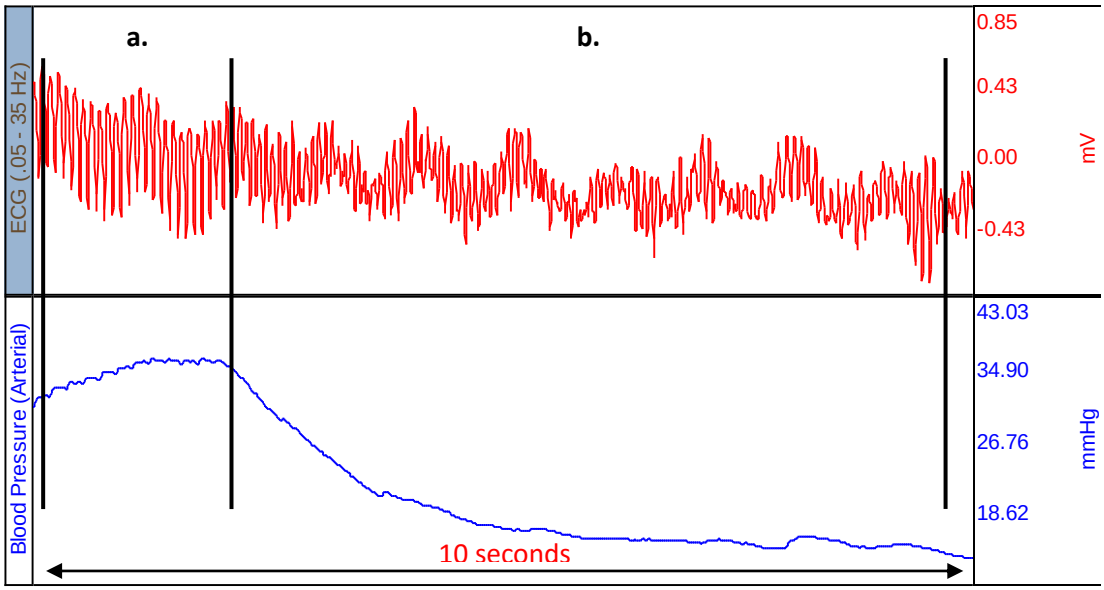

Figure 1.14. Ventricular tachycardia and ventricular fibrillation. **a.** ventricular tachycardia – heart rate: 705 beats/min, arterial blood pressure (systolic): 38,94 mmHg. **b.** ventricular fibrillation – heart rate: 705-1333 beats/min, arterial blood pressure (systolic): 13,31 mmHg.

1.2.2 Underlying Mechanisms of Arrhythmias

1.2.2.1 Abnormal Automaticity

In the normal heart, subsidiary pacemaker cells; AV node and His-Purkinje system, are suppressed by SA node, since SA node has the fastest firing rate. Suppression of SA node by any of the subsidiary cells or the enhancement of these cells cause abnormal automaticity which may lead arrhythmias. Abnormal automaticity is associated with ischemia and reperfusion, and may induce premature beats, atrial tachycardia and ventricular tachycardia (Gaztanaga, 2011).

1.2.2.2 Triggered Activity

Afterdepolarization is an oscillation which occurs during (early afterdepolarization) or after (delayed afterdepolarization) preceding action potential (Figure 1.15). When the oscillation is large enough to reach threshold level, it

initiates a new action potential. The impulse initiation caused by afterdepolarization is defined as triggered activity. Triggered activity may cause ectopic beats, and if it occurs repeatedly, may cause a sustained arrhythmia (Aaronson, 1999).

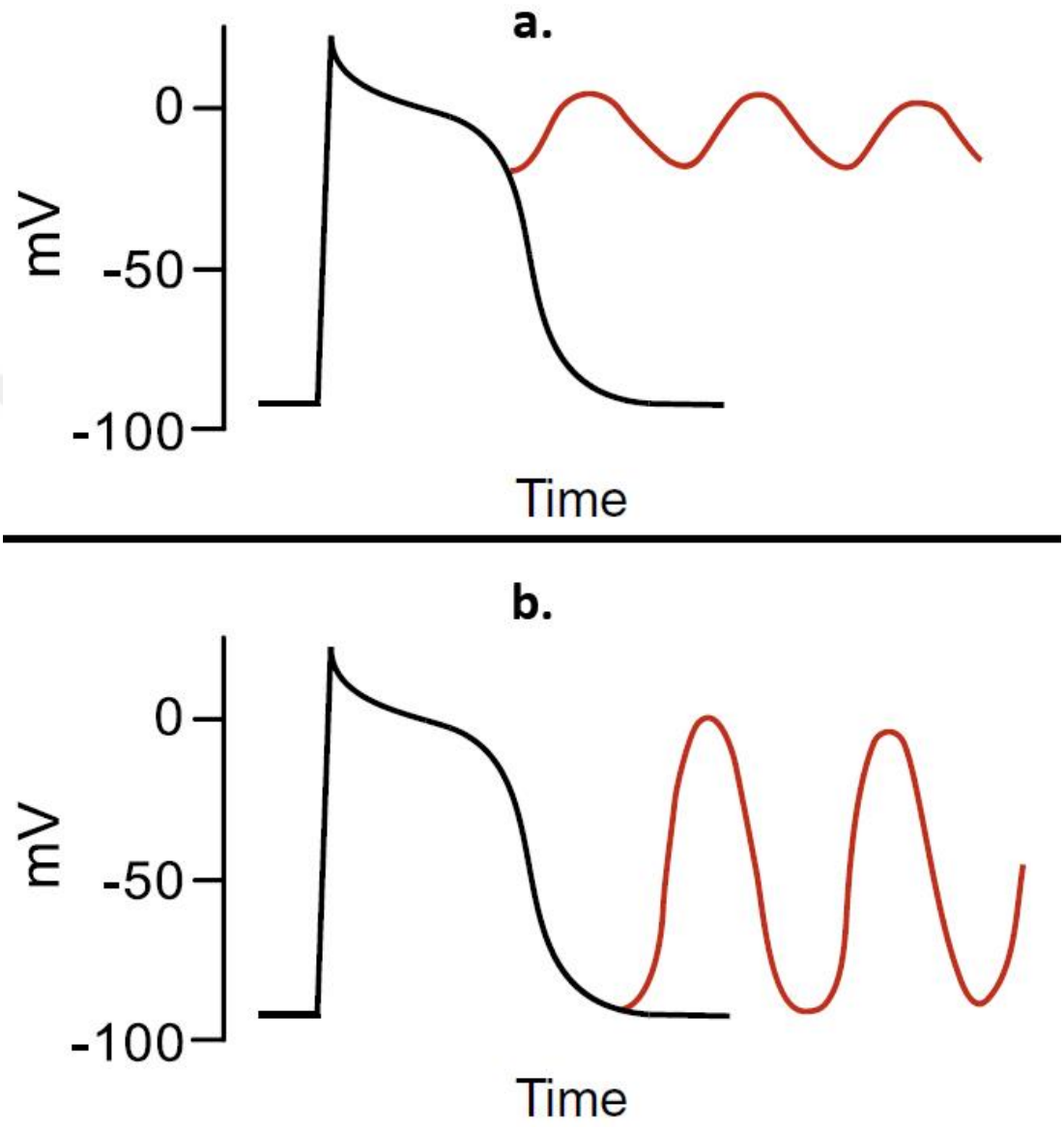

Figure 1.15. Afterdepolarizations. **a.** early afterdepolarizations. **b.** delayed afterdepolarizations (Klabunde, 2011).

Delayed afterdepolarizations (DAD) are caused by membrane depolarization in response to calcium release from sarcoplasmic reticulum (Marban et al., 1986). The events that are responsible for the DADs are; 1) cell becomes overloaded with calcium, 2) the sarcoplasmic reticulum takes up calcium after action potential, to a point where it sustains an overload-induced calcium release, 3) increase in calcium

opens Ca-dependent nonspecific cation channel or activates Na/Ca exchange system, causing Na entry. If the Na current depolarize the membrane potential to a threshold level, the action potential is triggered (Fozzard, 1992).

The events responsible for early afterdepolarizations (EADs) are; 1) depolarization activating the Ca^{2+} current, 2) repolarization bringing the voltage into the zone for recovery of L-type Ca^{2+} channels, 3) relaxation which reflects uptake of Ca^{2+} by the sarcoplasmic reticulum, decreasing the Ca^{2+} into the zone for recovery of the channels. If the range is within the availability of the Ca^{2+} current overlap, current will be reactivated and generate a depolarization (Fozzard, 1992).

1.2.2.3 Re-entrant Circuit

In the normal cardiac cycle, an impulse which is initiated by the SA node travels through the ventricles and after exciting all the muscle cells, it dies out. Since all of the muscle cells are at the refractory period the signal cannot excite any of the cells. However, under certain circumstances, for example during ischemia, an impulse can be delayed or conduction of impulses slow down in the ischemic region of the heart and the impulse origination from ischemic region re-excites the adjacent cells. Re-entry occurs when the re-excitement occurs in a circular region. Re-entrant circuit occurs only in the presence of; myocardial tissue with different electrophysiological properties, an area of block (anatomical or functional), a unidirectional conduction block (Figure 1.16).

1.2.3 Myocardial Ischemia and Infarction

Myocardial ischemia occurs when the myocardium cannot be supplied with the sufficient amount of oxygen. The cause of myocardial ischemia is mainly coronary artery occlusion. Under severe instances, such as permanent occlusion of coronary artery, ischemia may result in myocardial infarction and heart failure. The mechanism of myocardial infarction includes; decrease in oxygen level, loss of mitochondrial ATP production, anaerobic glycolysis which results in accumulation of hydrogen ions, lactate production that results in acidosis, increase in cytosolic and

mitochondrial calcium that results in the activation of proteases, and finally disruption of the sarcolemmal membrane and cell death (Fuster, 2011).

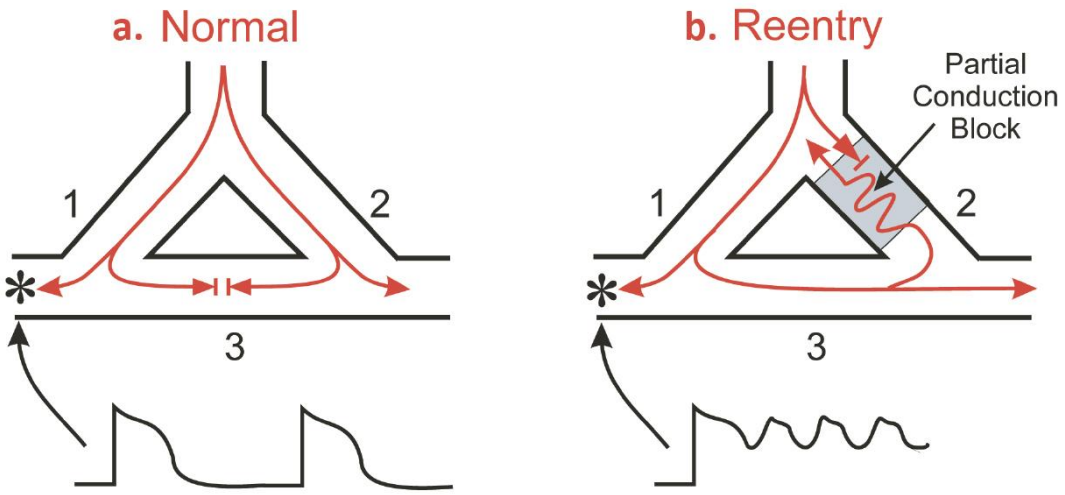

Figure 1.16. Re-entrant circuit. **a.** Normal conduction of action potential. Impulse travels along 2 branches (1 and 2) and they cancel out each other in branch 3. When the impulse reaches the marked location (*), it transmits 1 action potential each time a signal is conducted. b. Reentry. The partial conduction block in the branch 2 blocks orthograde impulses, however it transmits retrograde impulses. The impulse which traveled through branch 1 is transmitted slowly through the partial conduction block. If the impulse can reach an excitable tissue additional impulses can be conducted. Additional impulses increases action potential frequency (Klabunde, 2011).

1.2.3.1 Cellular Changes in Myocytes as a Result of Ischemia and Reperfusion

Basal ATP levels in myocytes are about 20-25 μmol/g tissue. Under ischemic conditions this level may drop by 70-80%. After reperfusion, ATP levels recover, however cannot reach preischemic levels (Murphy and Steenbergen, 2008).

Basal pH in heart is between 7.05-7.20, and after 15 minutes of ischemia pH decreases rapidly to about 6.0. This decrease is caused by anaerobic glycolysis and the protons released from ATP breakdown (Smith et al., 1993). After reperfusion, pH turns to normal level by the helps of proton extrusion mechanisms (e.g. Na+-H+ exchange) (Murphy and Steenbergen, 2008).

In a normal myocyte, the intracellular level K^+ is higher than the extracellular level, whereas the intracellular levels of Ca^{2+}, Na^+, and H^+ are lower than the extracellular levels of those. Energy consuming processes are needed to keep Ca^{2+}, Na^+, and H^+ out of the cell, and K^+ in the cell. As the ATP production ceases, these processes fail (Allen and Orchard, 1987).

Na^+-K^+-ATPase maintains higher intracellular K^+ and lower extracellular K^+ in basal conditions. After ischemia intracellular K^+ decreases, while extracellular K^+ increase. In ischemic conditions Na^+-K^+-ATPase are inhibited (Fuller et al., 2003). It has been suggested that the major cause of K^+ loss is the extrusion of accumulated anions from the myocardium (Gaspardone et al., 1986). Three mechanisms, which could be responsible for the increase in extracellular K^+, have been suggested; inhibition of the Na^+-K^+-ATPase (Wilde et al., 1988), activation of an inward Na^+ (Shickumar et al., 1997), and activation of ATP-sensitive K+ current (Wilde and Aksnes, 1995). The increase in extracellular K^+ contributes to the production of arrhythmias (Harris et al., 1954).

In basal conditions, Na^+-K^+-ATPase extrudes Na^+ out of the cell and keep the extracellular Na^+ level higher. In ischemic conditions, the activity of Na^+-K^+-ATPase is inhibited. As a result intracellular Na^+ level increases about fivefold (Pike et al., 1990). After reperfusion intracellular concentration of Na^+ is restored by the Na^+-K^+-ATPase.

In basal conditions, sarcoplasmic/endoplasmic reticulum $Ca2^+$ ATPase, the sarcolemmal $Ca2^+$ ATPase, and the sarcolemmal Na^+-$Ca2^+$ exchanger maintain intracellular Ca^{2+} below extracellular Ca^{2+}. In ischemic conditions Ca^{2+} enters the cell via Na^+-$Ca2^+$ exchanger and L-type Ca^{2+} channels, and intracellular Ca^{2+} increases. After reperfusion, restored Na^+ gradient allows Na^+-$Ca2^+$ exchanger, and restored ATP synthesis allows $Ca2^+$ ATPase to extrude Ca^{2+} out of the cell. After few minutes of reperfusion Ca^{2+} returns to preischemic level (Murphy and Steenbergen, 2008).

1.2.3.2 Ischemic Arrhythmias

Arrhythmias occurring as a result of coronary occlusion are called as ischemic arrhythmias. Following the coronary occlusion many biochemical and electrophysiological changes occur. ATP production by mitochondrial oxidative phosphorylation becomes interrupted, and leads cells to anaerobic glycolysis. As a result of anaerobic glycolysis H^+ and lactate accumulation occurs, then the accumulation causes acidosis. Extracellular K^+ level rises, and resting membrane potential falls. These changes trigger inhomogeneous refractory periods. The inhomogeneous electrophysiological properties of myocardial cells induce reentrant mechanism leading to arrhythmias (Mehta et al., 1997).

1.2.3.3 Reperfusion Arrhythmias

Arrhythmias occurring as a result of myocardial reperfusion are called as reperfusion arrhythmias. Life threatening arrhythmias, such as VT and VF, may occur just following the reperfusion. The frequency of VF after reperfusion is higher than it is after occlusion (Stephenson et al., 1960). After reperfusion there is an inhomogeneity in the action potentials of the cells within the ischemic area. Furthermore, there are substances, such as K^+, lactate and metabolites, accumulated in the extracellular space in the ischemic region. Accumulation of the substances and inhomogeneity may trigger reentry mechanism causing fibrillation (Wit and Janse, 2001). On the other hand, non-reentrant mechanisms; triggered activity and abnormal automaticity, contributes to the production of nonsustained VT which may lead to sustained tachyarrhythmias and sudden death.

1.2.4 Gender Differences and Arrhythmia

Many diseases are affected by gender, and in the basis of cardiovascular diseases; men are more susceptible to hearth diseases than women. Women tend to have a higher heart rate and a lower blood pressure than men. The percentage of cardiomyocyte apoptosis is more in men than in women (Mallat et al., 2001). Certain types of arrhythmias show differences between genders; atrial fibrillation and

ventricular fibrillation are more common in men then in women (Benjamin et al., 1994). Furthermore, sudden death related to coronary disease and morbidity are more common in men than in women (Lerner and Kannel, 1986). In female rats the incidence and duration of arrhythmias are lower than those in males (Gonca et al., 2004).

1.2.4.1 Effect of Estrogen

It is well known that some of the gender differences in arrhythmia are caused by estrogen (mostly 17ß-Estradiol), and its cardioprotective effect (Hale et al., 1996; Booth et al., 2003; Terrell et al., 2007). Cardioprotective mechanism of 17ß-Estradiol is not clear yet, however there are some different suggestions. It has been suggested that cardioprotective effect of 17ß-Estradiol might be produced by; the activation of myocardial mitochondrial K_{ATP} channels (Lee et al., 2000; Das and Sarkar, 2006), its antioxidant properties (McHugh et al., 1998; Barp et al., 2002), its effect on the production of nitric oxide, prostacyclin, and by inducing the Ca^{2+}-activated K^{+} channels (Node, et al., 1953), its effect on inhibiting tumor necrosis factor (TNF-α) and by limiting intercellular adhesion molecule-1 (ICAM-1) (Squadrito et al., 1997). Furthermore, the administration of 17ß-Estradiol also decreases blood pressure (Seely et al., 1999) by causing vasodilation (Oelkers, 1996).

1.3 Estrous Cycle

Estrous cycle is the reproductive cycle of non-primate female mammalians. There are different classifications of the phases of estrous cycle; proestrus, estrus, metestrus (also named as diestrus 1), diestrus (also named as diestrus 2), diestrus 3 (only classified in 5 day estrous cycle), and anestrus. Proestrus is the first phase of estrous cycle, in this phase ovarian follicle enlarges and secretes estrogens, which then absorbed into the blood and stimulates cell growth in genitalia (Husveth, 2011). Preceding corpus luteum degenerates in proestrus (Westwood, 2008). Proestrus is followed by estrus which is initiated by the elevation in estrogen level. Females become sexually receptive and the ovulation starts (Hebel and Stromberg, 1986) and

rats show a peak in voluntary activity during the estrus (Slonaker, 1924). The third stage of estrous cycle is metestrus which follows estrus. In the beginning of the metestrus, sexual receptivity ceases. In metestrus the function of corpus luteum and the secretion of progesterone increases (Husveth, 2011). The last phase of the estrous cycle is the diestrus. Large corpus luteum is maintained in the diestrus (Westwood, 2008).

There are three different forms of estrous cycle, depending on the appearance of the cycle: (1) polyestrous is a repeated cycling throughout the year. It is composed of proestrus, estrus, metestrus, diestrus, and the recent diestrus merges into proestrus of the following cycle. (2) Seasonal polyestrous shows multiple estrous cycles only during a season of the year, and the last diestrus merges into anestrus. Anestrus continues until the next breeding season. (3) Monoestrous shows only one estrous cycle per year. Unlike the seasonal polyestrous, anestrous is present between each cycle in monoestrous.

Rats are polyestrous animals, they show a repetitive estrous cycle throughout their lives until menopause. In rats puberty is related with the vaginal opening and the first estrous cycle. First estrous cycle starts about one week after the vaginal opening, which occurs 33-42 days after birth (Krinke, 2000). The average age of menopause of rats is 755 days, and until menopause rats my experience 140 estrous cycles (Slonaker, 1924).

There are different classifications of phases of estrous cycle, however in this study we considered 4 days (Mandle, 1951) classification which divides the cycle into proestrus, estrus, metestrus, and diestrus (Westwood, 2008). In rats, the duration of proestrus and estrus is 12 hours, metestrus is 21 hours, and diestrus is 57 hours (Paccola et al., 2013). Identification of these phases is achieved by observation of the cell types in the vaginal smears. The identification of proestrus is made by the observation of clusters of round and nucleated epithelial cells (Figure 1.17). Proestrus is followed by estrus, which can be identified by cornified cells. Estrus is followed by metestrus which is a transitional period during the early part of diestrus, and some researchers classify metestrus as “diestrus 1” (Maeda et al., 2000) or “early diestrus” (Mandle, 1951). Metestrus is identified by the presence of a combination of leukocytes and cornified and rounded epithelial cells (Goldman et al., 2007).

Diestrus (diestrus 2) is identified by the presence of large number of leukocytes and a small amount of larger cells.

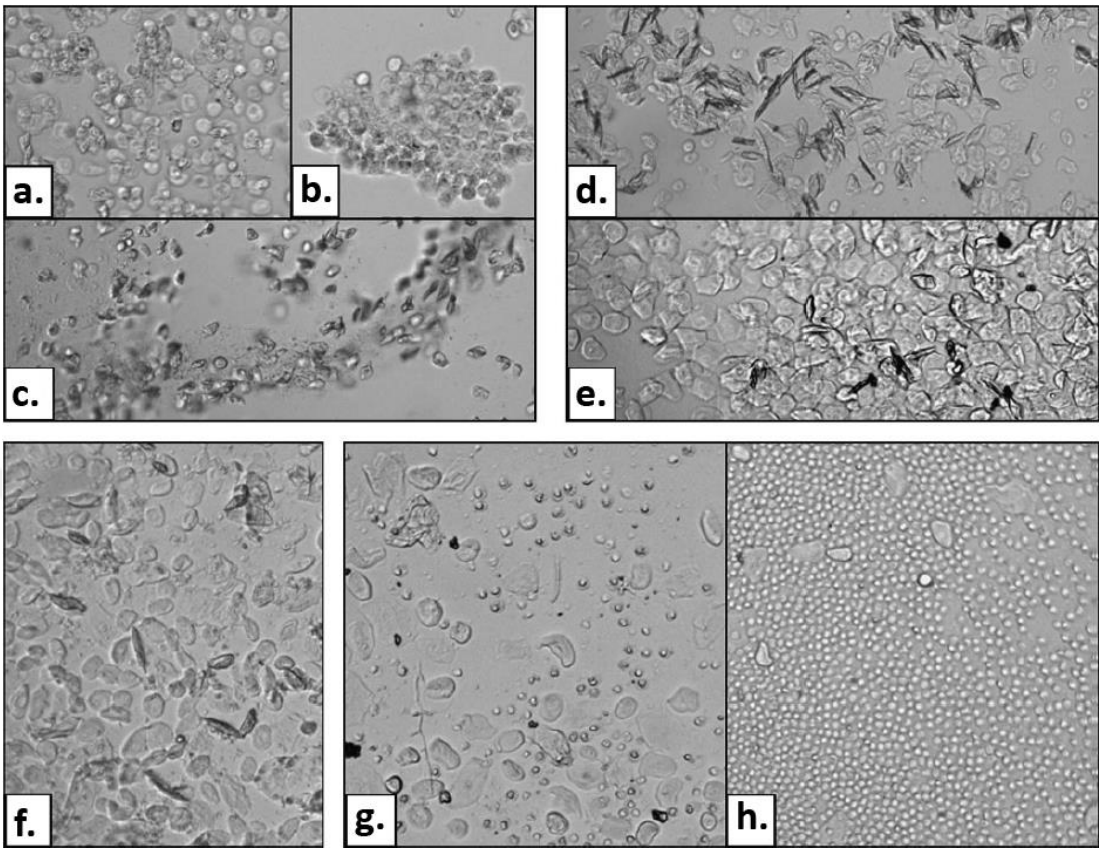

Figure 1.17. Unstained vaginal smears of different phases of estrous cycle. **a,b:** Proestrus; clusters of epithelial cells. **c.** Proestrus; epithelial cells can be found in strands instead of clusters. **d.** Estrus; keratinized, cornified cells. **e.** Estrus; cells can be appear rounded with edges. **f.** Metestrus; rounded cells, cornified cells and leukocytes are found together. **g.** Diestrus; leukocytes with other larger cells. h. Diestrus; large amount of leukocytes with a small amount of larger epithelial cells (Goldman et al., 2007).

1.3.1 Hormonal Changes in Estrous Cycle

Plasma concentrations of ovarian steroids (estrogen and progesterone), gonadotropins (FSH, and LH), and prolactin show fluctuations during the phases of estrus. During proestrus, luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, progesterone and 17-ß Estradiol levels increase to the peak level (Shaikh, 1971; Butcher et al., 1974; Kalra and Kalra, 1974; Smith et al., 1975) (Goldman et al., 2007; Krinke, 2000). Increased levels of estrogen (physiological

levels of estrogen is not sufficient, however exogenous estrogen may induce sexual responsiveness) and progesterone together induces sexual responsiveness (Powers, 1970). After proestrus, the levels of steroids and gonadotropins started to decrease to a baseline level (Smith et al., 1975).

The levels of endothelial-type nitric oxide synthase III (eNOS) and nitric oxide (NO) in the median eminence significantly increases during the proestrus. It is suggested that NO stimulates and controls gonadotropin-releasing hormone (GnRH) (Knauf, et al., 2001) which stimulates the synthesis and secretion of the gonadotropins (LH and FSH). Estradiol may be responsible for the increases on levels of eNOS and NO in the median eminence.

1.3.2 Physiological Differences Throughout the Estrous Cycle

A study have shown that, after injury, some cardiac functions; cardiac output, stroke volume and total peripheral resistance are depressed in rats in estrus, metestrus and diestrus, but these functions are maintained in rats in proestrus (Yang et al., 2006) (Jarrar et al., 2000). Yang et al. has suggested that the maintenance is associated with the high plasma levels of estradiol. Another study have shown that proestrus/estrus females has less myocardial contractile dysfunction after injury compared to diestrus females (Horton et al., 2004).

Internal or external conditions (e.g. drugs, stress, and isometric contraction) may result in an increase in arterial blood pressure. This increase is called as pressor response. It has been shown that the pressor response to vasopressin (a peptide hormone which increases peripheral vascular resistance) is higher in estrus rats than in diestrus, proestrus and metestrus rats (Crofton et al., 1988).

It has been shown that heart rate (HR) is lower in the dark phase of Proestrus than it is in estrus, metestrus and diestrus. However there were no differences in HR in light phases of estrous cycle (Takezawa et al., 1994). On the other hand, a study done in dairy cows has shown that HR was lower in Estrus than Proestrus, Diestrus, and Metestrus. After estrus HR started to increase (Lewis and Newman, 1984). In a recent study done in rats, the baseline HR of groups were Proestrus: 230 ± 8 bpm;

Estrus: 192 ± 16 bpm; Metestrus: 192 ± 14 bpm; and Diestrus: 220 ± 16 bpm, however there were no significant differences among the groups (Fraiser et al., 2013). In a study done with 26 women have shown that the resting heart rate in ovulatory and luteal phases was significantly higher than it was in the menstrual and follicular phases (Moran et al., 2000).

Besides the differences within phases on cardiovascular perspective, there are differences on cerebrovascular perspective. Lipopolysaccharide (LPS) induced proinflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are shown to be less in proestrus than in estrus after LPS injection (Sunday et al., 2006).

2. AIM AND SCOPE OF THE STUDY

Ischemia-reperfusion injury has been a vastly studied experimental scope for decades. It is well-known that there are differences between men and women in terms of ischemia-reperfusion injury. It is thought that the differences are derived from hormonal differences. Unlike men, women goes through menstrual cycle until menopause. In rats, estrous cycle substitutes menstrual cycle. During the estrous cycle, likewise menstrual cycle, hormone levels fluctuate. It is believed that these fluctuations may cause different responses to ischemia and reperfusion injury and leading different arrhythmic response, therefore using female animals in the experiments is avoided.

The aim of the study is to clarify the effect of estrous cycle on ischemia-reperfusion induced arrhythmias, the arrhythmic differences between the phases of estrous cycle, and the effect of endogenous 17ß-Estradiol on arrhythmia.

3. MATERIALS AND METHODS

3.1 Animals

In the present study 6-7 months old 28 female Sprague Dawley rats, weighing between 200-260g, were used. All the rats were fed ad libitum tap water and commercial rat pellets. Rats were kept in 12-h light (8 a.m. - 8 p.m.), 12-h dark (8 pm. – 8 a.m.) room and cages containing 4 rats. One week prior to operations estrous cycles of the rats were followed through the week. The animals showing irregular estrous cycle were removed from the operations. On the operation day estrous cycles of animals were determined again and 4 groups are produced; animals in Proestrus, Estrus, Metestrus and Diestrus. All study protocol was approved by ethical committee of Abant Izzet Baysal University, Bolu, Turkey and all animals were treated in adherence to all of the guiding principles in the care and use of animals together with the recommendation from the Declaration of Helsinki.

3.2 Determination of Estrous Cycle

Estrous cycle phases of each animal were determined by the cell types of the vaginal smears (Marcondes et al., 2002). Vaginal smears were collected with a plastic pipette, so that 10µl of saline (NaCl 0.9%) was injected into the rat vagina, and pulled back with the vaginal cells. One drop of the fluid was placed on a glass slide. The sample was observed under light microscope with 10 x and 40 x lenses. Three cell types were observed; epithelial cells which are round and nucleated, cornified cells which are irregular and lacking nucleus, and leukocytes which are round and smaller in size. In proestrus, epithelial cells are predominant in smear, cornified cells are predominant in estrus smear, all 3 cells are proportional in metestrus smear, and leukocytes are predominant in diestrus smear.

3.3 Coronary Artery Ligation and Reperfusion

Rats were anaesthetized with urethane (1.2g/kg) administrated intraperitoneally. Tracheotomy was performed by inserting a polythene cannula through an incision on the trachea for artificial respiration. An animal respirator (Ugo Basile Rodent Ventilator, Italy) was adjusted to 60 strokes/minute and 0.9ml/100gr. Carotid artery cannulation was performed by inserting a catheter filled with heparin (25.000 IU/100ml) diluted with saline (NaCl 0.9%) into left carotid artery, and arterial blood pressure was monitored with a transducer (Harvard Model 50-8952 transducer). Left thoracotomy was performed by cutting the 4^{th} rib and intercostal muscles. After pericardiotomy, the heart was exposed by gently pulling the intercostal muscles from 2 sides with retractors and gently pushing on the right side of the rib cage. An atraumatic 5-0 silk thread was placed around the left anterior descending coronary artery (LAD) about 2mm far from the origin of the vessel, and the heart is repositioned in the chest. Artificial respiration was started and subcutaneous needle electrodes were placed under the skin to record a standard electrocardiogram. The rats were allowed 5 minutes to stabilize their blood pressure and heart rate. 6 minutes of ischemia was made by tightening the thread with a bowknot, and 6 minute of reperfusion was made by loosening the bowknot.

The arterial blood pressure and bipolar ECG were recorded before ligation and during 6 minutes of ischemia and 6 minutes of reperfusion. Animals having arrhythmias or a blood pressure less than 70 mmHg before ligation procedure were discarded from experiment.

3.4 Determination of Area at Risk of Infarction

After recording 6 minutes of reperfusion, heparin (1ml/100g) was injected to the animals via the cannula that was inserted into left carotid artery at the beginning of the procedure. Respirator was turned off, and the heart was taken out by cutting the aorta, vena cava, pulmonary arteries and veins. Heart was submerged into saline in a beaker for 2 to 3 minutes. The silk thread was tightened again with a bowknot. Remaining vessels were cleaned from the heart, except a part of the aorta. A perfusion needle was inserted into the aorta and a thread was tied around the aorta to

keep the needle stable and prevent leakage. The heart, at first, was perfused with 10ml of saline (NaCl 0.9%), then 2ml of 80% ethanol. The silk thread around the LAD blocks the arteries, thus preventing saline and ethanol from reaching the cells in the area. The color of the area which was perfused with ethanol turns into white, whereas the color of non-perfused area does not change. Two area were separated from each other by cutting through the line between them, and atria were cleared from the ventricles (Figure 3.1). The non-perfused area was weighted and noted, then two areas were weighted together, and the percentage of the non-perfused area was calculated. The calculated percentage is called as the area at risk of infarction (AAR).

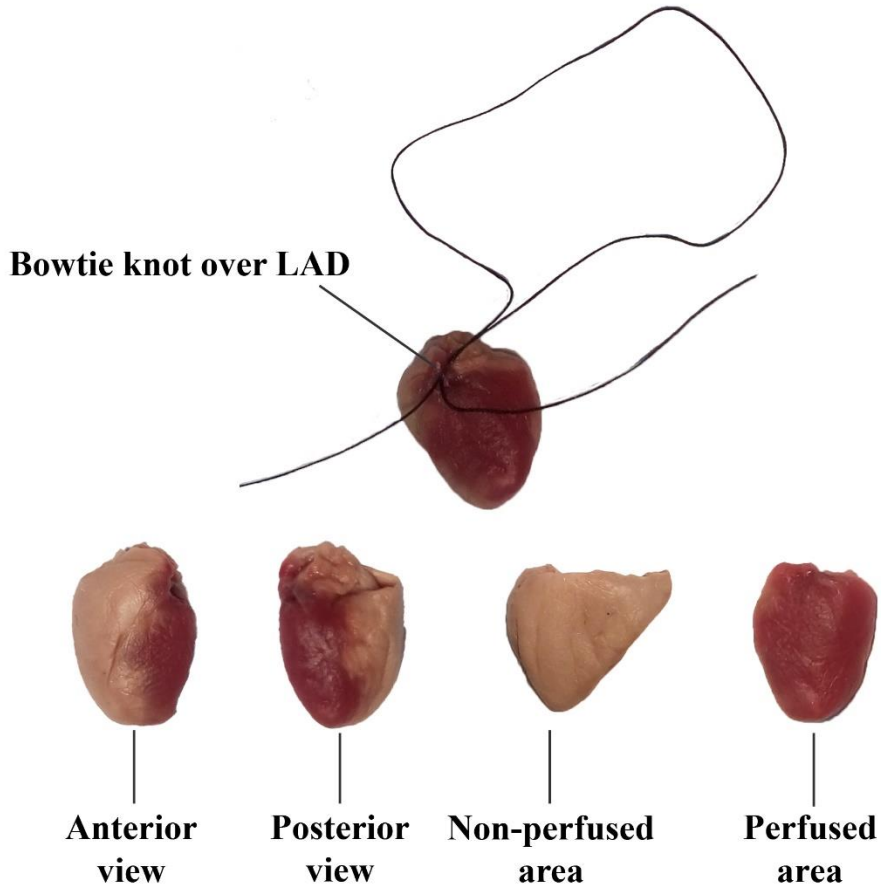

Figure 3.1. Ethanol perfused heart and bowtie over the LAD.

3.5 Evaluation of Arrhythmias, Heart Rate and Blood Pressure

Heart rate and blood pressure values before ligation (basal) and at 1st, 3rd, and 5th minute of both ischemia and reperfusion were determined from the records of ECG and blood pressure graph. The incidence and total length of arrhythmias during ischemia and reperfusion were calculated separately. Three types of classification were made for arrhythmias; ventricular tachycardia (VT), ventricular fibrillation (VF), and others including ventricular premature contractions, bigeminy, and salvo. Identification of arrhythmias were made according to the Lambeth Conventions II (Curtis et al., 2013). The onset and termination time of arrhythmias were recorded. Arrhythmia score for each animal was evaluated by grading the incidence and duration of arrhythmias (Figure 3.1) (Lepran et al., 1996).

Table 3.1. The chart used to evaluate arrhythmia score.

Arrhythmia Score	Duration of VT or other		Duration and type of VF
0	0 sec	and	0 sec
1	<10 sec	and	0 sec
2	11 to 30 sec	and	0 sec
3	31 to 90 sec	and	0 sec
4	91 to 180 sec	and/or	<10 sec reversible VF
5	>180 sec	and/or	>10 sec reversible VF
6	-	and	Irreversible VF

3.6 ELISA Protocol

At the end of the operation, 6-7 ml of blood were collected from random 4 animals in each group. Blood were collected directly from heart by using 10ml injectors. Blood were allowed to clot for 20 minutes at room temperature, and centrifuged at 2000 RPM for 20 minutes. Supernatant were collected and stored at -20°C. 17ß-Estradiol levels are measured using commercial Rat Estradiol (E2) ELISA kit (201-11-0175), the procedure was performed according to the instruction given by the manufacturer (Sunred Biological Technology, Shanghai China). The observed data was analyzed with one-way ANOVA combined with LSD post hoc test.

3.7 Statistical Analysis

The collected data were analyzed by SPSS (IBM SPSS Statistics, IBM Corp., ver. 20.0). Mean and standard errors of all parameter including body weight, heart rate, blood pressure, length of VT, VF and other arrhythmias during both ischemia and reperfusion, risk of infarct area, onset and termination time of arrhythmia, and arrhythmia score were determined. These data were analyzed by one-way ANOVA combined with LSD post hoc test. Survival rate and incidences of arrhythmia were evaluated by Fisher exact test. All data were presented as mean ± standard error.

4. RESULTS

An increase in amplitude of QRS and ST segment elevation and/or depression are seen in all groups of animals after the occlusion of coronary artery (Figure 4.1). Elevation and depression of the ST segment are accepted as indicators of ischemia.

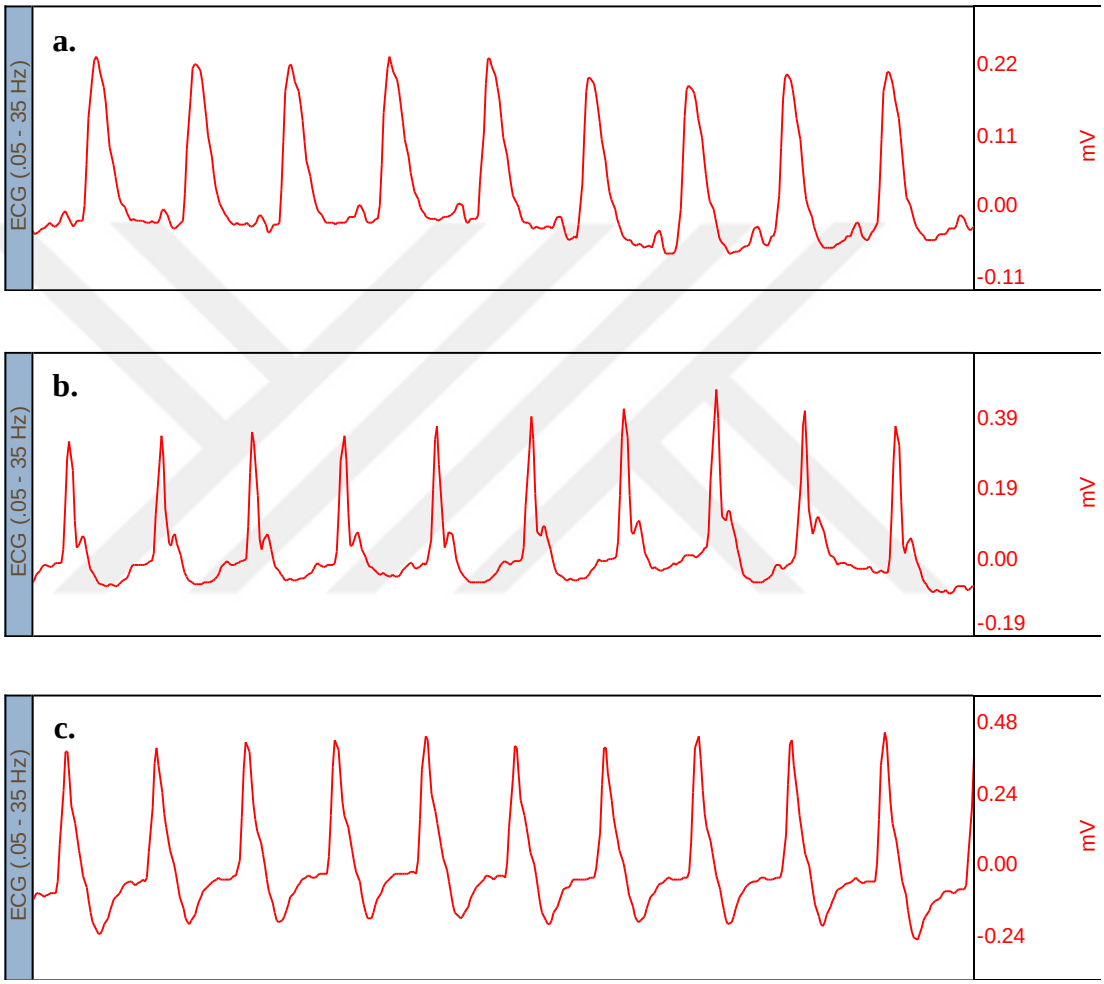

Figure 4.1. ST segment elevation. **a.** before occlusion, sinus rhythm. **b.** after occlusion, ST segment elevation, **c.** after occlusion, ST segment depression.

4.1 Heart Rates

Heart rates (HR) during 6 minutes of ischemia and 6 minutes of reperfusion are shown in the Table 4.1. There were no significant differences in heart rates

before the coronary artery occlusion among the groups including Proestrus (PRO), Estrus (EST), Metestrus (MET), and Diestrus (DIE). Basal heart rates were between 296 and 340. Highest heart rate were seen in proestrus and the lowest heart rate were seen in animals at estrus cycle.

Heart rate following occlusion was lower in estrus group than the other groups. At the following minutes of occlusion, heart rate tend to sustained in lower level in estrus in respect to other groups (Table 4.1).

There were no significant differences in HR between groups 1 minute after reperfusion (Rep1) and 3 minutes after reperfusion (Rep3). Heart rate in animals at estrus were significantly lower ($P<0,05$) than the HR of PRO (EST= 273,39 ± 23,64, PRO= 336,91 ± 24,76) at 5 minutes of reperfusion (Rep5).

Table 4.1. Heart rates during 6 minutes of ischemia and 6 minutes of reperfusion. α= significantly different from Proestrus, ß= significantly different from other groups, ε= significantly different from Metestrus (P<0,05).

Group	Basal	Ischemia (minutes)			Reperfusion (minutes)		
		1	3	5	1	3	5
Proestrus	340 ± 14	365 ± 21	344 ± 17	319 ± 14	306 ± 32	318 ± 16	336 ± 24
Estrus	296 ± 16	280 ± 23 $^{\alpha}$	269 ± 14 ^{ß}	283 ± 11 $^{\varepsilon}$	265 ± 16	269 ± 17	273 ± 23 $^{\alpha}$
Metestrus	321 ± 17	338 ± 21	334 ± 15	331 ± 20	305 ± 21	301 ± 16	294 ± 13
Diestrus	303 ± 10	326 ± 10	317 ± 10	326 ± 14	303 ± 10	299 ± 16	316 ± 14

4.2 Arterial Blood Pressures

Arterial blood pressures (BP) of all groups during 6 minutes of ischemia and reperfusion were shown in the Figure 4.2. No significant differences was found in BP during the operation between groups. Blood pressure before occlusion were in range between 90 mmHg to 100 mmHg.

Blood pressure in all animals rapidly decreased following the occlusion of coronary artery. Mean arterial blood pressure during occlusion were ranging from 57 mmHg to 81 mmHg, and during reperfusion were ranging from 52 mmHg to 71 mmHg. After reperfusion, BP of all groups started to increase slightly. Arterial blood pressures of all groups were higher at Rep5 than the pressure before the reperfusion. BP in all groups of animals at Rep5 were ranging from 64 to 71. 1 minute after ischemia (Isc1), blood pressure in animals at PRO were lower than the other groups, however the differences were not statistically significant.

Table 4.2. Blood pressures during 6 minutes of ischemia and 6 minutes of reperfusion.

Group	Basal	Ischemia (minutes)			Reperfusion (minutes)		
		1	3	5	1	3	5
Proestrus	93 ± 4	60 ± 5	57 ± 5	58 ± 4	53 ± 5	60 ± 5	64 ± 5
Estrus	94 ± 5	73 ± 7	62 ± 4	67 ± 3	68 ± 8	62 ± 9	71 ± 9
Metestrus	90 ± 6	61 ± 6	68 ± 5	69 ± 5	70 ± 5	70 ± 6	67 ± 5
Diestrus	100 ± 5	69 ± 5	63 ± 5	65 ± 6	62 ± 5	65 ± 7	69 ± 8

4.3 Arrhythmias

The onsets of arrhythmias were ranging from 80 to 111 seconds after occlusion and the ending times of arrhythmias were ranging from 142 to 260 seconds after occlusion in ischemic period. The onsets of arrhythmias were ranging from 65 to 96 seconds after reperfusion and the ending times of arrhythmias were ranging from 284 to 341 seconds after reperfusion period. Arrhythmic period of groups were ranging from 53 sec to 110 sec in ischemia and 174-260 seconds in reperfusion. No differences were found in the onset of arrhythmia, the ending time of arrhythmia, and the arrhythmic periods during ischemia and reperfusion (Figure 4.2).

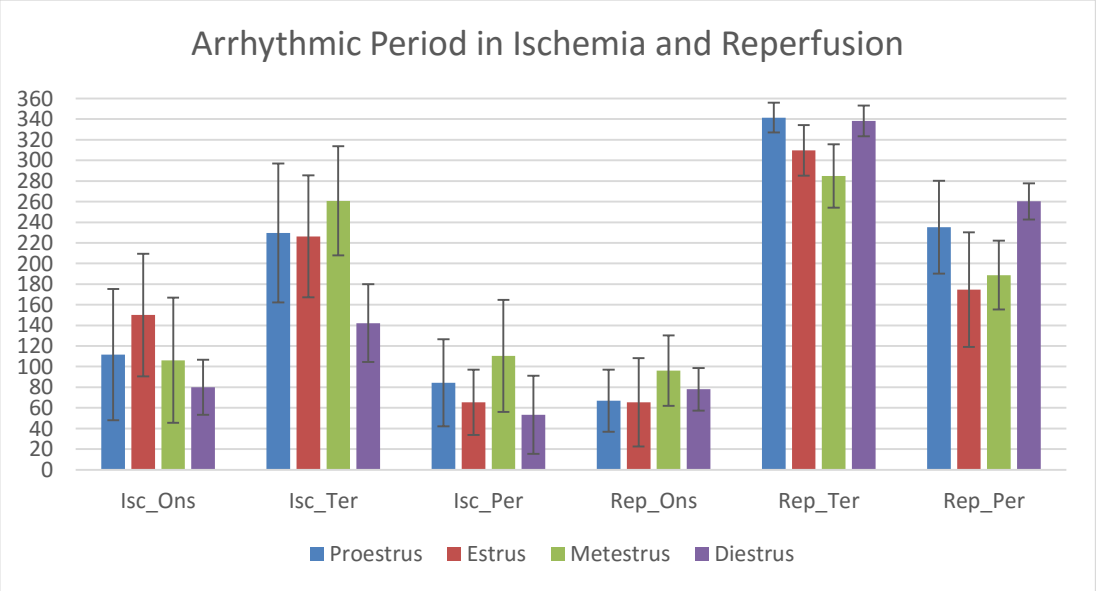

Figure 4.2. The arrhythmic period during ischemia and reperfusion. Isc_Ons= Onset of arrhythmia in ischemia, Isc_Ter= Ending time of arrhythmia in ischemia, Isc_Per= Arrhythmic period in ischemia, Rep_Ons= Onset of arrhythmia in reperfusion, Rep_Ter= Ending time of arrhythmia in reperfusion, Rep_Per= Arrhythmic period in reperfusion.

Area at risk of infarction (Figure 4.3) were ranging from 48,00 to 48,94 in groups. There were no differences found in AAR between groups.

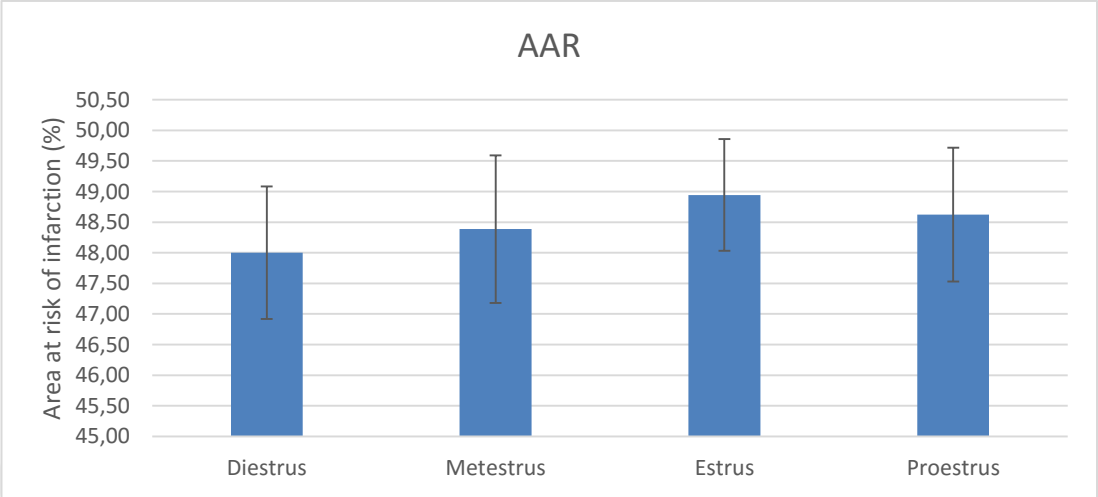

Figure 4.3. Area at risk of infarction.

The incidence of arrhythmia in ischemia, reperfusion, and the arrhythmia score were shown in Table 4.3, Table 4.4 and Figure 4.4. All animals have survived at the end of 6 minutes of reperfusion. VPC, salvo, and bigeminy were classified as "other". These types of arrhythmias were seen in 5/7 animals in both ischemia and reperfusion in PRO. The incidence of other arrhythmias was 6/7 in both ischemia and reperfusion in EST. The incidence was 5/7 in ischemia and 6/7 in reperfusion in MET, and it was 6/7 in ischemia and 7/7 in reperfusion in DIE. Ventricular tachycardia in ischemia was seen in only one animal and that animal was in PRO. There were not any animals which show ventricular fibrillation during ischemia. During reperfusion incidences of VT in PRO, EST, MET and DIE were 4/7, 5/7, 4/7, 6/7 significantly. During reperfusion VF was seen in only one animal, and that animal was in PRO. No significant difference were found in incidences of arrhythmia and arrhythmia score, in fact the data were nearly same.

Table 4.3. The incidences of arrhythmia in ischemia

Group	N	Survival (n/%)	Incidence of Arrhythmia (n/%)		
			Other	VT	VF
Proestrus	7	7/100	5/71	1/14	0/0
Estrus	7	7/100	6/86	0/0	0/0
Metestrus	7	7/100	5/71	0/0	0/0
Diestrus	7	7/100	6/86	0/0	0/0

Table 4.4. The incidences of arrhythmia in reperfusion

Group	N	Survival (n/%)	Incidence of Arrhythmia (n/%)			
			Other	VT	VF	Bradycardia
Proestrus	7	7/100	5/71	4/57	1/14	1/14
Estrus	7	7/100	6/86	5/71	0/0	0/0
Metestrus	7	7/100	6/86	4/57	0/0	1/14
Diestrus	7	7/100	7/100	6/86	0/0	0/0

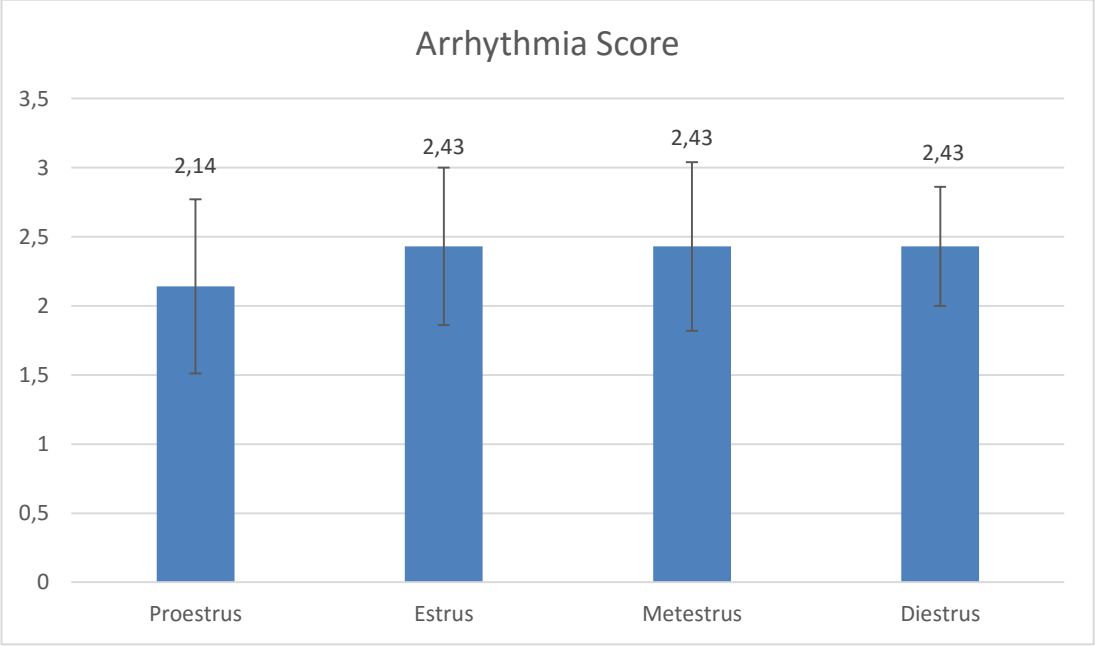

Figure 4.4. Arrhythmia score.

The duration of arrhythmias during 6 minutes of ischemia were shown in Figure 4.5. In ischemia, the duration of arrhythmias which were classified as “Other” were ranging from 2,19 sec to 8,74 sec. This type of arrhythmias were observed least in PRO, and most in DIE, however the difference was not statistically significant. Ventricular tachycardia was observed only in PRO, that was 0,97 ± 0,97 sec. In ischemia ventricular fibrillation was not observed in any of 28 animals comprising all groups.

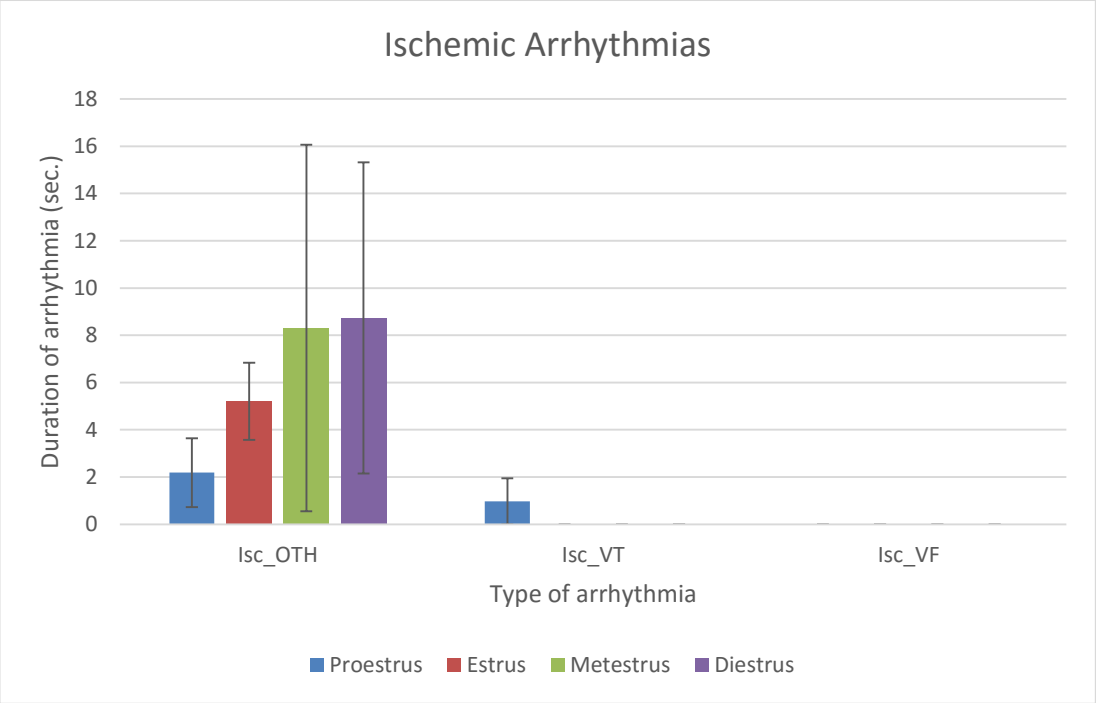

Figure 4.5. Duration of arrhythmias observed during ischemia. Isc_OTH= Other arrhythmia. Isc_VT= Ventricular tachycardia. Isc_VF= Ventricular fibrillation.

The duration of arrhythmias during 6 minutes of reperfusion were shown in the Figure 4.6. In reperfusion the arrhythmias classified as “Other” were ranging from 9,70 sec to 38,81 sec. This type of arrhythmias were observed least in MET and most in EST, however VT was observed least in EST and most in MET. Observed durations of VT were PRO= 23,53 ± 15,59, EST= 11,93 ± 4,16, MET= 44,05 ± 22,60, DIE= 24,29 ± 10,63. In reperfusion, VF was observed only in 1/7 animals in PRO, and the mean of duration of VT in PRO was 7,83 ± 7,83. According to the observed data there were no statistically significant differences in reperfusion arrhythmias among the groups.

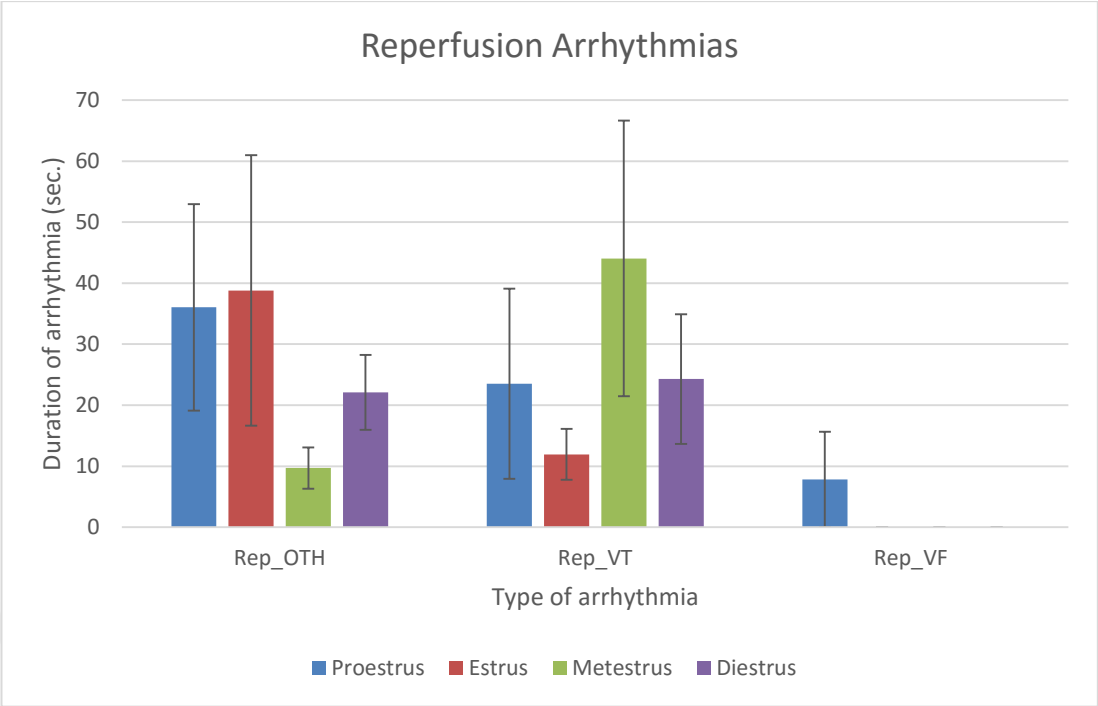

Figure 4.6. Duration of arrhythmias observed during reperfusion. Rep_OTH= Other arrhythmias. Rep_VT= Ventricular tachycardia. Rep_VF= Ventricular fibrillation.

Total durations of arrhythmias during 6 minutes of ischemia for PRO, EST, MET, and DIE were respectively; 3,16 ± 2,42, 5,21 ± 1,63, 8,31 ± 7,75, 8,74 ± 6,59 (Figure 4.7). During ischemia, total durations of arrhythmias (Isc_Total) were observed least in PRO and most in DIE. Total durations of arrhythmias during 6 minutes of reperfusion for PRO, EST, MET, and DIE were respectively; 67,40 ± 38,71, 50,74 ± 24,56, 53,75 ± 24,10, 46,37 ± 13,70. Unlike ischemia, during reperfusion, total durations of arrhythmias (Rep_Total) were observed least in DIE and most in PRO. Total durations of arrhythmias during both ischemia and reperfusion (TOTAL) for PRO, EST, MET, and DIE were respectively; 70,56 ± 39,00, 55,94 ± 25,13, 62,06 ± 31,20, 55,10 ± 18,54. TOTAL were observed least in DIE and most in PRO, however no statistically significant differences were found in Isc_Total, Rep_Total and TOTAL among the groups.

Bradycardia was only observed in 1/7 animals in PRO and 1/7 animals in MET. Durations of bradycardia observed in PRO and MET were respectively; 9,71 ± 9,71, and 31,71 ± 31,71 (Figure 4.8). There were no statistically significant differences in bradycardia among the groups.

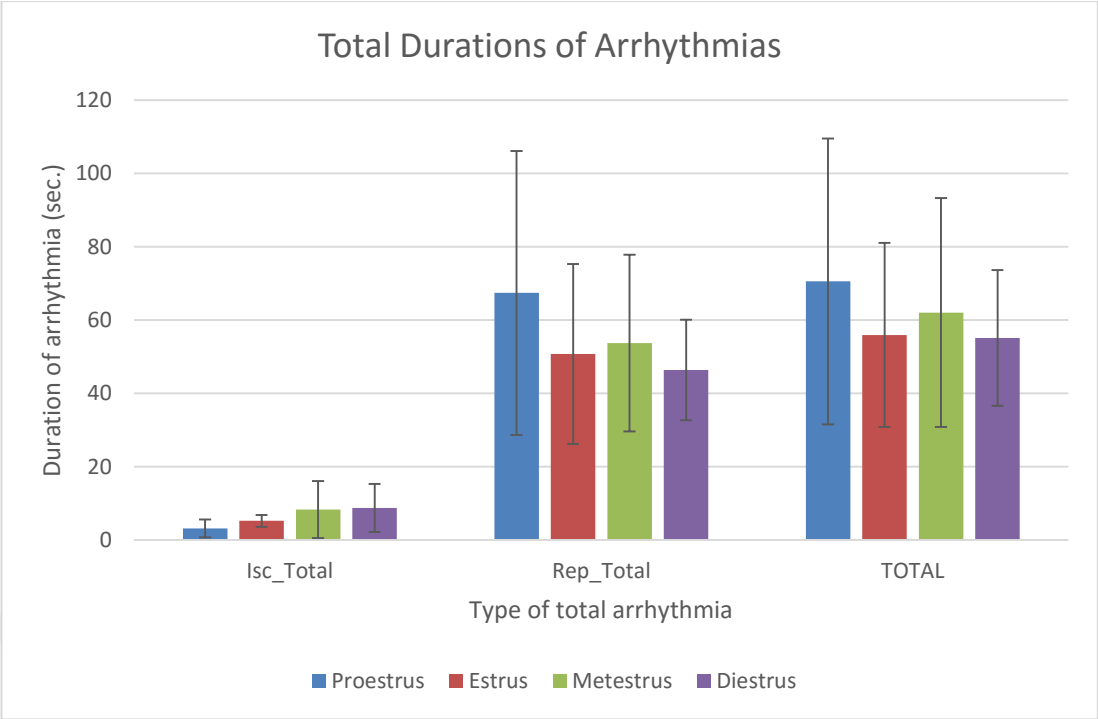

Figure 4.7. The duration of arrhythmias observed during ischemia and reperfusion. Isc_Total= total durations of arrhythmias during 6 minutes of ischemia. Rep_Total= total durations of arrhythmias during 6 minutes of reperfusion. TOTAL= summation of Isc_Total and Rep_Total.

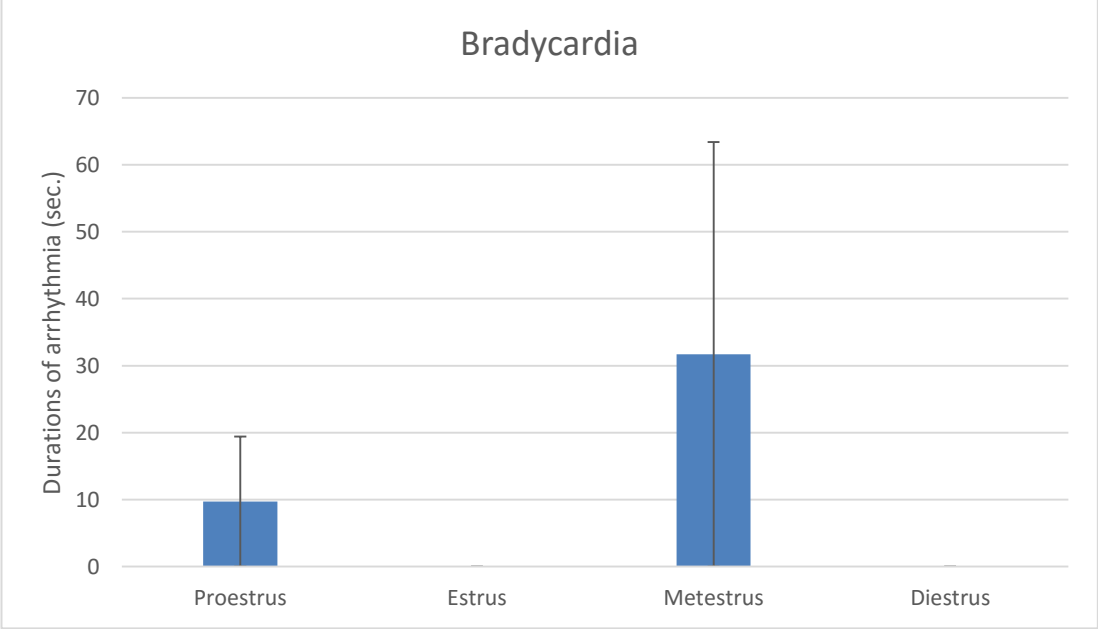

Figure 4.8. Durations of bradycardia.

4.4 Biochemical Analysis

Concentrations of 17ß-Estradiol of 4/7 animals in each group were determined by using a commercial Rat Estradiol (E2) ELISA kit. Data were obtained from ELISA Reader (Stat Fax® 4700 Microstrip Reader, Awareness Tech. Inc.). The highest level of 17ß-Estradiol was found in PRO (122,51 ± 6,11 ng/L), and the lowest level was found in EST (89,35 ± 1,28 ng/L) (Table 4.5 and Figure 4.9). 17ß-Estradiol level of PRO was significantly different from other groups (P<0,05). There were no significant difference among EST, MET, and DIE.

Table 4.5. Concentrations of 17ß-Estradiol. α= significantly different from other groups (P<0,05).

Groups	N	17ß-Estradiol (ng/L)
Proestrus	4	122,51 ± 6,11$^{\alpha}$
Estrus	4	89,35 ± 1,28
Metestrus	4	92,45 ± 4,62
Diestrus	4	93,74 ± 3,79

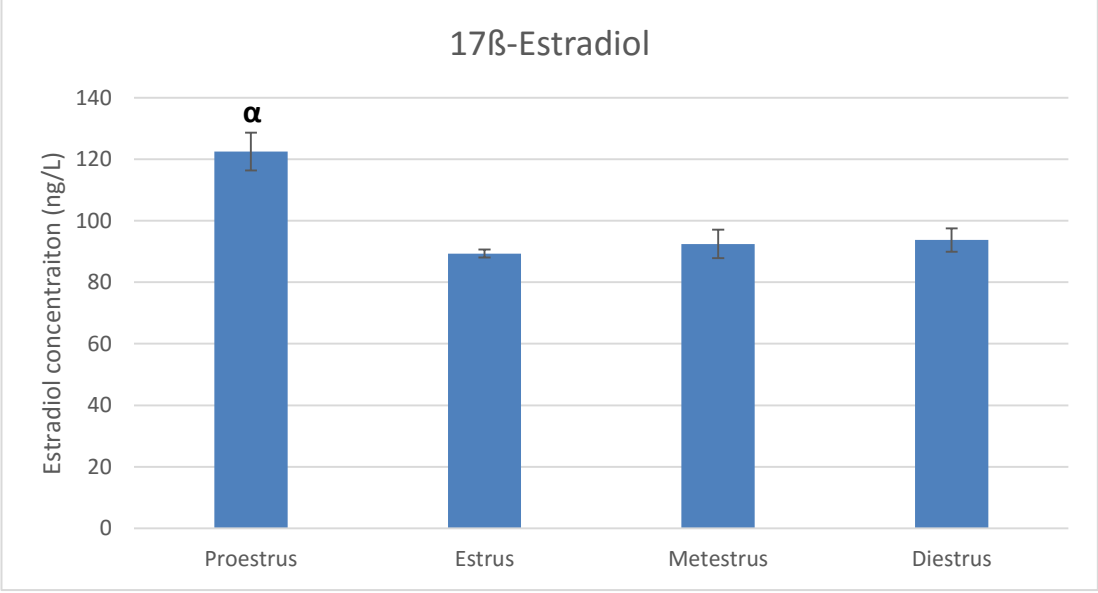

Figure 4.9. Concentrations of 17ß-Estradiol. α= significantly different from other groups (P<0,05).

5. DISCUSSION

ST segment elevation or depression were observed after coronary occlusion in every animals. ST segment elevation and depression were used as an indicator for ischemia (Chahine et al., 1976; Goldberg et al., 1981).

Area at risk of infarction (AAR) showed no differences among the groups. This means that, the ligation procedure was done from same point of coronaries in every animal, and there were no differences in the area which was supplied by the occluded artery.

Serum 17ß-Estradiol level of PRO was significantly higher ($P<0,05$) than those of other groups. Our findings are matching with former studies (Yoshinaga et al., 1969; Shaikh, 1971; Butcher et al., 1974).

Systolic blood pressure (BP) decreased after coronary occlusion in all animals in every group. The fall in the blood pressure is a sign of coronary occlusion (Levine, 1929). The decrease in blood pressure continued until reperfusion, and after reperfusion BP started to increase, however BP was not recovered fully after 6 minutes of reperfusion. At the 5^{th} minute of reperfusion, the blood pressure recovery for PRO, EST, MET, and DIE were $9,46 \pm 5,09$; $5,09 \pm 4,75$; $0,63 \pm 5,77$; $9,27 \pm 5,86$ respectively. The highest blood pressure recovery were seen in proestrus and diestrus groups, and the lowest recovery was in metestrus, however these differences were not statistically significant. To our knowledge, there was no study that specify the difference in blood pressure recovery. We have not observed any statistically significant differences in blood pressure among the groups during ischemia and reperfusion, however blood pressure in proestrus was lower than other groups after the 1^{st} minute of ischemia until the 5^{th} minute of reperfusion. Estradiol administration to postmenopausal women significantly decreases BP (Seely et al., 1999), and it causes both systemic and coronary artery vasodilation (Oelkers, 1996). The low blood pressure in proestrus during ischemia reperfusion may be caused by endogenous estrogen which was significantly higher in proestrus than other groups.

There was not a significant difference in heart rate (HR) among the groups before occlusion. The highest HR was found in PRO (340,23 ± 14,05) and the lowest HR was found in EST (296,86 ± 16,32), however the difference was not statistically significant. After occlusion the difference in HR between EST and other groups was statistically significant (P<0,05). The difference in HR and BP during estrous cycle have been shown before (Takezawa et al., 1994). Takezawa et al. have shown that the HR is lower in the dark phase of Proestrus than it was in other groups, and there were no differences in HR in light phases. In the present study, we have not studied dark phases of estrous cycle, therefore we do not support the results of Takezawa et al. (1994). However Takezawa et al. (1994) have shown that there were no differences in light phases, and in the present study we have not observed any statistically significant difference in HR in basal conditions. Frasier et al. (2013) also showed that there were no differences in baseline HR among the groups. Our study supports these findings, however Frasier et al. (2013) have not shown HR values after coronary occlusion. The difference in HR between the phases of estrous is known but the results are inconsistent. In the present study, we observed differences in HR among the groups during ischemia and reperfusion however there were not any statistically significant difference before the coronary occlusion.

In the present study, no differences were observed in ischemic arrhythmias, reperfusion arrhythmias, bradycardia, onset of arrhythmia, ending time of arrhythmia, arrhythmic period, incidences of arrhythmias, total durations of arrhythmias and arrhythmia score among the groups. The findings of arrhythmia score and the incidence of VF are matching with a previous study (Fraiser et al., 2013). However, other findings were shown the first time by the present study. The cardioprotective differences between female and male have been shown in the former studies (Lerner and Kannel, 1986; Benjamin et al., 1994; Mallat et al., 2001; Gonca et al., 2004; Brown et al., 2005; Johnson et al., 2006). Researchers have suggested different mechanisms of 17ß-Estradiol as a decisive molecule in the difference (Node, et al., 1953; Squadrito et al., 1997; McHugh et al., 1998; Lee et al., 2000; Barp et al., 2002; Das and Sarkar, 2006). The cardioprotective effect of 17ß-Estradiol administration is well known (Lee et al., 2000; Booth et al., 2003; Ohya et al., 2005; Sovershaev et al., 2006). In the present study, the plasma level of 17ß-Estradiol was found higher in PRO then EST, MET, and DIE, however, there were no differences

in arrhythmia parameters among the groups. Therefore, the results suggests that the physiological levels of 17ß-Estradiol does not trigger a cardioprotective effect against ischemia-reperfusion induced arrhythmias during the estrous cycle.

6. CONCLUSION

We have shown that the estrous cycle did not affect the ischemia-reperfusion induced arrhythmias in female rats. There were no differences in arrhythmia score, incidences of VT, VF and other arrhythmias among the groups. Onset of arrhythmia, the end of arrhythmia and the arrhythmic period also did not show any differences. However there were significant differences in heart rates during ischemia and reperfusion. The animals in estrus group showed significantly lower heart rates than the other groups besides the estrogen level is lower than the proestrus. Estrous cycle did not affect arrhythmias, however it may affect hemodynamic parameters. The effect of estrous cycle on hemodynamic parameters have been shown in the earlier studies, and our study supports the previous findings. Further studies are needed to clarify the results and the mechanism beneath these differences. This study has showed that the female rats can be used in arrhythmia studies without looking the phases of estrus cycle. Because there was no significant differences in ischemia and reperfusion arrhythmias depending on differences in phases of estrus cycle. Estrogen decreases blood pressure was also observed in this study. But although the level of estrogen is correlated with the decrease of arrhythmia in previous studies, this was not shown in this study. There was no difference in the severity of arrhythmia between proestrus animals having highest estrogen level and the estrus animals having lowest estrogen level. It is thought that not only estrogen but other factors might be also related with the severity of arrhythmia that increases in menopause. Further research should be performed to clarify the relation of other factors with the increased arrhythmia in menopause.

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