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Master's thesis

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Electrocardiography and measurements of the QT interval at different heart rates in a group of Icelandic horses

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Abstract

Cardiac repolarisation can be evaluated clinically by the QT interval in an ECG. Changes in the QT interval caused by diseases, drugs and genetic mutations can lead to fatal ventricular arrhythmias, syncope and sudden death in humans. In horses, cardiac repolarisation and QT interval is not well described and cannot yet be used diagnostically.

Objectives: Describe a sex-differentiated model for the QT interval in relation to HR/RR-interval in a group of healthy Icelandic horses, and to find the best descriptive model for HR-correction. Furthermore, to establish reference values for the QT interval at different HR/RR-intervals for Icelandic horses. T_{peak}–T_{end} (T_pT_e), a marker for transmural dispersion of repolarisation, will be measured in order to study its normal range and relation to a variation in RR-intervals.

Animals & Methods: 22 Icelandic horses of different sex (8 mares, 7 geldings and 7 stallions), with a mean age of 8.4 years (4-25 years) and weight 345 kg (269-385 kg). ECG was recorded during a 30 minute period including rest, warm-up, high intensity and recovery. QT and T_pT_e-interval were measured at seven different HR-intervals from each horse and plotted against the entire range of RR-intervals and fitted with a linear and a piecewise linear regression model. HR-correction were performed with the Bazett, Fridericia and a piecewise linear QT correction method.

Results: The relation between QT- and HR/RR-interval was best described by a piecewise linear regression model in all three groups ($0.93 < r^2 < 0.95$). On average the QT interval for geldings was longer than the other groups on most HR. T_pT_e was constant in geldings and mares, and only significant different from zero in stallions (p-value = 0.0386), but only 1.8 % were explained by variation in RR. A large variation in T-wave morphology was observed in the study both between horses and within one ECG recording. T-wave morphology appears to change with HR. A table of calculated reference values for the QT intervals with prediction intervals at different HR/RR Icelandic horses has been established.

Conclusions: The relation between QT interval and HR (RR) in the Icelandic horse is described with a piecewise linear regression, which is different from humans where it is described by linear regression. T_pT_e was constant and nearly RR-independent; a small difference was seen in stallions, possibly due to the higher athletic condition in this group. The calculated QT/RR-intervals can be used as reference values for Icelandic horses.

Key words: Electrocardiography, QT interval, heart rate, horse/equine, T-wave morphology .

Resumé

”Elektrokardiografi og målinger af QT-interval ved forskellige hjertefrekvenser i en gruppe Islandske heste”.

Hjertets repolarisering kan evalueres klinisk som QT-intervallet i et EKG. Ændringer i QT-intervallet som følge af sygdom, medicin eller genetiske mutationer kan føre til fatale ventrikulære arrytmier, besvimelse og pludselig død. Der er begrænset viden om QT-intervallet og hjertets repolarisering i heste, hvorved den diagnostiske værdi er sparsom.

Formål: At beskrive en model for hvordan QT-intervallet varierer i forhold til hjertefrekvens (HF)/RR-intervallet i en gruppe Islandske heste af forskellige køn, samt at finde den metode, der bedst korrigerer for varierende HF. Yderligere, udarbejdes der reference værdier for QT-intervallet for de ulige køn. T_{peak}–T_{end} (T_pT_e), en markør for den sidste del af repolariseringen, måles for at studere værdien i relation til HF/RR-interval.

Dyr og metoder: 22 Islandske heste af forskelligt køn (8 hopper, 7 vallakker og 7 hingste) var inkluderet i studiet. Gruppens gennemsnitsalder var 8,4 år (4-25 år) med en gennemsnitsvægt på 345 kg (269-385 kg). EKG blev optaget under en 30 minutters periode inklusive hvile, opvarmning, høj intensitet og restitution. QT- og T_pT_e blev målt ved syv ulige HF-områder fra hver hest og vurderet i mod RR-interval. En lineær og en to-delt lineær regressionsmodel blev testet. Korrektion for HF blev udført med Bazzett, Fridericia og en to-delt linær QT korrektionsmetode.

Resultat: Forholdet mellem QT- og HF/RR-interval blev bedst beskrevet med den to-delte lineære regressionsmodel i alle tre grupper ($0,93 < r^2 < 0,95$). Vallakker havde gennemsnitlig længere QT-interval end de to andre grupper. T_pT_e var konstant for hopper og vallakker og kun signifikant afvigende fra 0 for hingste (p-værdi=0,0386), men kun 1,8 % var forklaret af variation i RR-interval. Der blev observeret en stor variation i T-bølge-morfologi i studiet. Der blev udarbejdet en tabel med referenceværdier for QT-interval ved forskellig HF/RR for hvert køn.

Konklusion: Forholdet mellem QT- og HF/RR-interval i den Islandske hest er bedst beskrevet ved en to-delt lineær regression, hvilket adskiller fra humant, hvor QT/RR beskrives ved en lineær regression. T_pT_e var konstant og uafhængig af RR, med undtagelse af hingstene hvor T_pT_e var noget forlænget, muligvis forklaret af at denne gruppe var meget godt trænet. De udarbejdede QT-intervaller kan benyttes som referenceværdier for Islandske heste.

Nøgleord: Elektrokardiografi, QT-interval, hjertefrekvens, hest, T-bølge morfologi

Preface

This Master's Thesis has been written at the Department of Clinical Veterinary Medicine and Animal science at the University of Copenhagen, as part of the last year of the veterinary education. The report was written in the period from February to August, 2012. The ECG of Icelandic horses were recorded in Norway and Denmark in May 2012. Planning the practical work, collection of data and measurements of QT intervals, were all done by the author.

The idea for this Master's Thesis came from a Ph.D. study at the Department of Clinical Veterinary Medicine and Animal science that studies the QT interval in relation to heart rate (HR) in horses. The purpose of this project is to study how the QT interval varies at different HR in healthy Icelandic horses. The project will be of interest for any clinicians and non-clinicians with interest in equine cardiology and ventricular repolarisation.

I would like to thank everyone who has contributed to this Master Thesis, without whom the project would not have been possible.

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Date:

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List of abbreviations

2AVB	Second degree AV block
APC	Atrial premature complex
APD	Action potential duration
Bpm	Beats per minute
BWT	Body weight
BWT_e	Estimated body weight
Ca^{2+}	Calcium
Cav	Voltage gated calcium channel
CICR	Calcium induced Calcium release
Cl^-	Chloride
ECG	Electrocardiogram
HERG/KCNH2	Rapid, delayed potassium rectifier channel
HR	Heart rate
I	Current through a ion channel
IC	Intracellular
K^+	Potassium
KCNQ1	Slow, delayed potassium rectifier channel
Kv	Voltage gated potassium channel
LQTS	Long QT syndrome
LVH	Left ventricular hypertrophy
Na^+	Sodium
Nav	Voltage gated sodium channel
NSR	Normal sinus rhythm
PE	Prediction error
QT hysteresis	Different QT intervals at the same RR depending on an increasing or decreasing HR
QT interval	Time interval from onset of Q-wave to end of T-wave
QT lag	Delayed adaption of QT interval when HR change
QTc	Heart rate corrected QT-interval
RMP	Resting membrane potential
RR interval	Time from one R-peak to the next R-peak. Equal to one cardiac cycle
Rrbend	Bending point of the piecewise linear regression
SCD	Sudden cardiac death
SD	Sudden death
SQTS	Short QT syndrome
TdP	Torsade de Pointes - fatal ventricular arrhythmia
TpTe	Time interval from Tpeak to end of T-wave
VPC	Ventricular premature complex

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1. Introduction

Sudden death (SD) in apparently healthy horses is a well-known phenomenon in all types of horses, but has been given the most attention among racehorses (Gelberg *et al.* 1985; Kiryu *et al.* 1999; Lyle *et al.* 2011). The horse is a large and valuable animal, and when SD happens it affects animal welfare, rider safety, public relations and finances (Pedersen *et al.* 2012). An exact cause of SD in horses is often undefined, but presumably related to sudden cardiac death (SCD) (Gelberg *et al.* 1985; Kiryu *et al.* 1999; Lyle *et al.* 2011).

In human medicine we know of short and long QT syndromes (SQTS/LQTS), which are associated with an increased risk of the polymorphic tachycardia Torsade de Pointes (TdP), ventricular fibrillation (VF) and SCD (Anderson *et al.* 2002). The conditions are characterised by a shortening or prolongation of the ventricular repolarisation and are manifested clinically by changes of the QT interval on the surface electrocardiogram (ECG) (Finley *et al.* 2003; Hedley *et al.* 2009). Genetic mutations in genes encoding ion channels responsible for the ventricular repolarisation or proteins related to the regulation of these channels are known causes of congenital SQTS/LQTS in humans. Drugs that block repolarisation-channels, electrolyte disturbances and heart failure can result in acquired forms of SQTS/LQTS (Anderson *et al.* 2002; Kramer & Zimetbaum 2011).

The QT interval is very heart rate (HR) dependent and many formulas have been published to correct for this variation in humans (Luo *et al.* 2004). In the horse the HR is very variable ranging from 25 to 250 beats per minute (bpm), and pulse adaption of repolarisation is assumed to be more efficient in horses than in humans (Pedersen *et al.* 2012). Earlier ECG studies and QT interval measurements in horses are obtained from horses at rest, hence, at very limited ranges of HR intervals (Buss *et al.* 1975; Bakos & Lohne 2009; Schwarzwald *et al.* 2012). The relationship between the QT interval and HR in horses have until recently been unknown. Therefore a Ph.D. study at the University of Copenhagen including 30 racefit Standardbreds (SB) (mean weight 510 ± 34 kg) of different sex (stallion, gelding and mare) is presently looking into the correlation between QT and HR in horses. Interesting, it has here been found that horses display a non-linear relationship between the QT interval and the RR interval (60/HR) (Pedersen *et al.* 2012). This is different from the linear relationship seen in humans (Rajappan *et al.* 2003). The present study will further build a platform, which will make it possible to search for SQTS/LQTS in horses and use the QT interval as a marker of cardiotoxicity of drugs, which acts as possible inducers of acquired SQTS/LQTS.

The methodology and findings in the study by Pedersen *et al.* (2012) is also the background for this study. Here the Icelandic horse was chosen because it is a very homogeneous group of horses (pure breed) and because it represents a smaller horse (normal weight 380-400 kg) than the horses used in the above mentioned study, which could potentially have influence on the normal QT interval and the relationship between the HR and QT interval.

$T_{peak} - T_{end}$ (T_pT_e) is a marker for transmural dispersion in repolarisation (Pedersen *et al.* 2012). T_pT_e has been showed to increase with left ventricular hypertrophy (LVH) in humans (Porthan *et al.* 2007) and prolonged T_pT_e has been related to increased mortality in humans (Panikkath *et al.* 2011). The T_pT_e will be measured in this study and plotted against the RR interval in order to study the normal range in Icelandic horses.

The first part of this paper includes a literature study necessary for understanding the analysis, discussion and conclusion. Books, reviews and primary articles have been used in the literature study.

2. Literature study

2.1 Anatomy and physiology of the equine heart

The heart is a muscular pump that drives the blood around the cardiovascular system by systematically contracting and relaxing (Cunningham & Klein 2007; König & Liebich 2007). Blood circulates and transports oxygen and nutrients to every tissue and organ in the body, and transports carbon dioxide and metabolic waste products from cells to lungs, liver and kidneys, where it can be excreted (Cunningham & Klein 2007).

The heart consists of four chambers: the right and left atria and ventricles. The chambers are separated longitudinally by *septum interatriale et. interventriculare.* A transverse septum divides the blood receiving atria from the blood pumping ventricles (König & Liebich 2007). The equine heart weighs about 0.7 per cent of the body weight (BWT) (Holmes 1968), approximately 3.5 kg in a 500 kg horse.

A cardiac cycle consists of a period of relaxation and a period of contraction of the heart, called the *diastole* and the *systole*. Every systole happens in two stages; first the atrial systole, followed by the ventricular systole (Cunningham & Klein 2007). In the horse, the atrial contribution to diastolic filling of the ventricles is essential for maximal performance during exercise, but negligible during rest (Bonagura *et al.* 2010).

In every cardiac cycle the blood circulates through the heart in a specific manner, and two sets of heart valves ensure the one-way direction of the blood flow (figure 1). These valves are the *valva atrioventriculare dexter et. sinister* (AV valves) between the atria and ventricles and the *valva semilunares dexter et. sinister* between the ventricles and the arteries (Schaller 2007; Silverthorn 2007). The right and left AV valves are also known as the tricuspid and bicuspid/mitral valves, respectively, and they prevent back flow of blood into the atria during ventricular systole. The semilunar valves prevent blood from flowing back from the pulmonary and systemic circulation system, and in to the ventricles during ventricular diastole (Silverthorn 2007; Cunningham & Klein 2007).

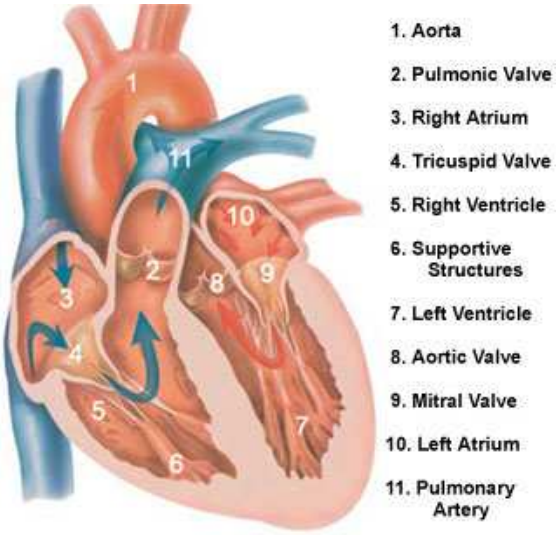


Figure 1: The one-way direction of the blood flow through the heart. Adapted from http://www.communitymemorial.com/services/heart/working-heart.cfm

Deoxygenated blood flows in to the right atrium (*atrium dexter*) from *vena cava cranialis et caudalis* and from the heart itself (*sinus coronarius*). The blood flows via the right ventricle and is pumped through the *truncus pulmonalis* to the lungs, where it becomes oxygenated. The oxygenated blood returns to the left atrium (*atrium sinister*) via *venae pulmonales*. The blood flows into the left ventricle (*ventriculus sinister*), where it is pumped with a rapid injection into the aorta and the systemic circulation (Cunningham & Klein 2007; Schaller 2007; König & Liebich 2007).

The wall of the heart is composed of an *endocardium*, *myocardium* and *epicardium*. The myocardium, which is the middle layer of the cardiac muscle, is the thickest layer. The thickness of the wall differs, being thickest in the ventricles and thinnest in the atria (Bacha & Bacha 2000). The myocardium consists of cardiac muscle cells (fibres). Most of the cardiac muscle fibres are contractile fibres, but about 1 % of them are specialised conduction cells (Silverthorn 2007), whose function will be described in detail later.

The cardiac muscle fibres branch and connect directly to neighbouring cells via *intercalated disks* (ID). Within the IDs there are small cytosolic channels termed *gap junctions*. These channels form a functional syncytium, which is important for fast transmission of action potentials between cells (Cunningham & Klein 2007; Rohr 2004). Each contractile cardiac muscle fibre is built up like a skeletal muscle fibre, with strictly organized arrangements of the contractile proteins (myofilaments) *actin* and *myosin*.

2.2 Electrophysiology of the heart

2.2.1 The conduction system

The conduction system is essential to describe because it is the electrical pathway through the heart, which leads to depolarisation and repolarisation of the cardiac muscle fibres and results in coordinated contraction and relaxation of the heart. It is necessary to understand the origin of depolarisation and repolarisation of the cardiac cells, in order to understand how the QT interval is a marker for these events in the ventricles.

About 1 % of the cardiac muscle fibres are specialised conduction cells that are able to depolarise spontaneously. In the horse, similar to other species, the conduction system consists of the sinoatrial (SA) node, the internodal atrial tracts, the atrioventricular (AV) node, the bundle of His, the right and left bundle branches and the Purkinje network, as showed in figure 2 (Bishop & Cole 1967; Tilley 1992). The conduction (transmission of electricity) follows a predictable pathway in the normal heart, leading to a coordinated contraction of atrial and then ventricular muscle (Patteson 1999). The SA node is the primary pacemaker of the heart and its' cells are able to depolarise spontaneously and initiate a heartbeat. The SA cells are therefore known as pacemaker cells. The SA node is located in the right atrium, near the entry of the cranial vena cava (Keith & Flack 1907; Bishop & Cole 1967; Tilley 1992).

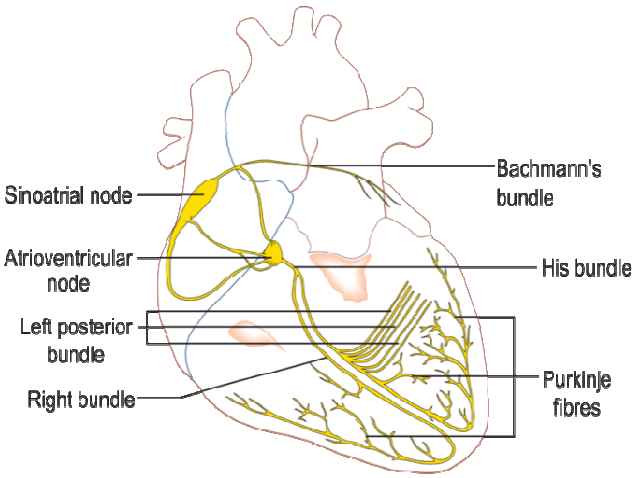


Figure 2: Schematic figure of the conduction system. Adapted from (http://commons.wikimedia.org/wiki/File:Conductionsystemoftheheart.png)

The conduction follows three inter-nodal tracts through the atria and to the AV node: the anterior, middle and posterior tracts (James 1963; Tilley 1992). The AV node lies in the lower part of the

right atrial septum, just above the tricuspid valve. The conduction is slowed down through the AV node, due to a longer refractory period in the AV nodal cells (Cunningham & Klein 2007). This allows complete filling of the ventricles before they contract, and protects the ventricles from being stimulated to contract to fast (Bishop & Cole 1967; Tilley 1992; Cunningham & Klein 2007).
The AV node continues into the bundle of His, which penetrates the fibrous AV ring. This is the only electrical connection between the atria and the ventricles, and it ensures coordinated ventricular contraction from bottom to top (Silverthorn 2007). The bundle of His branches into a left and a right bundle branch. The left bundle branch courses through the interventricular septum and to the left ventricle where it joins a network of Purkinje fibres, whereas the right bundle branch joins a Purkinje network in the right ventricular myocardium (Meyling & Borg 1957; Bishop & Cole 1967; Tilley 1992). In the horse, the Purkinje network has a widespread distribution in both ventricular free walls, which in turn lead to activation of the ventricles from multiple sites (Hamlin & Smith 1965; Patteson 1999).

In the normal heart, the cells of the AV node and Purkinje fibres are also able to spontaneously depolarise. Under normal circumstances the SA nodal cells depolarise faster, and therefore the SA node is the primary pacemaker of the heart. Under certain abnormal conditions, where the SA node is injured and unable to initiate depolarisation, the pacemaker activity of the AV node is critical for survival. The heart rate will in those cases be abnormal low (Patteson 1999; Cunningham & Klein 2007). Injured cardiomyocytes can also create abnormal automacity and arrhythmias, which will be discussed in section 2.4.3.

2.2.2 Resting membrane potential

The electrical activity in the heart, as described in the previous section, is created from different ion currents across the cell membrane at different times. To understand how electrical activity is created in the heart, it is essential to understand that all cells have an electrical potential difference across their cell membrane, which is called the *resting membrane potential* (RMP). The RMP is an indirect result of a relative permeability across the plasma membrane for specific ions (K^+, Na^+, Ca^{2+} and Cl^-) (Silverthorn 2007; Cunningham & Klein 2007).

Ion movements are determined by the electrochemical gradient. The ions are charged and their movements across the membrane will depend on both their concentration and the electrical field

across the membrane, which is created by the different ions (Ashcroft 2000). The typical ion concentrations across the membrane are shown in table 1.

Ion	**Extracellular concentration (mM)**	**Intracellular concentration (mM)**
Sodium (Na^+)	135 – 145	12
Potassium (K^+)	3.5 – 5	140
Calcium (Ca^{2+})	2.25 – 2.52	10^{-7} M (free)
Chloride (Cl^-)	115	2.5 – 50

Table 1: Typical ion concentrations in extracellular and intracellular fluid (Ashcroft 2000).

The potential at which the electrical force on the ion, balances the opposing force of the concentration gradient is called the *equilibrium potential* for the ion, and is given by the Nernst equation. For a given ion,

$$E_{ion} = \frac{RT}{FZ} \ln \frac{[Ion]_e}{[Ion]_i}$$

Where E_{ion} is the equilibrium potential (in volts), R is the gas constant (8.314 $JK^{-1}mol^{-1}$), T is the absolute temperature (in degrees Kelvin), Z is the valency (positive or negative charge) of the ion, and F is the Faraday constant (96,500 C mol^{-1}). $[Ion]_e$ and $[Ion]_i$ is the extra- and intracellular ion concentrations (Ashcroft 2000).

From Nernst equation, the calculated potassium (K^+) equilibrium potential (E_K) is –84mV. This negatively charged intracellular fluid is close to the RMP of most cells (-60 to -100 mV), which indicates that the potential is primary determined by K^+ selective channels (Ashcroft 2000). The Na/K-ATPase plays an important role in maintaining the concentration gradients, by pumping 3 Na^+ ions out and 2 K^+ ions in to the cell (Ashcroft 2000).

When the pacemaker cells of the SA node spontaneously depolarise, it creates an electrical signal that spreads through the conduction system and creates voltage changes in the membrane potentials of the cardiac cells. These changes result in opening and closing of voltage gated ion channels important for depolarisation and repolarisation, and their structure and function will be described in the next section.

2.2.3 Structure and function of voltage gated potassium (K^+) channels

The voltage gated K^+ channels (Kv-channels) are the primary determinants of repolarisation in the mammalian myocardium. The basic structure and function of these channels will be described in this section, in order to understand how genetic mutations in genes encoding these ion channels or proteins related to the regulation of the channels can result in changes in the QT interval (SQTS/LQTS) (Nerbonne & Kass 2005).

Kv-channels are made up of pore-forming α- subunits, which may associate with one of many different β- subunits (Ashcroft 2000). The α-subunit contains four homologous repeats (I to IV) (figure 3 B), where each of these repeats contains six transmembrane segments (S1 to S6) (figure 3 A). A functional Kv channel is composed of four α-subunits, with the amino- (NH_3^+) and carboxyl (COO^-) termini of the segments sited on the cytoplasmic side of the membrane (Ashcroft 2000; Nerbonne & Kass 2005). The fourth segment (S4) is positively charged and is involved in voltage-dependent activation of Kv channels (Pongs 1992; Ashcroft 2000).

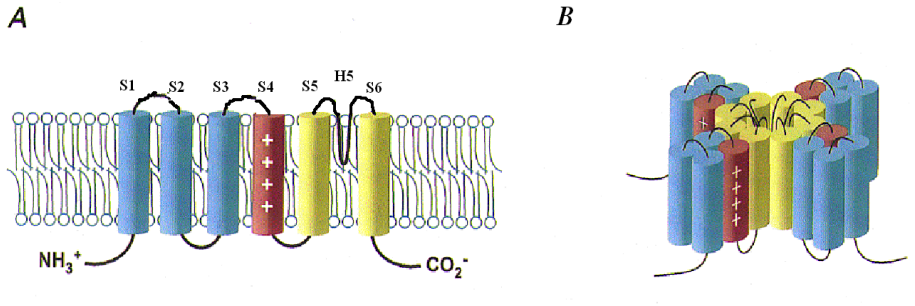


Figure 3: Structure of a voltage gated K^+ channel pore forming α subunit. A, Kv α-subunits are made up of six transmembrane domains (S1-S6), a positively charged S4 and a highly K^+ selective H5 region. B, Schematic diagram of a Kv channel illustrating the four α-subunits creating the K^+ selective pore (modified from Nerbonne 2000).

The mechanism by which the S4 segment serves as a voltage sensor is not completely understood (Ashcroft 2000), but there is a voltage driven change in the protein conformation that results in opening of the ion pore. This opening mechanism has been explained by a “sliding helix” model. In response to a change in voltage, the S4 helixes move across the membrane in a spiral path and thereby lead to a conformational change and opening of the ion pore (Ashcroft 2000).

The H5 region between the S5 and S6 segments participates in formation of the channel pore, and enters and exits the membrane from the extracellular side (Pongs 1992; Ashcroft 2000). The Kv-channel is highly selective for K^+ ions, and the H5 regions are responsible for this selectivity filter due the specific combination of amino acids of the region (Doyle *et al.* 1998; Ashcroft 2000).

Positively charged K^+ ions are attracted to the channel pore by negatively charged amino acids in the entrance of the channel (Doyle *et al.* 1998). The walls of the channel are lined with hydrophobic residues, and the hydrated and positively charged K^+ ions move easily into the water filled cavity of the channel pore (Ashcroft 2000). The selectivity filter is narrow and can accommodate two K^+ atoms at the same time, located at the opposite ends of the filter. When a second K^+ enters the selectivity filter it creates an electrostatic repulsive force that pushes the first K^+ ion through the pore and out into the extracellular fluid (Doyle *et al.* 1998; Ashcroft 2000).

The inactivation of Kv channels is displayed in two main modes; fast and slow inactivation (Ashcroft 2000). The cytoplasmic amino terminus is responsible for the fast voltage-dependent inactivation, which is called N-type inactivation. Some Kv channels do not undergo fast inactivation and they are referred to as *delayed rectifiers*. The most distal part of the N-terminus serves as an "inactivation ball" which can swing and bind to a receptor site on the S4-S5 linker. This is close to the inner opening of the channel pore, and the binding inactivates the channel, as showed in figure 4 A. (Ashcroft 2000). Some Kv channels have a slow inactivation, which is called C-type inactivation because it involves the C-terminus of the protein (Ashcroft 2000). In C-type inactivation it appears to be a conformational change in the external part of the pore, which leads to constriction an occlusion of the pore (figure 4 B) (Yellen 1998; Ashcroft 2000).

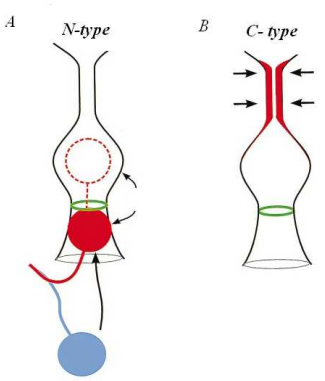


Figure 4: N- and C-type inactivation. A: N-type inactivation, **B**: C type inactivation. Modified from Yellen (1998).

β-subunits are small peptides in the cytosol, which may bind to the α- subunits. Not all combinations of β- and α-subunits are possible, since a single type of β-subunit does not associate with all types of α-subunits, and *vice versa*. The binding can either increase the function and expression of the Kv channel or increase the rate of inactivation (Ashcroft 2000).

The structure and function of voltage gated Na^+ - and Ca^{2+} ion channels (Nav- and Cav-channels) is similar to what is described for Kv-channels in this section (Ashcroft 2000). Ion currents through Kv, Nav and Cav-channels in the cardiac cell membranes give rise to the depolarisation and repolarisation phases of the cardiac action potential, which will be described in the next section.

2.2.4 The cardiac action potential

The cardiac action potential can be described in five phases, where sequential activation and inactivation of depolarising and repolarising ion channels and currents contributes to voltage changes in the cardiac cells (Nerbonne & Kass 2005). The five phases are showed in figure 5, and described shortly below.

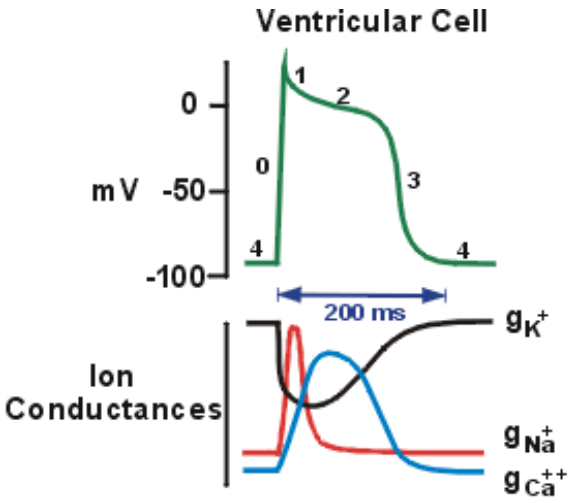


Figure 5: The five phases of the cardiac action potential and main ionic currents. An upward deflection represents inward ion current and downward deflection represents an outward ion current. Adapted from http://www.cvpharmacology.com/antiarrhy/cardiac_action_potentials.htm.

Phase 4: Resting membrane potential

During rest there is a negative membrane potential in the cardiac cells at about –80-90mV, as described in section 2.2.2. This negatively resting membrane potential is mostly a result of K^+ efflux through open K^+ channels. Most Na^+ and Ca^{2+} channels in the cell membrane are closed in this phase (Cunningham & Klein 2007).

Phase 0 Rapid depolarisation

This phase involves depolarisation and firing of the cardiac action potential. Fast, voltage gated Na^+ channels (Nav) in the cell membrane open when the cardiac cell is depolarised to the threshold voltage for opening at about –55 mV (Nerbonne & Kass 2005). This leads to a rapid influx of Na^+ ions and the membrane potential becomes positively charged on the inside.

Phase 1: Short repolarisation

The Nav-channels inactivate quickly when the cell is depolarised. Rapid opening and closing of voltage gated K^+ channels lead to a rapid efflux of K^+ ions and a short repolarisation phase (Silverthorn 2007; Cunningham & Klein 2007).

Phase 2: The plateau of depolarisation

The plateau of depolarisation is unique to the cardiac action potential, and does not exist in skeletal muscle fibres or nerve cells. Opening of many voltage gated Ca^{2+} channels and closing of some of the K^+ channels result in increased intracellular K^+ and Ca^{2+} concentrations that keep the cell in this prolonged depolarised state for about 200 msec. Ca^{2+} influx triggers Ca^{2+} release from sarcoplasmatic reticulum (SR) (Bers 2002; Cunningham & Klein 2007). The Nav-channels are inactivated until the cell reaches its resting potential, which means that a new action potential cannot be initiated in this period. The period is called the absolute refractory period, and is critical for adequate diastolic filling and prevents the heart from continuous contraction (Finley *et al*. 2002; Cunningham & Klein 2007).

Phase 3: Repolarisation

Other Kv-channels are activated slowly by the depolarisation and open at this point. K^+ ions exit rapidly through the open Kv-channels, and together with closing of Cav-channels, the cardiac cell is repolarised to its resting membrane potential (phase 4) (Cunningham & Klein 2007; Silverthorn 2007).

Under normal conditions, each cardiac action potential is triggered by an SA node pacemaker cell. The cells of the SA node demonstrate a less negative membrane potential, at about – 60 mV (Patteson 1999; Silverthorn 2007; Bonagura *et al*. 2010). As seen in figure 6, the morphology of the SA and AV nodal cells lack the steep depolarisation phase 0. This is due to the lack of the fast, voltage gated Na^+ channels. Instead pacemaker cells have so called "funny channels" (I_f), which are closed during an action potential, and spontaneously open at the end of the action potential (Silverthorn 2007; Cunningham & Klein 2007). The spontaneous opening results in an increase in Na^+ influx and decrease in K^+ efflux. An increase in Ca^{2+} permeability makes a late contribution to depolarisation toward threshold, and the action potential is primarily driven by this large, prolonged increase in Ca^{2+} permeability (Cunningham & Klein 2007; Bonagura *et al*. 2010).

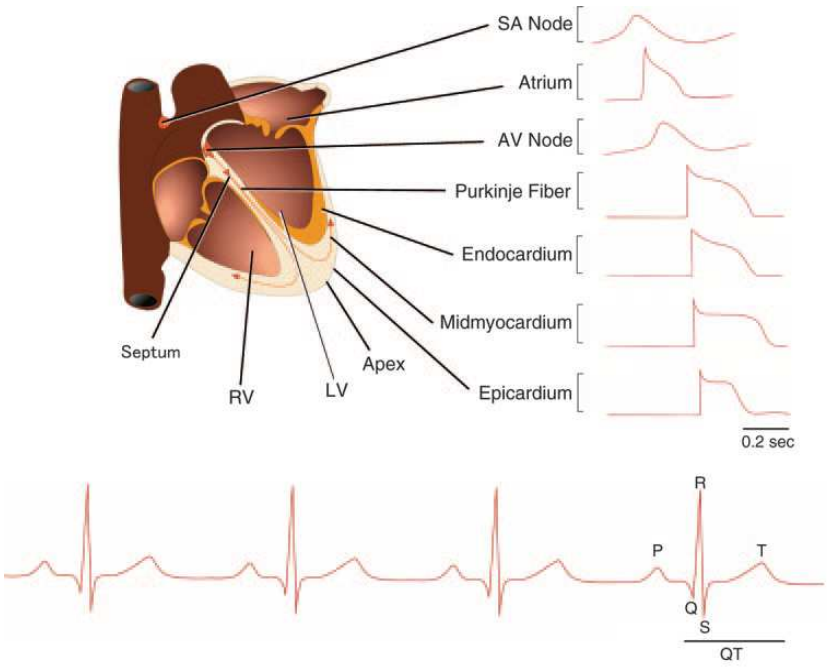


Figure 6: Electrical activity in the myocardium. *Top*: schematic of a human heart with illustration of typical action potential waveforms recorded in different regions. *Bottom*: schematic of a surface electrocardiogram; three sequential beats are displayed (Nerbonne & Kass 2005).

As seen in figure 6, the action potential morphology and duration is different in the distinct areas of the myocardium. For example is the atrial action potential shorter than the ventricular, a mechanism which ensures atrial systole and emptying before the ventricular systole. The differences in action potential durations in difference areas of the heart have been explained by heterogeneous expression of the repolarising K^+ ion channels in the atria and the ventricle, which will be further discussed in section 2.3.1 (Nerbonne & Kass 2005).

2.2.5 Excitation-contraction coupling

The different ion currents described above is essential for the cardiac muscle cells to contract, a process known as “cardiac excitation-contraction coupling” (Bers 2002). Influx of Ca^{2+} through ion channels in the cardiac cell membrane is essential for the plateau phase of the action potential and for the contraction of the cardiac muscle fibres.

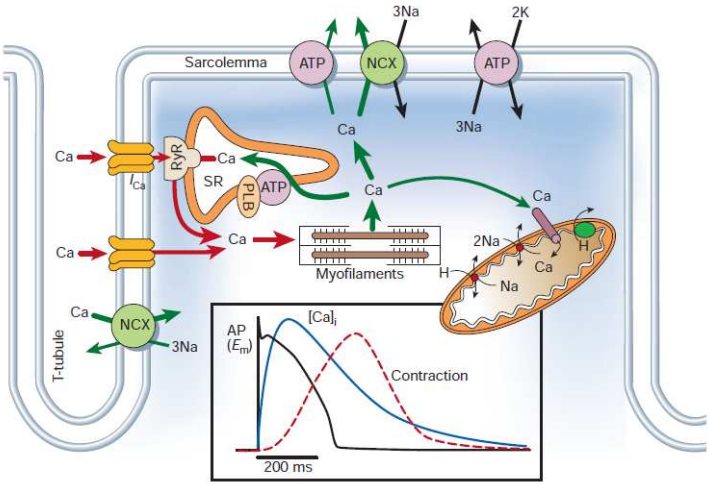


Figure 7: Excitation-contraction-coupling. The figure shows how Ca^{2+} is transported over the cell membrane and triggers Ca^{2+} induced Ca^{2+} release from the SR. Adapted from Bers (2002).

Figure 7 describes how Ca^{2+} enters the cardiac cell and triggers opening of ryanodine-receptor (RyR) channels on the sarcoplasmatic reticulum (SR), which in turn leads to a Ca^{2+} induced Ca^{2+} release from the SR (CICR). Ca^{2+} influx and SR-Ca^{2+} release result in a raised free intracellular Ca^{2+} concentration. As mentioned in section 2.1, the cardiac muscle fibres are built up with organized arrangements of the contractile proteins actin and myosin. The cytosolic free Ca^{2+} binds to Troponin C which is attached to the actin filaments. The binding enables myosin to bind to the actin filament, and movement of the actin filaments result in contraction of the cardiac muscle fibres (Silverthorn 2007; Bers 2002).

The intramembranous Ca^{2+} channels are an important difference between cardiac and skeletal muscle cells because skeletal muscle cells do not have Ca^{2+} channels in their cell membrane and only receive Ca^{2+} from the SR (Cunningham & Klein 2007).

2.2.6 Innervation of the heart

The heart is innervated by the sympathetic and parasympathetic nervous system (Patteson 1999). The parasympathetic nerves affect the SA and AV nodal cells, by decreasing the heart rate (SA), slowing down conduction and lengthen the refractory period (AV). The sympathetic nerves affect all regions of the heart; SA node, AV node and all myocardial cells. Sympathetic stimulation leads to higher, shorter action potentials and to stronger and quicker contractions. In quietly resting horses there is often a very strong parasympathetic activity and AV node refractory period is so long, that some atrial beats are not conducted to the ventricles. This is called AV block and the horse will have some "missing" beats. The arrhythmia will disappear after exercise (Cunningham & Klein 2007, Verheyen *et al*. 2010b).

2.3 Molecular aspects of electrophysiology in the heart

2.3.1 Important ventricular ion currents

As mentioned in section 2.2.4 and showed in figure 6, there are differences in the action potential morphology and duration in the distinct areas of the heart. The differences can be explained by a variety in ion channel expression levels (Nerbonne & Kass 2005).

The ion flow through an ion channel is described by its current (I). Voltage gated Na^+ - and Ca^{2+} ion channels (Nav- and Cav-channels) and their respective currents (I_{Na} and I_{Ca}), are similarly expressed in human atrial and ventricular myocytes. In contrast, multiple types of repolarising K^+ currents (I_k) particularly through voltage gated channels (Kv-channels) are expressed differently in atrial and ventricular cells and give rise to different action potential morphologies (Nerbonne & Kass 2005). Between species, variations in action potential morphology and duration have also been explained by differences in ion channel expression levels (Rosati *et al*. 2008).

Figure 8 illustrates the ion currents and channels that are important for the ventricular depolarisation and repolarisation. Only these channels will be discussed here, because mutations in the respective channels are associated with changes in the QT interval and increased risk of severe ventricular arrhythmias (Finley *et al.* 2003; Kramer & Zimetbaum 2011). In the following text the long QT syndrome will be in focus.

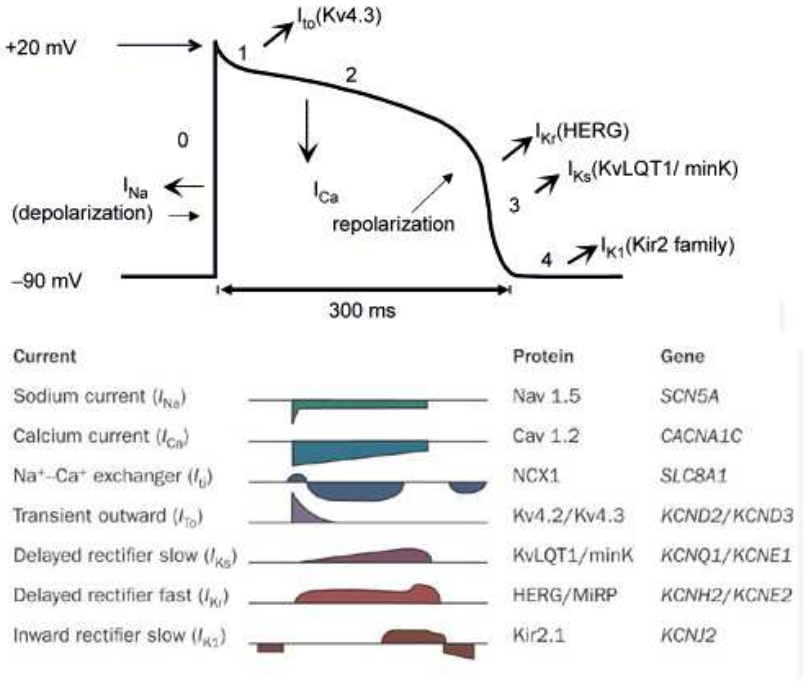


Figure 8: Ionic currents and channels of the cardiac action potential. Coordinated opening and closing of ionic channels mediate the action potential. Depolarising inward (downward deflection) and repolarising outward (upward deflection) ion currents are shown (modified from Ruan *et al*. 2009 and Vaz *et al*. 2005).

Voltage gated sodium (Na^+) channel – Nav1.5 (I_{Na})

The voltage gated Na^+ channel (Nav) show an overall topology similar to the voltage gated Kv-channels, which structure and function is described in section 2.2.3. The Na^+ current (I_{Na}) is responsible for the rapid depolarisation (phase 0) in ventricular and atrial cells (figure 8). There are a number of different Nav-channels, but the α-subunit that is most expressed in the mammalian myocardium is Nav1.5 encoded by the SCN5A gene (Nerbonne & Kass 2005). Both the activation and inactivation of these channels are rapid and voltage dependent (Catterall 2000a). The threshold for activation is at approximately –55 mV, and during the plateau phase about 99% of the I_{Na} channels are inactivated (Nerbonne & Kass 2005). The channel is inactivated by the fast, N-type inactivation (figure 4A) (Ashcroft 2000; Catterall 2000a).

Voltage gated calcium (Ca^{2+}) channel – Cav1.2 ($I_{Ca,\,L}$)

Depolarisation leads to activation of voltage gated Ca^{2+} channels (Cav) in the cardiac cell membrane (Cunningham & Klein 2007; Bers 2002; Nerbonne & Kass 2005). The I_{Ca} results in Ca^{2+} induced Ca^{2+} release (CICR) and high intracellular Ca^{2+} concentrations which switches on the contractile machinery, as described in section 2.2.5 (Bers 2002; Cunningham & Klein 2007). There are two known types of Cav-channels; high- and low-voltage gated channels. The high-voltage-dependent L-type Ca^{2+} channel is responsible for the I_{Ca} in the heart and is activated at membrane potentials above –20 mV (Nerbonne & Kass 2005). The channel is called Cav1.2 and is encoded by the gene CACNA1C (Catterall 2000b; Nerbonne & Kass 2005). L-type Ca^{2+} channels are responsible for the plateau phase 2, which is long in ventricular myocardial cells, as seen in figure 6 (Nerbonne & Kass 2005).

Inactivation of L-type Ca^{2+} channels is both voltage and Ca^{2+} dependent. The Ca^{2+} dependent is more rapid and results from binding of Ca^{2+} and calmodulin to the C-terminus of the Cav1.2 channel (Catterall 2000b; Ashcroft 2000). Inactivation of the Ca^{2+} channels and removal of Ca^{2+} from the cytoplasm is essential for muscle relaxation and diastolic filling of the heart (Bers 2002). Ca^{2+} is cleared from the cytoplasm by several mechanisms, where the main routes are via the SR-ATPase pump that transports Ca^{2+} back into the SR, and extrusion of Ca^{2+} via Na^{+}/Ca^{2+} exchange. The Na^{+}/Ca^{2+} antiporter (NCX1) exchanges 3 Na^{+} and 1 Ca^{2+} ion, and extrusion of Ca^{2+} is favoured by relatively high intracellular Ca^{2+} concentrations and negative repolarising membrane potentials. The relative amounts of Ca^{2+} being removed by the different mechanisms varies between species (Bers 2002).

Potassium (K^{+}) inward rectifier channel – Kir (I_{K1})

The name may confuse, but the resting membrane potential (section 2.2.2) is stabilised near the K^{+} equilibrium potential (E_K) by an outward current of K^{+} ions through the inward rectifier channels (Kir) at potentials just above the E_K (Kubo *et al*. 1993; Ashcroft 2000). Molecular studies have shown that the K^{+} current (I_{K1}) flows best through the channel in an inward direction and that the flow is largest at action potentials below the $E_{K,}$ but such potentials will normally not exist. However, the inward current has been suggested to have an effect in preventing excessive hyperpolarisation of the cardiac cell (Kubo *et al.* 1993). The current is blocked during depolarisation by intracellular cations (Mg^{2+}) and polyamines, and the channel opens on hyperpolarisation due to fast Mg^{2+} unblocking (Ashcroft 2000; Hibino *et al.* 2010).

Voltage gated potassium (K^+) channels – Kv

There is a huge variety of voltage gated K^+ ion channels, originating from a number of genes in the mammalian genomes encoding this channel type (Ashcroft 2000). In most myocardial cells, several Kv channels are expressed and each channel type contributes to shaping the wave forms of the action potential (Nerbonne 2000). The Kv4.2/4.3, KCNQ1 and HERG channels, and their respective K^+ currents (I_K), will be described in this section, because they serve important roles in the cardiac action potential repolarisation (Nerbonne 2000). In some species, there are marked differences in the channel densities and K^+ currents expressed in different myocardial cell types, which give rise to a variation in the morphology of the action potential (Liu *et al.* 1993; Liu & Antzelevitch 1995; Nerbonne 2000).

Fast, transient outward K^+ current – Kv4.2/Kv4.3 ($I_{to,\,f}$)

The transient outward current $I_{to,\,f}$ activate and inactivate rapidly and underlie the early phase of repolarisation (phase 1 in figure 7) (Nerbonne 2000; Nerbonne & Kass 2005). $I_{to,\,f}$ is expressed in both atrial and ventricular tissue in many different species. Robust expression of the $I_{to,\,f}$ in epicardial and mid-myocardial cells give rise to the "notch" seen on the action potential in these cells (Liu *et al.* 1993; Nerbonne & Guo 2002).

Slow, delayed rectifier K^+ channel – KCNQ1 (I_{Ks})

KvLQT1 is a slow, delayed rectifier potassium channel, and it is encoded by the gene KCNQ1. The I_{Ks} is important for the repolarisation phase 3 (figure 8). Mutations in this gene give rise to the most common form of long QT syndrome (LQTS) in humans (LQTS1), and the ion channel was found through genetic analysis of human patients with LQTS. KCNQ1 mRNA is strongly expressed in the heart, and lower levels are found it pancreas, kidney, lung and placenta. In similar manner, a small peptide called minK, is expressed in the mentioned tissues. minK is encoded by the gene KCNE1 (Ashcroft 2000). Experiments have showed that expression of KCNQ1 alone yields rapidly activating and non-inactivating outward K^+ currents, unlike any known cardiac K^+ currents. Co-expression of minK with KCNQ1 induced a slowly activating delayed-rectifier current that was much larger than the currents from KCNQ1 alone, and similar to properties of I_{Ks} in the heart (Sanguinetti *et al.* 1996; Barhanin *et al.* 1996; Nerbonne & Kass 2005). minK may therefore be considered to serve as a β-subunit for the I_{Ks} channel in the heart (Ashcroft 2000). Stimulation of β-autonomic receptors (sympathetic nervesystem) activates KCNQ1 and increases the slow outward

I_{Ks} (Marx *et al.* 2002). Another modulator is cytosolic Ca^{2+}, which seem to increase the expression and effect of the KCNQ1/KCNE1 via Ca^{2+} sensitive receptors (Gamper et al. 2005; Pedersen 2010). At high heart rates there will be a large amount of Ca^{2+} in the cytosol, and the effect of I_{Ks} seem to be important for the fast repolarisation at high heart rates.

Different action potential duration in epi- endo- and mid-myocardial cells has been explained by different expression of the KCNQ1 channels (Viswanathan *et al.* 1999). Liu & Antzelevitch (1995) showed that a low density of I_{Ks} in mid-myocardial cells resulted in a longer action potential in these cells.

Rapid, delayed rectifier K^+ channel – HERG (I_{Kr})

HERG (human-*eag*-related gene) is the α-subunit of the rapid, delayed rectifier potassium current, I_{Kr}, encoded by the gene KCNH2 (Nerbonne & Kass 2005). The channel will be referred to as HERG, or ERG in other species. A K^+ channel was found to be responsible for a "shaking-legs" (go-go dancing) behaviour demonstrated by mutant fruit flies (*Drosophila*) when they were anesthetised with ether, and thereby given the name eag- (ether-à-go-go) K^+ channel family. The gene was isolated from the fruit fly and a related gene was later demonstrated in human cDNA and given the name HERG (Warmke & Ganetsky 1994; Ashcroft 2000).

HERG was demonstrated to have nearly identical properties to the I_{Kr} in cardiac myocytes, but it was suggested that there might be an additional subunit required in the native I_{Kr} (Sanguinetti *et al*. 1995). MiRP1/KCNE2 (minK-related peptide 1) is a small integral membrane subunit and co-expression of MiRP1 with HERG has been demonstrated to alter HERGs' function and give it properties that resemble the native I_{Kr} in the heart (Abbott *et al*. 1999). Curran *et al*. (1995) demonstrated that that HERG was strongly expressed in the heart and that mutations in HERG caused LQTS2.

The HERG channel, as other Kv-channels, has a K^+ selective pore with two "doors" (the activation and the inactivation gates) that open and close in a voltage- and time-dependent matter, as demonstrated in figure 9 (Witchel *et al*. 2002).

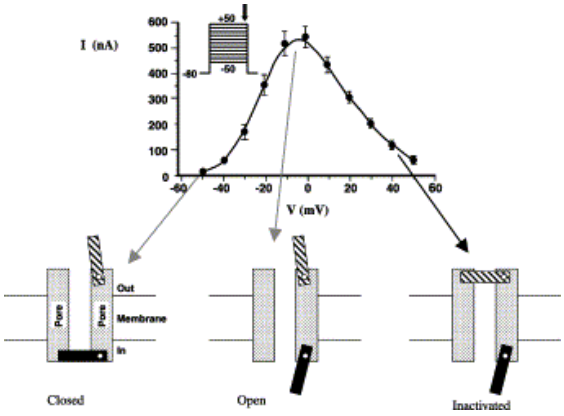


Figure 9 The "two gate" model of the HERG channel. Both gates must be open for a current to flow through, and the current is shown in the graph. The black "door" is the activation gate which is closed at repolarised membrane potentials. The striped "door" is the inactivation gate, which opens at a faster rate than the activation gate closes during repolarisation, resulting in a large current through the channel. Adapted from Witchel *et al.* (2002).

Both gates must be open to enable K^+ to flow through the channel. Depolarisation opens the activation gate, but in contrast, closes the inactivation gate. On the other hand, repolarisation closes the activation gate and opens the inactivation gate. Unlike other Kv-channels, the inactivation gate of HERG responds to voltage changes faster than the activation gate. When the membrane is at depolarised potentials, much of the HERG channel population is in an inactivated state and there is no current through the channel. When the membrane repolarise, the inactivation gates open at a faster rate than the activation gates close. This results in a large current through the open channel during the repolarisation (Witchel *et al*. 2002), phase 3 (figure 8).

2.3.2 Voltage gated potassium channels in the equine heart

Finley *et al.* 2002 demonstrated the expression of both ERG (I_{Ks}-α-subunit) and KCNQ1 (I_{Kr}-α-subunit) in the equine heart. The channels are expressed in a generalised distribution pattern throughout the atrium and the ventricular endo- and epicardium. By gene sequencing, the same authors found 83 % shared identity between the equine KCNQ1 gene and the human clone. The β-subunits KCNE1, KCNE2 and KCNE3 are expressed in the equine atrium and ventricle, and KCNE1 associate with both KCNQ1 and ERG in the horse (Finley *et al.* 2002).

In the same experiment, a specific I_{Ks} antagonizer, Chromanolol 293B, was used to establish the function of I_{Ks} in the equine heart. It was demonstrated that blocking of I_{Ks} resulted in prolonged epicardial action potential duration (APD), which indicated that I_{Ks} is an important, repolarising potassium current in the equine ventricle (Finley *et al*. 2002). MK-499 (class III antiarrhythmic agent) and cisapride was used to demonstrate the function of I_{Kr} in the horse, both of which resulted in prolongation of the equine ventricular APD. In conclusion, both the rapid and slow components of the delayed rectifier contribute to the action potential repolarisation in the horse (Finley *et al*. 2002). An ultrarapid, delayed rectifier potassium current, I_{Kur}, is also expressed in both equine atrial and ventricular tissues and contributes to phase 2 and 3 of cardiac repolarisation. I_{Kur} activates very rapidly and inactivates very slowly, and has been suggested to contribute to the rate-related adaption of the action potential duration required for the wide range of heart rates in horses (Finley *et al*. 2003).

In two master thesis studies on this faculty, the equine KCNH2/ERG has been successfully sequenced and large parts of the equine KCNQ1 and the gene sequences share a large similarity with the human sequences (Pedersen 2010; Olander 2012).

2.4 Clinical aspects of electrophysiology in the heart

It is essential to describe the principles of electrocardiography and the electrocardiogram (ECG), in order understand evaluation of the electrophysiology of the heart in a clinical situation. Mutations in the ion channels described in the previous section will lead to changes in the ECG. Understanding of how the normal ECG is created is important to be able to draw any conclusions regarding the electrophysiology of the heart of a patient.

2.4.1 Electrocardiography - Lead system and function

The surface electrocardiogram (ECG) is widely used to measure electrical activity in the heart and is the gold standard for diagnosing arrhythmias (Verheyen *et al*. 2010a).

Einthoven's triangle is a bipolar lead system in which the heart is assumed to sit in the centre of a triangle formed by the two forelimbs and the left hind limb, as demonstrated in human (figure 10, left) (Patteson 1999; Tilley 1992).

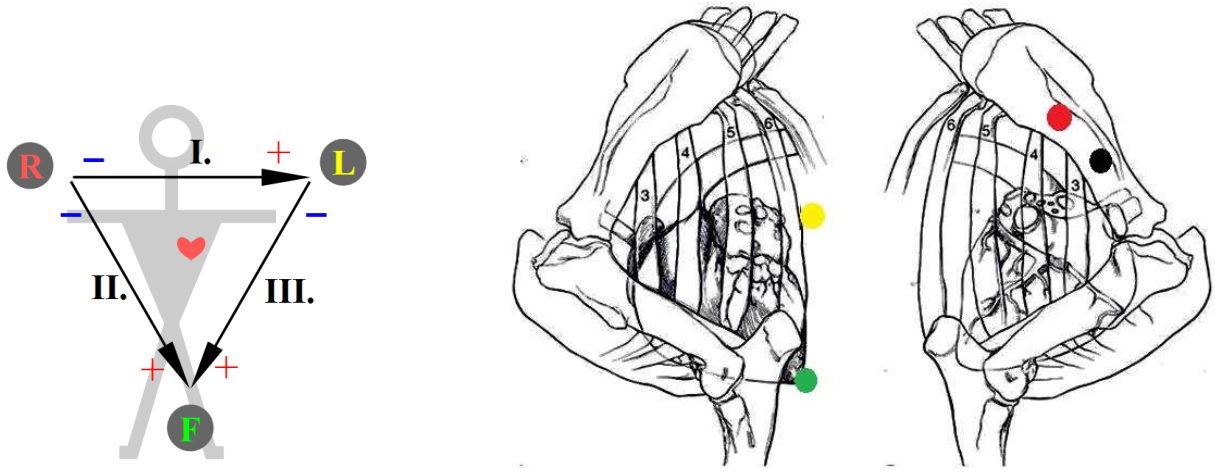


Figure 10: Left: Einthovens' triangle. Adapted from https://commons.wikimedia.org/wiki/File:ECG-Einthoven-triangle.svg. **Right: The equine heart and its placement in thorax with electrodes as they were placed in this study.** (modified from image by Ph.D. stud. Mette Flethøj).

This system is commonly used in human and small animals, and can also be used for ECG recording in horses at rest. The ECG complexes of the traditional limb leads are sometimes small in the horse, and over the years, many different ECG lead systems have been developed for use in horses to accommodate the fact that, in the horse, the heart does not sit in the centre of a triangle formed by the limbs (figure 10, right). A base-apex lead and modifications of this lead are examples

of lead systems used in horses, to determine heart rate and rhythm (Patteson 1999; Miller 1987; Lannek & Rutqvist 1951; Fregin 1982).

As described in section 2.3.1, different currents of ions are flowing across the cell membrane at various points during the depolarisation and repolarisation. These currents result in an electrical field around the cardiac cells, which creates a dipole (Tilley 1992; Patteson 1999). Gap junctions between the cardiac cells create a functional syncytium and they behave electrically like one large cell. In simplified terms; the heart can be seen as one dipole. The sum of electrical effects creates an electrical field around the heart that can be recorded from the body surface (Verheyen *et al*. 2010a; Cunningham & Klein 2007; Patteson 1999).

The sum of the electromotive forces has a direction and magnitude, which is called the cardiac vector. The electrocardiograph records the differences in electrical activity between a positive and a negative electrode (bipolar lead system). The ECG is a graph that shows the variations in voltage produced by the cardiac muscle cells over time (Miller & Bonagura 1985b; Tilley 1992). A positive deflection will be seen if the cardiac vector is towards the positive electrode, and a negative deflection is seen if it is towards the negative electrode (Tilley 1992; Patteson 1999).

2.4.2 The electrocardiogram (ECG)

The P-QRS-T deflections seen on an ECG (figure 11) represent the wave of depolarisation and repolarisation that spreads throughout the heart and releases the contractile forces of the heart (Tilley 1992).

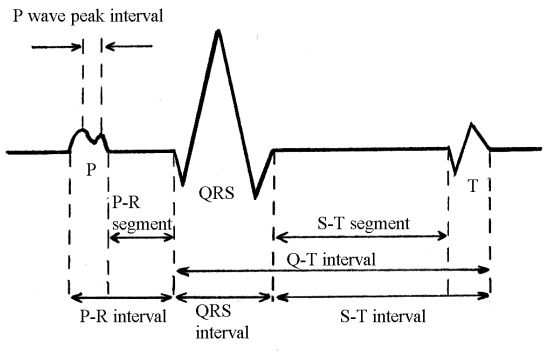


Figure 11: Determination of electrocardiographic waves, segments and intervals for measurments. Adapted from Bakos & Lohne (2009).

The **P-wave** is created by atrial depolarisation (Miller & Bonagura 1985a). The morphology of the equine P wave is variable, but often bifid or notched (Buss *et al.* 1975; Verheyen *et al*. 2010a). Single peaked, biphasic and polyphasic P-wave patterns can be seen in normal horses (Miller & Bonagura 1985a). The first peak of the bifid P wave has been described to represent depolarisation of parts of the right atrium, whereas the second peak represents activation of the atrial septum and parts of the left atrium (Miller & Bonagura 1985a). Atrial repolarisation has been described by a downward deviation of the P-R interval (T_a-wave), but is often not seen because of a large QRS complex (Miller & Bonagura 1985a; Verheyen *et al*. 2010a).

The **P-R segment** represents the time it takes for an impulse to travel from the SA node to the ventricle (Tilley 1992). The specialised cells of the AV node conduct the impulse slowly, and a small number of cells are depolarised, therefore no deflection is seen on the ECG (Patteson 1999).

The **QRS complex** represents the depolarisation of the ventricular myocardium, and is composed of various components, defined according to international agreements (Verheyen *et al*. 2010a). The R-wave is the first positive deflection, the Q-wave is the first negative deflection that precedes an R-wave, and the S-wave is the first negative deflection that follows an R-wave. Subsequent positive and negative deflections are termed R'- and S'-waves, respectively (Tilley 1992; Verheyen *et al*. 2010a). According to these definitions, the horse usually does not have a "QRS" morphology, whereas the rS or rSr' morphology is most common (Verheyen *et al*. 2010a). In the horse, the Purkinje fibres spread widely in the free wall of the heart and the ventricular myocardium will be depolarised from multiple sites. Simultaneous depolarisation results in cancelling of much of the electromotive force, and the "QRS"-deflection seen on the ECG are mostly created by depolarisation of the ventricular septum and the left ventricular wall (Miller & Bonagura 1985a; Hamlin 1965). Figure 12 shows a typical ECG recording in a horse at rest.

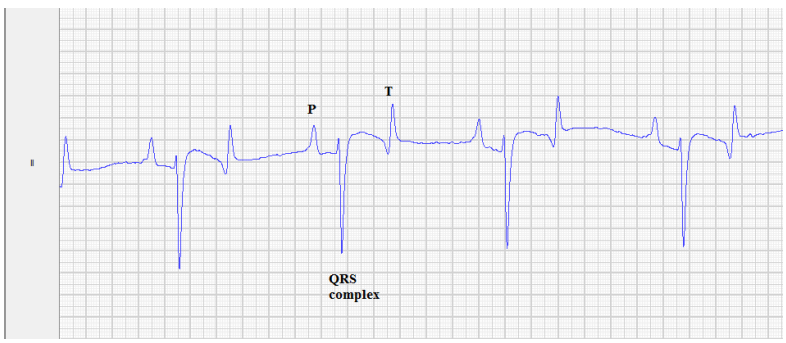


Figure 12: A normal ECG (lead II) from an Icelandic horse at rest. Gain 40 mm/mV, Feed 50 mm/sec. The recording demonstrates four consecutive beats (four P-QRS-T-complexes). The P wave appears to be positive, the "QRS"-complex demonstrate "rS" morphology and the T wave is biphasic with a larger positive deflection (from a recording in the present study).

All the ventricular cells are at their plateau phase (phase 2), hence no deflection is seen in the **S-T segment** (Cunningham & Klein 2007). The **T-wave** represents the repolarisation of the ventricles (Tilley 1992). In horses, the T-waves' size and morphology is variable (Holmes & Rezakhani 1975; Verheyen *et al*. 2010a). Positive, negative and biphasic T-wave morphology have been described in literature for both horses and ponies (Buss *et al.* 1975; Fregin 1982; Ayala *et al.* 1999), and the T-wave can also be variable within one recording (Reef & McGuirk 2009). $\mathbf{T_{peak} - T_{end}}$ (T_pT_e) is the interval from the peak to the end of the T-wave. It has been described in human medicine as a measure of transmural dispersion of repolarisation (Antzelevitch *et al*. 2007). The T-wave is a result of repolarisation of the endo-, myo- and epicardium of the ventricles, which all have different action potential durations (figure 6, section 2.2.4). Repolarisation of the epicardium give rise to the ascending limb of the T-wave and T_p is the peak where the epicardium is fully repolarised. Repolarisation of the myocardial cells gives rise to the descending limb of the T-wave (T_pT_e) (Antzelevitch 2001). In humans, the T_pT_e seems to be independent of HR, sex and age (Haarmark *et al*. 2010).

The **QT interval** is the time from the beginning of ventricular depolarisation to the end of ventricular repolarisation. It represents the ventricular systole, and is typically measured in seconds or milliseconds (msec) (Tilley 1992; Cunningham & Klein 2007). Prolongation of the QT interval (long QT syndrome) is associated with serious ventricular arrhythmias, syncope and sudden death, which will be described in section 3. Shortening of the QT interval (short QT syndrome) is a very rare condition in humans, and is associated with atrial fibrillation and increased risk of sudden death (Hedley *et al.* 2009), but will not be further discussed in this rapport. As mentioned, the ventricular depolarisation in the horse often has a different morphology than the typical "QRS"-complex, due to different types of leads that are used. The term "QT interval" will be used in this rapport when discussing the time interval from the start of the deflection of ventricular depolarisation to the end of the T-wave.

2.4.3 Clinical approach to the ECG and arrhythmias

It is important that the clinician uses a systemic approach when analysing an ECG recording. First the QRS-complexes should be identified. Every QRS-complex should be followed by a T-wave, and preceded by a P-wave. The P-waves should always be followed by a QRS-complex. The quality of the ECG recording should be evaluated and checked for the presence of artefacts (Reef & McGuirk 2009). The morphology and time intervals for the ECG-deflections, segments and

intervals should be evaluated, and normal values for the horse can be found in literature (Bakos & Lohne 2009; Patteson 1999; Bonagura *et al.* 2010). These values most often represent ECG values in horses at rest.

The morphology of the P-waves and QRS-complexes should be evaluated for identical or normal morphology. As mentioned in the previous section, the morphology of the T-waves can change during an ECG recording. The atrial and ventricular rates can be evaluated by measurements of PP- and RR- intervals. In a normal ECG, these intervals should be regular and the atrial and ventricular rate should be identical. The rate is categorised as either too slow (bradycardia), normal, or too fast (tachycardia) (Reef & McGuirk 2009).

Cardiac arrhythmias are often categorized by their site of origin (ventricular, "junctional" (AV-nodal or Bundle of His) or supra-ventricular) and by their rate (bradyarrhythmias or tachyarrythmias). In horses, most bradyarrhythmias are physiological, without pathologic or functional cardiac disorders, and are associated with a high vagal (parasympatethic) tone. Most tachyarrhythmias are considered to be pathologic (Bonagura & Miller 1985; Miller 1987; Reef 1999). Tachyarrhythmias usually result in poor performance, exercise intolerance, syncope or sudden cardiac death (Miller 1987).

Bradyarrhythmias are variations in normal sinus rhythm, often seen in the horse. Examples of such arrhythmias are sinus bradycardia, sinus arrhythmia, sinus block, or AV block. Second-degree AV block (2AVB) is the most common bradyarrhythmia detected in resting horses (Miller & Bonagura 1985c; Reef 1999). In the ECG this can be seen as occasional P-waves not followed by a QRS-complex (Reef 1999). It is important that the clinician recognise these physiological arrhythmias, and know that these vagal induced irregularities will disappear during exercise (sympathetic activity) (Miller 1987).

Tachyarrhythmias are the most serious and life-threatening arrhythmias. The tachyarrhythmia can either be a result of abnormal SA node pacemaker activity, or from an ectopic pacemaker. An ectopic pacemaker can develop as a result of damage to a few localised cells which act as a focus of abnormal depolarisation (Patteson 1999). Slow conduction through the damaged cardiac cells can result in re-entry of an action potential, and thereby initiate extra beats (Cunningham & Klein 2007).

Supra-ventricular arrhythmias are common in the horse, and are a result of abnormal automacity or disturbed intra-atrial conduction (re-entry) before SA nodal automacity. Occasional isolated atrial premature complexes (APCs) (extra beat) are generally well tolerated, and therapy is rarely indicated because the ventricular rate is seldom affected. However, frequent APCs may lead to atrial fibrillation, which may result in poor performance and exercise intolerance, which will require treatment. In these cases the horse is presented with an ECG with irregular RR intervals, no P-waves, normal appearing QRS-complexes, and a rapid baseline fibrillation ("f"-waves) (Miller & Bonagura 1985d; Reef 1999).

Ventricular tachycardia is a life-threatening arrhythmia because it results in ventricles that do not relax long enough for adequate filling, and it stops the heart from pumping blood, regardless of normal atrial function (Cunningham & Klein 2007). Ventricular arrhythmias can either result from one or multiple ectopic focuses. Multiple ventricular premature complexes (VPCs) are associated with an increased risk of developing life-threatening ventricular tachyarrhythmias, like ventricular fibrillation and *torsade de pointes* – a phenomenon which will be described in section 3.2 (Bonagura & Miller 1985; Reef 1999).

3. The QT interval

3.1 Factors affecting the QT interval

It is interesting to study the QT interval because it is a way to assess the ventricular depolarisation and repolarisation clinically (Kramer & Zimetbaum 2011). Changes of the QT interval can lead to fatal arrhythmias, as described shortly in section 2.4.2, but it is important to know that many (physiological) factors can have an influence on the QT interval. Little is known about the QT interval in horses, but in humans some of the factors known to affect the QT interval are; heart rate (HR), autonomic nerve system, electrolyte disorders, age, sex and drugs affecting important cardiac ion channels (Funck-Brentano & Jaillon 1993; Al-Khatib *et al*. 2003).

The QT interval is prolonged at slow heart rates (HR) and shortened at faster HR, and many formulas have been proposed to adjust for these variations (Al-Khatib *et al*. 2003). The formulas attempt to "correct" the QT interval to a value "QTc", which is HR independent. The Bazett and Fridericia are two formulas widely used for HR-correction in humans, but they do have some limitations. In humans, the Bazett formula often give much higher values of QTc at higher HR and

generally lower values for QTc at lower HR. The Fridericia formula is more precise than Bazett in humans (Luo *et al.* 2004).

In humans, the QT interval has a negative linear trend with HR (Rajappan et al. 2003; Luo *et al.* 2004), whereas in horses, the QT interval seems to be best corrected with a non-linear regression model (Pedersen *et al*. 2012). When the HR changes there is a delayed adaption of the QT interval, which is called QT lag (Lang *et al*. 2001). QT hysteresis is another phenomenon described in human medicine, which is characterised by longer QT intervals at a given RR interval while the HR is increasing and shorter QT intervals at the same RR interval while the HR is decreasing. The mechanism behind is uncertain, but the autonomic nerve system appear to have an influence (Pelchovitz *et al.* 2012).

3.2 Long QT syndrome

Articles describing long QT syndrome in animals and its potential relevance to veterinary patients are sparse (Finley *et al.* 2003).

LQTS in humans can be of both acquired and hereditary origin, and are both important causes of sudden cardiac death (SCD) (Kramer & Zimetbaum 2011). Some factors that predispose to prolongation of the QT interval in humans are high age (Reardon & Malik 1996) and female gender (Reardon & Malik 1996; Bidoggia *et al*. 2000). Electrolyte disturbances, heart failure and drugs can cause an acquired LQTS (Al-Khatib *et al*. 2003; Anderson *et al.* 2002; Kramer & Zimetbaum 2011). In horses, stallions have been suggested to have longer QT intervals in relation to HR, than mares, but very few studies have been done (Pedersen *et al*. 2012).

Congenital LQTS is often caused by “loss of function”-mutations in genes encoding the subunits responsible for the rapid (I_{Kr}) or slow (I_{Ks}) delayed rectifier K^+ currents, or by “gain of function”-mutations in the genes encoding the fast voltage gated Na^+ channel (I_{Na}) (Anderson *et al*. 2002). LQTS is characterised by abnormally prolonged cardiac repolarisation, presented in the ECG as prolonged QT intervals (Kramer & Zimetbaum 2011). LQT1 is associated with a mutation in the KCNQ1-gene, which encodes the I_{Ks} potassium channel α-subunit (KvLQT1). LQT2 is associated with a mutation in the KCNH2-gene, encoding the I_{Kr} potassium channel α-subunit (HERG). Mutations in the SCN5A-gene, encoding the α-subunit (Nav 1.5) of the cardiac I_{Na} sodium channel

are responsible for the LQT3 syndrome (Kramer & Zimetbaum 2011). LQT1 and LQT2 account for the majority of LQTS in human patients (>90 %) (Anderson *et al.* 2002).

Torsade de Pointes (*TdP*) is a polymorphic ventricular tachycardia, which is associated with LQTS, and can result in SCD (Kramer & Zimetbaum 2011). On the ECG, *TdP* is characterised by QRS and T complexes that are twisting around the base line (Reef 1999). Delayed inactivation of calcium channels, due to prolonged cardiac action potential, results in a late inflow of Ca^{2+}. This contributes to formation of early after-depolarisations (EADs) in phase 2 or 3 of the action potential (Viskin 1999; Anderson *et al.* 2002). After-depolarisations of sufficient magnitude can trigger repetitive action potentials and be the initiating cause of *TdP*, which can result in ventricular fibrillation and SCD (Viskin 1999).

4. Sudden death in horses

As described shortly in the introduction, sudden death (SD) is a well-known phenomenon in all types of horses, although given the most attention among racehorses. In a study of *post mortem* findings in 268 cases of SD in race horses, a definitive diagnosis was made in only 53 % (143/268) of the cases. The major pathological findings among these horses were primarily cardiac or pulmonary failure and haemorrhagic shock. In the remaining 47 % (125/268) of the cases, the causes of SD remained unexplained or got a presumptive diagnose often related to cardiac or cardiopulmonary failure (Lyle *et al.* 2011). In a similar, but smaller study, the cause of SD was undetermined in 68 % (17/25) of the cases. The cause of death in these cases was thought to be due to myocardial lesions leading to ventricular fibrillation and sudden cardiac death (SCD), but histological evidence of such lesions was seldom found (Gelberg *et al.* 1985).

Often there is an absence of gross pathological or histological cardiac lesions, and Lyle *et al*. (2011) explained this by the fact that arrhythmias leading to SD may reflect a functional rather than a structural cell abnormality. Kiryu *et al.* (1999) managed to record an ECG from a race horse during the terminal event of SD shortly after intensive training. The recording exhibited a variety of arrhythmias, like atrial premature complexes (APCs), multiple ventricular premature complexes (VPCs), followed by the “R on T”-phenomenon that deteriorated into ventricular fibrillation (VF), which resulted in cardiac arrest.

5. The Icelandic horse

Horses were brought to Iceland in the second half of the ninth century by settlers from Scandinavia. Living on Iceland without horses would have been impossible for the new settlers; transport and communication depended on them (Björnsson & Sveinsson 2006). Natural disasters and many centuries of selective breeding have resulted in a very small gene pool in the Icelandic horse (Campana *et al*. 2011). For similar reasons, the Icelandic horse is one of very few species in the world that has both tölt and pace, in addition to the traditional gaits; walk, trot and gallop (Björnsson & Sveinsson 2006). Import of horses to Iceland has been illegal since the mid-1800s, in order to protect the horses from foreign diseases (Trolle 1997; Campana 2011).

The average height of the modern Icelandic horse is about 138 cm, and its ideal weight is about 380-400 kg (Trolle 1997; Björnsson & Sveinsson 2006). The Icelandic horse has a widespread international distribution, and it is used as a riding horse for leisure, sport, competion and breeding shows. Sport competitions are in either four- or five-gate competitions on an oval track, tölt competition, „gæðinga“-competition or pace-racing. The horses are judged in every gate, and among the judging criterias are; clear rhythm, long strides, high movements, collection, spirit and form under rider (Björnsson & Sveinsson 2006).

To the authors’ knowledge, there are no specific cardiac diseases in the Icelandic horse and sudden cardiac death in this breed has not been described in literature.

6. Hypotheses and Aims of project

Based on the findings in the study of Pedersen *et al.* (2012) and knowledge of the QT interval in humans, the following hypotheses will be investigated:

6. 1 Hypotheses

- The QT/RR curve in the Icelandic horse follows a piecewise linear regression model.
- There is a difference in the QT interval between geldings, mares and stallions. Longer QT intervals in stallions are expected.
- The TpTe is not dependent on HR/RR or sex.

6. 2 Aims of project

1. Obtain electrocardiographic recordings of Icelandic horses during variable intensity levels.
2. Measure QT intervals at different HRs in order to study the relationship between the QT- and RR intervals in the Icelandic horse. All three sexes (mares, geldings and stallions) will be included in the study in order to create a sex-differentiated model, where any possible differences can be detected.
3. Find the best descriptive model for HR-correction of the QT interval in Icelandic horses, and establish reference values for the QT interval at different heart rates in Icelandic horses.
4. Measure $T_{peak} - T_{end}$ interval (T_pT_e) to study its normal range and relation to RR interval in Icelandic horses.

7. Materials and methods

Horses

32 Icelandic horses were used in the present study. Name, age, sex and exercise level were obtained. The horses were recruited from 4 different stables, and 22 of them were from the same stable. A physical examination with auscultation of the heart and lungs were obtained. Normal heart rhythm was classified as normal sinus rhythm (NSR), and any sinusarrhythmias or 2nd degree AV blocks (AVBII) were registered. The heart rate, respiratory rate and body temperature were measured. The weight of the horse was estimated (BWT_e) with a special weight measure band around the cranial part of thorax, caudal to the withers and the elbows.

Placement of electrodes

The ECG recordings were obtained with a veterinary telemetric ECG system (Krutech-Televet 100, Kruuse Denmark)[1] (Televet users Guide V5.0.0). A four lead ECG was used, and the electrodes (KRUUSE ECG Electrodes)[2] were placed in a modified base-apex lead as, showed in figure 13 (figure 10 shows the electrodes in relation to the heart).

Right side: Red electrode (negative -/-) equal to right arm (RA) placed on the upper third of the shoulder. Black electrode (neutral) placed approximately 10 cm cranio-ventral to the red electrode, along the axis of the scapulae.

Left side: Yellow electrode (positive/negative +/-) equal to left arm (LA) placed at the middle part of the thorax approximately between the 6th and 7th costa (in the girth area). The green electrode (positive +/+) equal to left leg (LL) placed approximately 3 cm to the left of the ventral midline.

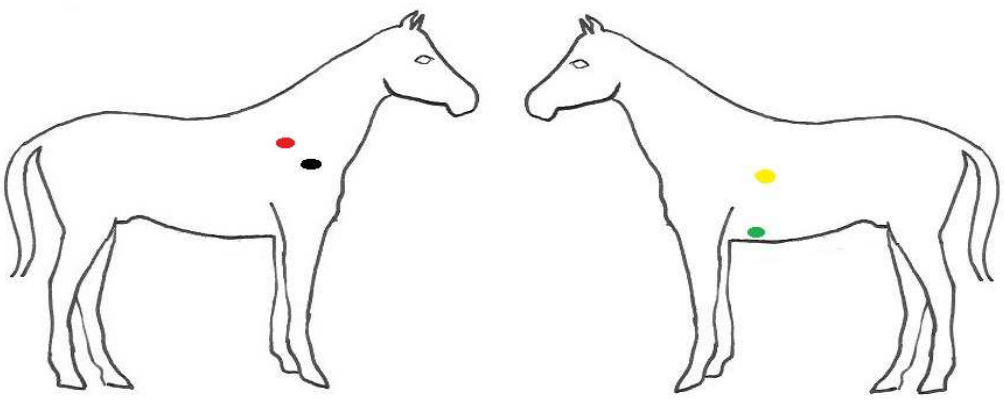


Figure 13: Placement of electrodes. Modified from http://www.allaboutdrawings.com/pencil-horse-drawings.html (idea from Almind & Petersen 2010)

For optimal contact, the skin was clipped and rinsed at the areas of electrode connection. A sticky patch (Snögg, Kruuse, Denmark)[2] was used to attach the electrodes to the skin. After the ECG wires were connected to the electrodes, a piece of tape (Durapore, Kruuse, Denmark)[2] was covering the electrode for further stability of the connection. An elastic girth was placed above the green and yellow electrodes. The ECG device was secured to the girth with an elastic bandage (Co plus, Kruuse, Denmark)[2] on the left side of the wither. The ECGs were recorded and stored on a SD-card in the ECG Televet device and data were transferred to a USB stick and a computer afterwards.

ECG recordings

The ECGs were recorded during a 30 min period of different intensity levels; including a period of rest, warm up, exercise at increasing level until the horse was not able to run faster under the given physical circumstances and recovery. Electrode placement was followed by;

1. 10 minutes rest in box.
2. 5 minutes hand-walk, starting slowly and gradually increase the speed.
3. Run by hand for a couple of minutes.
4. Lunge in trot, gradually increase the speed.
5. Canter for approximately 4 minutes in lunge.
6. Run in free gallop for a couple of minutes in an arena.
7. 5 minutes recovery in box.

QT interval measuring and measuring of T_pT_e

The ECGs were analysed using the provided software (Televet 100 Version 4.2.0)[1]. RR analysis was performed with maximal deviation of 10 %. The "heart rate view" of the program, was used to find areas where the heart rate was constant for at least 15 beats. Lead II between the green (+) and red (-) electrode was used (figure 10 and 13). The heart rate within each period was measured manually with an on screen electrocardiogram tool (Cardio Calipers 3.3)[3], as an average of 10 consecutive beats. The RR was calculated via the formulae: RR = 60/HR. In the selected area on the ECG the QT intervals were then measured manually with the Cardio Calipers on the following 5 heart beats. In total, 35 QT intervals were measured from each of the included horses (from seven different HR areas).

The time interval from the onset of the Q-wave to the peak of the T-wave (T_{peak}) was measured and used to calculate the $T_{peak}T_{end}$ (T_pT_e) interval at different RR intervals. T_p was measured in the

positive peak of the T-wave or in the negative peak, when this was the largest deflection.

QT interval measurements depending on variation in Q- and T-wave morphologies.

Table 2 shows the variation in Q- and T-wave morphologies seen in the present study.

Wave	Type 1	Type 2	Type 3
Q-wave	+ peak	Flat or tilted	Round

Wave	Type 1	Type 2	Type 3	Type 4
T-wave	Biphasic	+/–	–/+	+

Table 2: Q- and T-wave types. –/+ indicates a larger negative deflection than positive deflection, the opposite for +/–.

"Q-and T-wave atlas": Figures 14-17 illustrate the variety of Q-wave morphologies, and figures 18- 22 illustrate the variety of T-waves morphologies. The figure texts provide descriptions of how the QTe-intervals are measured in this study. All images show at least one P-QRS-T-complex and the measuring tool Cardio Calipers. The Cardio Caliper shows how QTend is measured. The paper speed is 200 mm/sec and gain is set to 40 mV/sec in all images. The T-wave morphologies appear to change with heart rate and the images are from different HR areas. The QTe-interval will from now on be referred to as "QT interval".

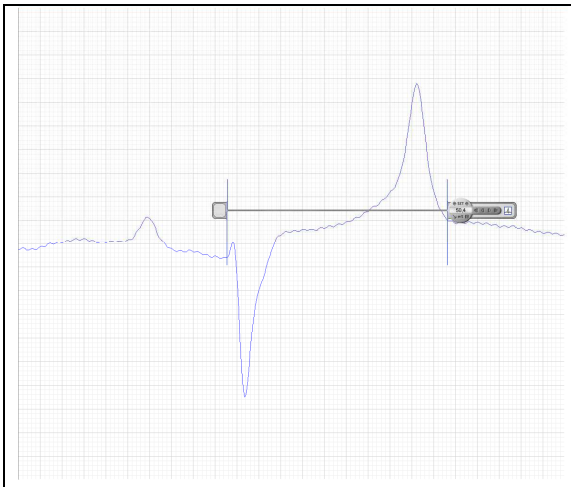


Figure 14: Q-wave type 1, positive peak. The QT interval is measured from where the onset of the peak to the end of the T-wave.

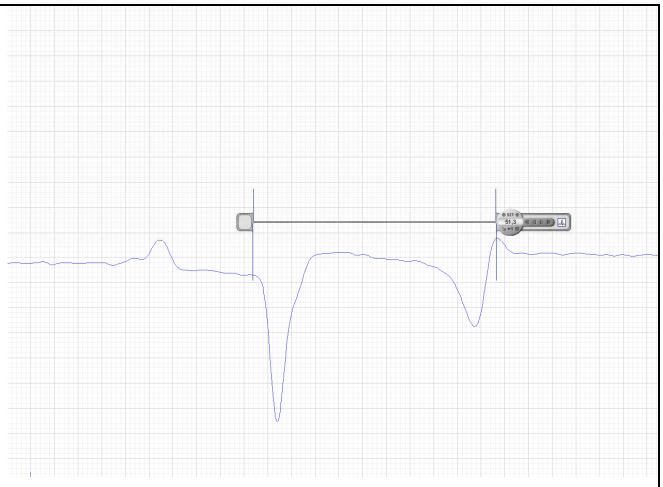


Figure 15: Q-wave type 2, flat. HR 43.3 bpm. In type 2 and 3 Q-waves (figure 15 and 16) with no clear Q-wave the QT interval is measured 1.5-2 small squares (0.3-0.4 cm) from the onset of the negative "QRS"-deflection.

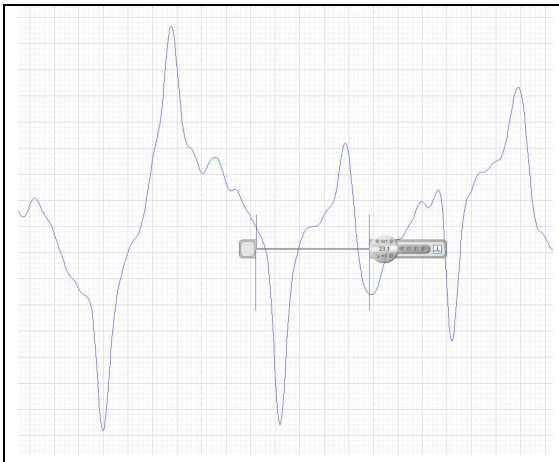


Figure 16: Q-wave type 2, "tilted". See figure 14 for description.

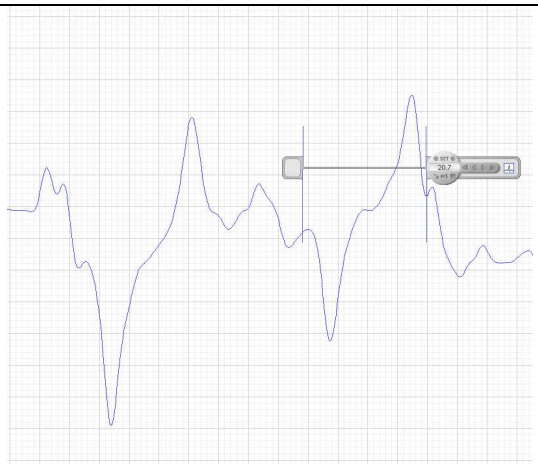


Figure 17: Q-wave type 3, round. See figure 14 for description.

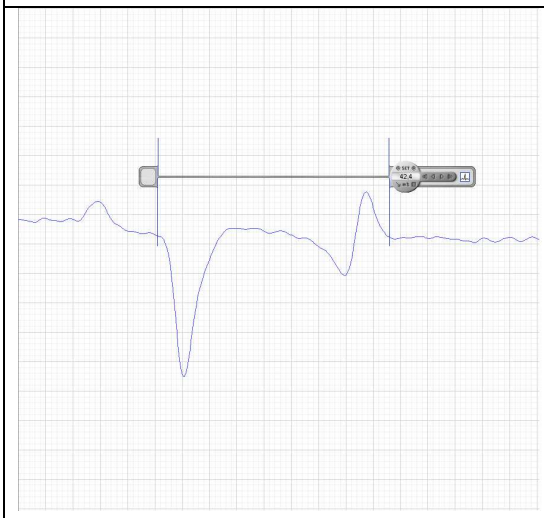


Figure 18: T-wave, type 1 (biphasic). Te is measured after both deflections.

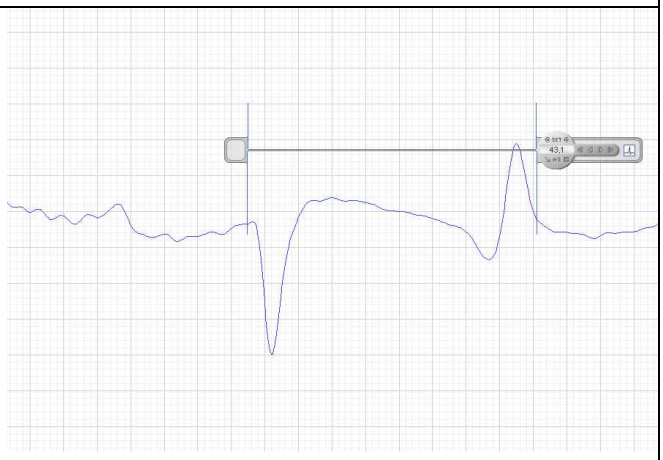


Figure 19: T-wave, type 2 (+/-) Te is measured after the positive deflection where it reached the base line level. (HR 53.7 bpm)

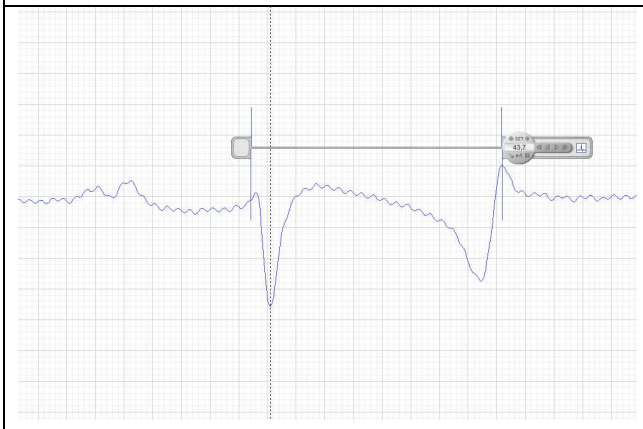


Figure 20: T-wave, type 3 (-/+). Te is measured in the peak of the small positive deflection. (HR 41.1 bpm).

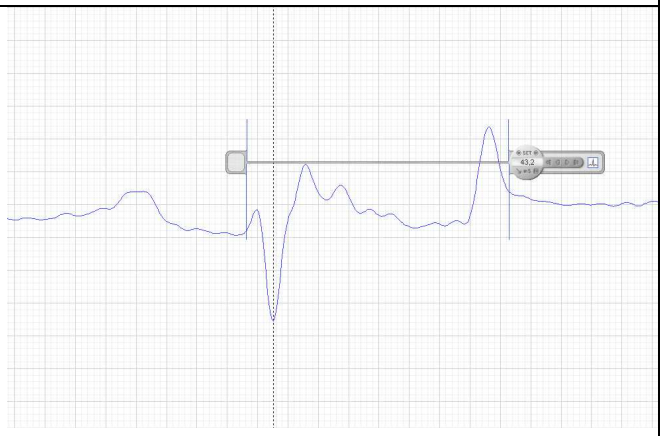


Figure 21: T-wave, type 4. Positive. Te is measured after the positive deflection where it reached the base line level. (HR 61.1 bpm).

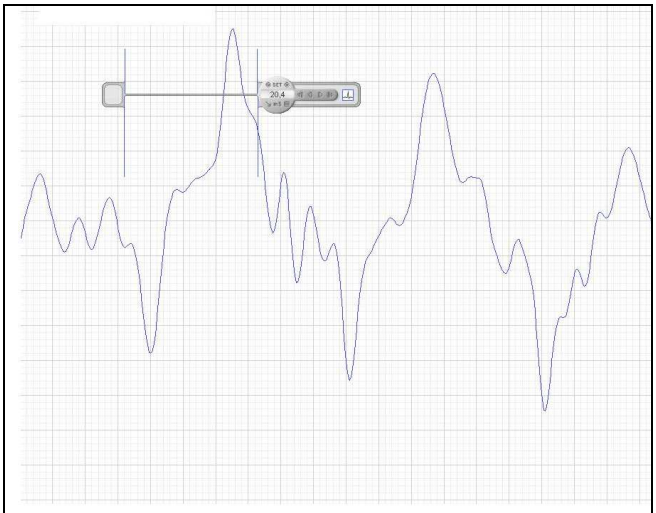


Figure 22: T-wave with a "bend", measured like this in cases were measuring in the bottom of the T-wave would give a very long QT intervals compared to the previous QT interval, Te difficult to measure (HR 199 bpm).

Statistical analysis

QT-correction: To estimate the best description of QT correction on the entire RR range data was plotted and corrected with the two most commonly used HR-correction formulas used in human medicine, namely the Bazett ($QTC = QT/\sqrt{RR}$) and Fridericia ($QTC = QT/\sqrt[3]{RR}$). Furthermore a piecewise linear regression model and a linear regression model was tested. The piecewise model is described as two straight lines joined in a bending point (RR_{bend}), given by the equations:

$QT = Slope_1 * RR + intercept_1$ for $RR \leq RR_{bend}$

$QT = Slope_2 * RR + intercept2$ for $RR \geq RR_{bend}$

Since the bending point is given, the intercept is redundant and the model can be reduced to three parameters ($Slope_1$, $Slope_2$ and RR_{bend}).

To assess the best method for HR-correction of the QT interval, an average prediction error (PE) was calculated on the Bazett, Fridericia and the piecewise linear QT correction models. PE's were plotted over the entire RR-range using a locally weighted scatterplot smoothing (LOWESS) plot. Here a low-degree polynomial was fitted to a local subset of the data, giving more weight to points near the point that is being estimated and less weight to points further away. Hence the LOWESS

plot is very flexible, making it ideal for modelling complex processes for which no theoretical model exist.

T_pT_e analysis: To assess the timing of the late phase of repolarisation T_pT_e from each horse was plotted against RR for each sex. A linear regression fit was performed on these data to estimate the T_pT_e RR dependence.

The results were considered significant if $P < 0.05$. The statistical analysis programs SAS[4] and Graph Pad Prism[5] were used for all statistical analysis in this study.

8. Results

Of the 32 horses, 22 horses were included in the final results of the present study. These are 8 mares, 7 geldings and 7 stallions. 10 horses are excluded from the study, either as a consequence of bad ECG quality or because of a shortage of stabile HR areas and thereby too few measured QT intervals. The mean age and BWT_e of the horses were: 6.4 years and 358 kg, 10 years and 321 kg and 8.7 years and 356 kg in mares, stallions and geldings, respectively. Mean age and BWT_e for all horses were 8.4 years and 345 kg (ranging from 269 to 385 kg). There was no history of cardiopulmonary disease in any of the included horses, and they were all healthy at the clinical examination. Normal physiological arrhythmias (2AVB and sinusarrhythmia) were auscultated in some of the horses.

The QT interval appears to have a clear piecewise linear relationship with RR in Icelandic horses. From the data analysis in this study the best descriptive model was the piecewise linear regression fit ($0.93 < r^2 < 0.95$) for all three sexes. Figure 23 demonstrates the sex-differentiated model of the QT intervals in relation to RR intervals found in this study. The fitted piecewise regression lines are drawn in the figure. The linear regression fit was a less descriptive model for all sexes ($0.82 < r^2 < 0.87$). An enlarged model of figure 23 and individual QT/RR plots with piecewise regression lines for each sex can be found in Appendix III (A-D).

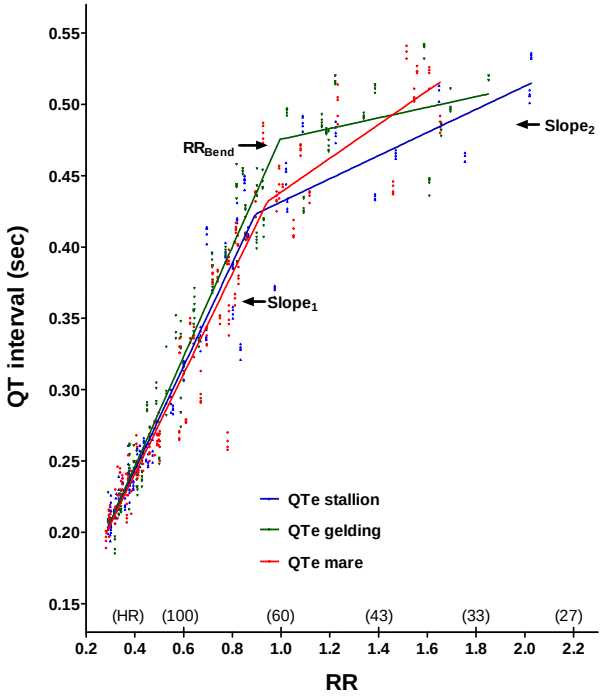


Figure 23: Sex-differentiated plots of QTe/RR intervals with a piecewise linear regression fit.

The values for $Slope_1$, $Slope_2$ and RR_{bend} of the piecewise linear models for each sex of Icelandic horses included in this study are shown in table 3.

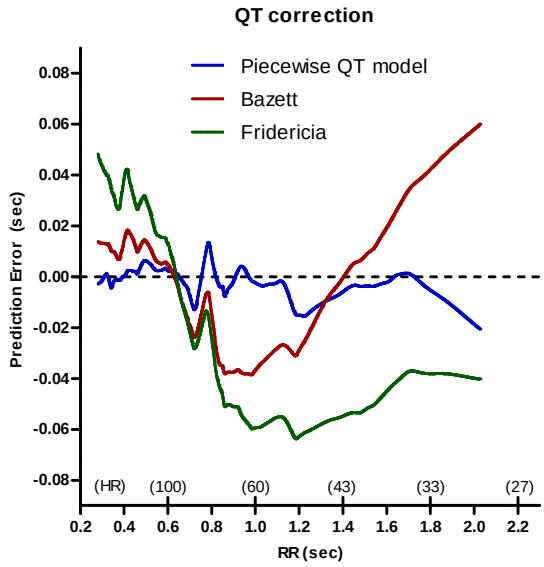
	$Slope_1$	$Slope_2$	RR_{bend}
Stallion	0,362 [0,345 - 0,379]	0,081 [0,065 - 0,097]	0,894 [0,849 - 0,939]
Gelding	0,385 [0,369 - 0,401]	0,037 [0,016 - 0,058]	0,995 [0,960 - 1,031]
Mare	0,351 [0,334 - 0,368]	0,118 [0,093 - 0,143]	0,944 [0,888 - 1,00]

Table 3: Sex differentiated values for $Slope_1$, $Slope_2$ and RR_{bend} from the Icelandic horses in this study.

$Slope_2$ of the geldings in this study appears more flat than $Slope_2$ of the other sexes, as can be seen in figure 23 and table 3. The geldings of this study appear to have longer QT intervals, particularly close to RR_{bend}. Appendix V provides a table of calculated reference QT intervals with 95 % prediction intervals at different HR/RR-values for the different sexes of Icelandic horses.

To determine the best method for HR-correction of the QT interval in Icelandic horses, the average prediction error (PE) of the QT measurements were calculated with the three different methods: Bazett, Fridericia and the piecewise linear QT models. A LOWESS plot which illustrates the tendencies of systematic miscorrection of the formulas over the entire RR-range can be seen in figure 24. The piecewise model performed best in almost all HR (RR) areas, whereas the two other

correction methods systematically tend to miscorrect (deviate from zero). The average miscorrection for the piecewise model is 17.2 msec, while the average miscorrections of Bazett and Fridericia are 23.5 and 39.8 msec, respectively.



Figur 24: LOWESS plot of the prediction errors (PE) of all corrected QT measurements based on correction method (Bazett, Fridericia and a piecewise QT model). The plot illustrates systematic tendencies of miscorrection (deviations from zero) of the formulas.

The T_pT_e/RR plots with linear regression fits for all three sexes are shown in figure 14. Only the slope of the linear regression fit for T_pT_e in the stallions is significantly different from zero (P-value = 0.0386), but RR only explains 1.8 % of T_pT_e dependence on RR in stallions (R^2 = 0.018).

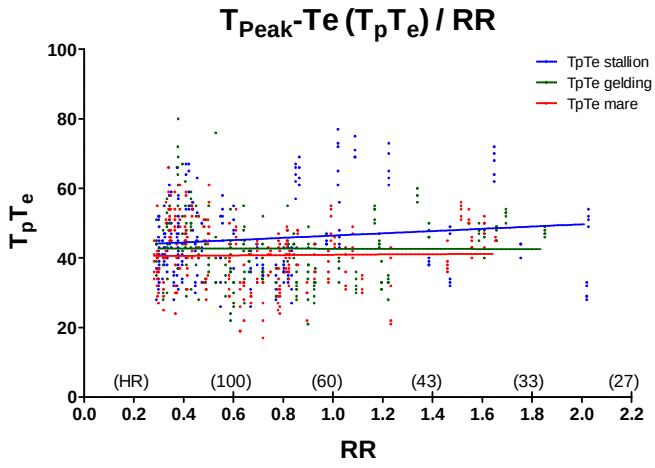


Figure 25: TpTe/RR plots with a linear regression fit.

9. Discussion

This is the first study of the QT interval at different heart rates in Icelandic horses.

8 mares, 7 geldings and 7 stallions were included in the study. Arrhythmias, age, body weight (BWT) and athletic condition were not included in the analysis of the results. Occasional physiological arrhythmias were observed in some of the horses, but the QT intervals were only measured in areas with a constant heart rate (HR) with no arrhythmias. The mean age of the horses included in the study was 8.4 years. However, one of the stallions included in the study was 25 years old. Old age has been related to prolongation of the QT interval in humans (Reardon & Malik 1996), but when comparing the QT interval of this stallion to the rest of the stallions, it was not different. All QT intervals from this stallion were close to the regression line for the group (not significantly tested), and the horse was therefore not excluded from the study.

The athletic condition of the horses included in this study differed between highly trained and untrained. As physiological left ventricular hypertrophy (LVH) is well known in human athletes (Pluim *et al*. 2000; Rajappan *et al*. 2003) and has been seen in horses as well as part of the "athletes heart syndrome" (Buhl *et al.* 2005; Gehlen *et al.* 2007) it could be speculated if this would potentially introduce bias to the results, due to the fact that LVH has been associated with prolonged QT intervals in humans (Sharma *et al.* 1999). However, the QT interval has been shown to shorten similarly due to HR in both athletes and untrained controls in humans (Rajappan *et al.* 2003), and therefore we believe that athletic condition could be excluded in the analysis of the results in the present study.

The body condition of the included horses was normal. Studies have shown that body weight (BWT) (varying from ~50-1000 kg) seems to have very little influence on the QT interval in horses (Schwarzwald *et al.* 2012), and therefore the BWT was not included as a factor of relevance in this study.

One limitation of the chosen method for ECG recording was that some of the horses were not used to being lunged in a large arena, and hence, the flow of the exercise was sometimes interrupted. The purpose of slow and stable changes of gait and speed was to gradually increase the HR. Fright, stress or excitement led to a sudden increase in HR resulting in fewer constant HR areas of the ECG recording. The highest intensity level under the given physical circumstances was probably

submaximal due to difficulties in keeping the horses galloping for the wanted period of time. A way of eliminating some of these problems in future studies could be to do the ECG recordings while the horses are being ridden in different gates and intensity levels. Walk sessions between the intensity levels could be suggested since the HR tends to stabilise more at different levels while decreasing. It is important that the rider is instructed in how to ride the horse when recording, since stress and disturbances of the horse appear to reduce the quality of the ECG.

It is relevant to point out why the QT measurements were done using the on screen electrocardiogram tool (Cardio Calipers 3.3)[3] instead of the built-in measuring tool of the Televet, as it is much more adjustable and can easily be placed at exact start and endpoints of the wanted ECG complexes. Thereby the measured QT intervals will be more precise.

The QT intervals were measured in areas of constant HR. A "live HR-view" was not possible to use during the ECG recordings in this study, due to lack of necessary equipment. The HR of the horses was therefore not "controlled" and the stable HR-areas varied between the horses, and hence the QT intervals were measured at different HR in each horse. Appendix IV shows the number of horses representing the QT-measurements within each HR-area. The HR-intervals ranging from 30 to 90 bpm were often stable and QT intervals could easily be measured for most of the horses. The HR-intervals from 110 to 130 bpm were seldom stable, and hence, the QT intervals measured at these HR are only from a small number of horses within each sex. QT intervals measured at the same HR in a large number of horses may represent a more "true" QT interval for the group at that HR, compared to QT intervals only measured in a small number of horses. If all horses should have had QT intervals measured from the same HR-areas, it would have been necessary to use a live HR-view during the ECG recording to ensure stable HR. The use of treadmill-exercise could have made this possible, but would have complicated the present study, as horses must be trained to run on a treadmill and must be transported to its location.

The chosen technique for measurement of the QT intervals in this study can be discussed. During the study it became clear that the different Q- and T-wave morphologies that appeared created difficulties determining the correct onset of the Q-wave and end point of the T-wave, which made measurement of the QT interval difficult. This led to creation of the "Q-and T-wave atlas" seen in section 7, to document how the QT intervals were measured. The Q-wave variations can be seen in figures 14-17 in section 7. In type 2 and 3 Q-waves, the QT intervals were measured 1.5-2 small squares (0.3-0.4 cm) from where the negative "QRS"-deflection started and to the end of the T-

wave. Whether this was the correct method can be discussed. It might have been more correct to measure exactly from the edge where the change from base line to negative "QRS"-deflection started. This would have resulted in RR intervals approximately 0.015-0.020 seconds shorter than measured in the present study. Most of the "QRS"-complexes in this study had type 2 or 3 Q-wave morphology. Appendix V provides a table of calculated reference QT intervals with 95 % prediction intervals for all HR/RR. The prediction interval is an estimate of an interval in which future QT interval measurements will fall (with a 95 % probability), and is based on all QT intervals measured in this study. Even if the chosen method in this study had given QT intervals that are 0.015.0.020 seconds too long, they would still be within the predicted QT intervals and it can be assumed that the chosen measuring technique has not had too much influence on the results.

Variations in T-wave morphology, as seen in this study, created some challenges. T_e was measured as shown in figures 18-22 in section 7. T_e was measured after both deflections in the biphasic type 1 T-wave and after the positive deflection in type 2 and 4 T-waves. In type 3 T-waves, the T_e was measured in the peak of the small positive deflection. It might have been more correct to measure T_e after both deflections in all cases. If done so, the QT intervals with type 3 T-waves in this study have been measured too short, but as described for Q-waves, the measured QT interval will probably still be within the QT prediction interval for the respective HR (Appendix V).

The variations in T-wave morphology are seen with changes in HR. T-wave-variation within one ECG recording at rest, and HR-related changes in T-wave morphology have been described as a normal phenomenon in horses (Holmes & Rezakhani 1975; Buss *et al.* 1975; Verheyen et al. 2010a). Accurate determination of the QT interval, especially the T-wave termination (T_e) is described as challenging in humans (Luo *et al.* 2004), and especially at high heart rates where the T and P-waves "collide" (Kramer & Zimetbaum 2011). This is also observed at high heart rates in this study. When the HR increases towards the maximal, the ECG is more difficult to interpret. In this study, the T-waves seem to become positive (type 4) at HR from 90 bpm to the peak HR. The biphasic T-waves tend to occur at HR at about 50-80 bpm, but the exact occurrence of all T-wave morphologies has not been analysed in detail.

It would be a good idea to evaluate the different Q and T-wave morphologies found in this study with an experienced cardiologist, to decide exactly how the QT interval should be measured in any future studies, in order to eliminate the possibilities of inconsistent measuring.

This is the first study of QT intervals at different HR-levels, from rest to high intensity, in Icelandic horses. The relation between QT- and RR intervals in this study was best described by a piecewise linear regression model. This is similar to the findings of Pedersen *et al.* (2012) in race-fit Standardbreds and it confirms the first hypothesis of this study. The finding is different from findings in healthy humans, where the QT interval in relation to RR is described by linear regression (Kligfield *et al*. 1996; Rajappan *et al*. 2003), although a non-linear model of QT- interval in relation to RR intervals has been suggested in humans (Hodges 1997; Luo *et al*. 2004). The bending point (RR_{bend}) of the piecewise regression was found to be 0.894 [0.849 – 0.939], 0.995 [0.960 – 1.031] and 0.944 [0.888 – 1.0] in stallions, geldings and mares, respectively (included 95 % confidence intervals). In HR this means that the curves bend between 60 and 67 bpm (HR=60/RR) in the three groups. Within this HR-interval the QT intervals have been measured from a large number of horses, as discussed earlier (Appendix IV), and the T_e is usually unproblematic to measure.

With the aim of finding the best method for HR-correction of the QT interval in Icelandic horses, an average prediction error (PE) was calculated on the Bazett and Fridericia and the piecewise linear QT correction models. The piecewise linear QT correction model performed best at almost all HR (RR) areas, with an average miscorrection of 17.2 msec. The two other models show tendencies to systematically miscorrect. The Bazett method undercorrects at the higher HR and overcorrects at the lower HR, whereas the Fridericia method tends to undercorrect the QT intervals over the entire RR-range. The Bazett method seems to perform better than the Fridericia method in these horses, with an average miscorrection of 23.5 and 39.8 msec, respectively. This is different from what is seen in humans, were the Fridericia method generally performs better method than Bazett (Luo *et al.* 2004). However, it was similar to the findings of Pedersen *et al*. (2012) in Standardbreds, were the piecewise linear QT correction model performed best and the Bazett method also performed better than Fridericia.

$Slope_1$ represents the QT intervals from RR_{bend} (60-67 bpm) to peak HR. This slope is steeper than $Slope_2$, which means that the QT interval shorten relatively faster as the HR is increasing (RR decreasing) beyond the bending point. $Slope_2$ represents the QT intervals at the lower HR from rest (approximately 30 bpm) to RR_{bend}, and the QT intervals seem to be more spread around the regression lines. This can also be seen in Appendix V, were the prediction intervals are larger at the lower HR (longer RR intervals).

The biophysical explanation for the piecewise linear QT/RR relationship in horses is only speculative at this point. It has been suggested to be explained by the point where contribution of the slow, delayed rectifier potassium channels (KCNQ1) become significant (Pedersen *et al.* 2012). The KCNQ1 is expressed in equine ventricular myocytes (Finley *et al*. 2002), and is activated upon β-adrenergic stimulation and increased intracellular Ca^{2+} concentrations (Marx *et al*. 2002; Gamper *et al*. 2005). The ultrarapid, delayed rectifier potassium current, I_{Kur}, has also been suggested to be important for the rate-related adaption of action potential duration in ventricular repolarisation in the horse (Finley *et al.* 2003), and RR_{bend} might reflect an increased contribution of this current.

The second hypothesis of this study was that there could be a difference in the length of the QT intervals based on gender. It was expected that the stallions would have longer QT intervals than mares and geldings, based on the findings of Pedersen *et al*. (2012) in race-fit SBs. This could not be confirmed in this study, although some differences were seen between the sexes. $Slope_1$ from all three sexes follow each other closely from HR ~150 bpm (RR = 0.4 sec) to peak HR. The QT intervals are very similar between the groups at these HR. At HR below ~150 bpm the geldings in this study have on average longer QT intervals than the mares and stallions, being most pronounced around the RR_{bend} for geldings (seen from figure 23 and appendix V. Not significantly tested). Mares appear to have longer average QT intervals than the stallions from HR ~60 bpm, as well as the geldings at HR around 40 bpm. $Slope_2$ of the mares is steeper compared to the other two. As mentioned earlier in this discussion, LVH is described in race-fit Standadbreds (SB) (Buhl *et al.* 2005), and has been associated with prolonged QT intervals in humans. Most of the geldings in the present study were not in good athletic condition and the difference is presumably not explained by LVH (the stallions were actually in the best athletic condition). One explanation could be physiological differences in the QT interval or mistakes introduced during measuring; creating a few outlying QT-plots which make the RR_{bend} for geldings occur at a longer QT/RR interval. This possibly "wrong" placement of RR_{bend} in geldings could also be a reason for the apparently flat regression line of $Slope_2$ in this group, compared to the two other groups. The apparently longer QT intervals in the mares could possibly be caused by the same reasons as for the geldings; either physiological differences in the QT intervals or measuring mistakes that has created some outlying long QT intervals. In humans, women are known to have longer QT intervals than men and castrated men have longer QT intervals than intact men, and hormones are thought to have an influence on the QT interval in humans (Bidoggia *et al.* 2000). The results from the present study

could be comparable to the findings in humans, but more research should be done in horses before final conclusions about sex-related differences in the QT interval in horses can be done.

An influence of QT lag (delayed adaption of the QT interval in response to HR-changes) on the results of this study is probably minimised since the QT intervals are only measured from periods of constant HR. QT hysteresis (different QT intervals at the same RR depending on an increasing or decreasing HR) could potentially have an influence on the results of this study (Pedersen *et al.* 2012). No conclusive explanation of the mechanisms behind QT hysteresis exists, but the autonomic nerve system appears to have an influence. Little is known about beat-to-beat dynamics in horses, but since it is known that the autonomic nerve system has a strong impact on the equine heart, it could be assumed that QT hysteresis could be present in horses.

$T_{peak} - T_{end}$ (T_pT_e) is a marker for the late phase of repolarisation, were the mid-myocardial cells of the ventricle are being repolarised. The third hypothesis of this study was that the T_pT_e did not show sex or HR-dependence. This was confirmed when the T_pT_e was plotted against RR and fitted with a linear regression line for each sex. The T_pT_e was constant and not strongly influenced by the RR, which is similar to studies of T_pT_e in relation to RR intervals in healthy humans (Haarmark *et al*. 2010). An exception was though the slope of the linear regression fit for T_pT_e in the stallions, which was significantly different from zero (P-value = 0.0386), but RR only explains 1.8 % of the T_pT_e variation ($R^2 = 0.018$). The stallions in this study were in the best athletic condition of all horses and the apparently longer T_pT_e intervals might reflect some LVH (Porthan *et al.* 2007) caused by training (Buhl *et al.* 2005; Gehlen *et al.* 2007).

10. Conclusion

The study included 22 healthy Icelandic horses (8 mares, 7 geldings and 7 stallions) from which electrocardiograms were recorded at rest and during exercise of increasing intensity. QT intervals were measured at different heart rates in order to study the relationship between the QT- and RR interval in Icelandic horses. The main finding of this study is that the relationship between the QT- and RR interval was best described by a piecewise linear regression model, which is different from in healthy humans, were the QT/RR-relation is described by a linear regression model. A large variation in T-wave morphologies was seen in the ECGs and appears to be normal for these horses.

The geldings in the present study appeared to have longer QT intervals than stallions and mares at most HR (RR), and the mares had longer QT intervals than both geldings and stallions at the lowest HR. An explanation of the differences is not conclusive. One could be actual physiological differences in the QT interval or errors from the measuring method. More research of sex-related differences in the QT interval in horses should be done before a final conclusion can be made.

HR-correction was performed with both the Bazett and Fridericia formulas on each horse individually. The Bazett correction performed better than the Fridericia, which is in contrast to what is seen in humans. The best method for HR-correction was the piecewise linear QT correction method which on average miscorrected with 17.2 msec, compared to 23.5 and 39.8 msec with the Bazett and Fridericia formulas, respectively.

Calculated reference values for QT intervals with 95 % prediction intervals at different HR/RR-intervals were established for geldings, stallions and mares of the Icelandic horse breed.

$T_{peak} - T_{end}$ interval (T_pT_e), a marker for transmural dispersion in repolarisation, was measured and plotted against RR. The T_pT_e was constant and nearly independent from HR/RR, similar to studies of T_pT_e in healthy humans. Only the slope of the linear regression fit for T_pT_e in the stallions is significantly different from zero (P-value = 0.0386), but RR only explains 1.8 % of the T_pT_e variation ($R^2 = 0.018$). The stallions in this study were in the best athletic condition of all horses and the apparently longer T_pT_e intervals might reflect some LVH caused by exercise.

11. Perspectives

The results of this study can be used to support the piecewise linear model of QT-/RR-relation in horses. The purpose of this study was to contribute to more knowledge about the HR-dependence of the QT interval in normal horses. The calculated values for QT intervals with 95 % prediction intervals at different HR/RR from this study, can be used in future studies with for example in evaluation of the potential cardiotoxity of a drugs in horses, were the QT interval can act as an important marker. More research should be done before final conclusions about differences in QT intervals between sexes can be made. It could be interesting to do similar studies in another type of horse, for example in endurance horses or thoroughbreds.

It could be interesting to study the contribution of important ventricular repolarising currents in horses. Studies could be done by injecting horses with drugs known to block a specific channel, while recording an ECG and study the effect on the QT interval. It could also be interesting to evaluate the T-wave morphology after drug injection to study if any specific ion current may have an influence on the morphology of the T-wave. Many anti-arrhythmic and non-cardiac drugs block the ERG/KCNH2 channel (I_{Kr}), and it would be of great importance that great care should be taken, since dangerous ventricular arrhythmias could occur.

It became clear during this study that variation in T-wave morphologies sometimes made it difficult to decide the exact termination of the T-wave. It would be a good idea to evaluate the different Q- and T-wave morphologies found in this study with an experienced cardiologist, to decide exactly how the QT interval should be measured in any future studies, in order to eliminate the possibilities of inconsistent measuring.

The KCNQ1 and KCNH2 are expressed in the equine ventricular myocardial cells and that the I_{Ks} and I_{Kr} are important repolarising currents in the horse. The possibilities of acquired LQTS could definitely exist in horses by the use of drugs which are known to prolong the QT intervals in humans. Quinidine is an example of an anti-arrhythmic drug used as therapy for atrial fibrillation in horses and is known to block I_{Kr}. More knowledge about the equine QT interval can be used both for monitoring during treatment, and as a diagnostic tool in horses which have experienced syncope. Sudden death without any pathological findings could possibly be due to fatal ventricular arrhythmias resulting from mutations in genes encoding important repolarising ion channels. This can’t be either denied or confirmed without genetic testing for mutations.

Manufacturer's addresses:

[1] Engel Engineering Services GmbH, 63073 Offenbach am Main, Germany

[2] Jørgen Kruuse A/S, Havretoften 4, 5550 Langeskov, Denmark

[3] ICONICO, New York, USA

[4] SAS system version 9.2 for Windows, SAS Institute Inc., Cary, NC, USA

[5] Graph Pad Prism, GraphPad Software, Inc. 2236 Avenida de la Playa, La Jolla, CA 92037 USA

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APPENDIX I

Owner agreement/Ejers samtykkeerklæring

Angående hesten _____

Ejer _____

Studiets titel	EKG og måling af QT afstand ved forskellig hjertefrekvens hos islandske heste. (ECG and measurements of the QT interval during different heart rates in icelandic horses).
Baggrund	Elektrokardiografi (EKG) er en teknik, der bruges til at måle hjertets elektriske aktivitet. Det er meget brugt til undersøgelse af hjertets rytme og funktion, ved mistanke om hjertelidelser. Hos mennesker findes et syndrom som kaldes "langt QT-syndrom", som medfører ændringer i hjertets elektriske aktivitet. Blandt andet kan det føre til besvimelse, kramper og pludselig død. En dansk PhD studerende, Philip Pedersen, er nu i gang med at undersøge hvorvidt dette syndrom eksisterer hos heste, og om det kan være en årsag til pludselig død. For at undersøge dette nærmere, ønskes der grundige EKG optagelser af heste, for at kunne fastsætte en normalværdi for QT-afstanden. Den islandske hest er valgt som race, da det er en meget renracet gruppe heste, samt at den størrelsesmæssig er mindre, end de heste der tidligere er lavet undersøgelser på.
Formål	Formålet med dette specialet er at lave EKG på islandske heste. Fokus er på at måle QT afstanden, og studere hvordan den ændrer sig i forhold til hestens hjertefrekvens. Resultaterne skal sammenlignes med tidligere undersøgelser lavet på travheste. Derfra kan det vurderes om heste, uanset størrelse og race, viser lignende resultater. Derudover vil man se, om man kan bestemme en normalværdi for QT afstanden hos islandsheste.
Studiet udføres af	Elin Hestmann Vinjerui, veterinærstuderende, under supervision af vejlederne: *Rikke Buhl, Dyrlæge* *Dan Klærke, Professor* *Philip Pedersen, PhD studerende*
Undersøgelser	EKG-optagelserne laves mens hesten lejes/longeres. Der optages under forskellige hjertefrekvenser, fra hvile til hårdt arbejde. Udstyret påsættes og hesten står i fred i 10 minutter, hvor den tilvænnes udstyret. Derefter lejes hesten ud af stalden, hvorved pulsen langsomt øges. I de 10-15 efterfølgende minutter lejes hesten i trav, longeres i trav og senere galop. Afsluttes med en frisk galop. Hesten skrittes ned og tages ind i stalden, hvor udstyret tages af. Det er vigtigt at hesten går i et *jævnt, flydende tempo*, uden for meget korrektion. Dette giver de bedste resultater.
Beskrivelse af undersøgelsesmetoden	
EKG	Der laves en EKG optagelse: Elektroderne (i form af 4 små klistermærker) limes fast på hesten med hudlim udviklet til dyr. Ledninger knappes fast på elektroderne og forbindes til en EKG-optager. Elektrodene holdes på plads med en elastistikgjord, og EKG-optageren bandageres fast til denne. Det vil måske være nødvendigt at klippe lidt, for at skabe bedst mulig kontakt mellem hud og elektrode. Det tager ca 5 min at sætte udstyret på hesten og derefter mærker hesten intet til

	optagelserne. Efter EKG optagelsen fjernes elektroderne igen ved at opløse limen. Det er muligt, at der efterlades små rester af lim og klistermærke på hesten. Limen afstødes naturligt i løbet af 5-10 dage.
Forventet varighed	21.-31. maj 2012
Fortrolighed	Alle informationer om din hest behandles fortroligt. Dette gælder også billede- og videomateriale optaget i forbindelse med studiet, som dog kan blive anvendt i anonymiseret form til undervisning og forskning. Billed- og videomateriale tilhører Institut for Produktionsdyr og Heste ved Københavns Universitet, Danmark
Mulige bivirkninger	Der er ingen kendte bivirkninger ved de anvendte materialer og metoder. Der gives ikke injektioner i forbindelse med undersøgelserne.
Ansvarsforhold	Deltagelse i studiet sker på eget ansvar. Institut for Produktionsdyr og Heste ved Københavns Universitet, Danmark, kan ikke drages til ansvar for eventuelle skader, der opstår i forbindelse med undersøgelserne af hesten, med mindre Institut for Produktionsdyr og Heste har handlet forsætligt eller groft uagtsomt. Institut for Produktionsdyr og Heste er ikke i noget tilfælde ansvarlig for driftstab, følgeskader eller andre indirekte tab.

Jeg har læst ovenstående oplysninger samt modtaget mundtlig information om studiet "EKG målinger på islandsheste ". Jeg giver hermed tilladelse til at min hest må deltage i undersøgelsen. Jeg forstår, at deltagelse er frivillig, og at jeg til enhver tid kan tage min hest ud af undersøgelsen. Jeg giver også tilladelse til at eventuelt billede- og videomateriale taget af min hest må anvendes til undervisningen og forskning uden yderligere kompensation.

Navn _____

Adresse _____

Postnr./By _____

Tlf. _____

Email _____

Sted/Dato	Underskrift ejer	Underskrift projektansvarlig

Elin Hestmann Vinjerui
Veterinærstuderende
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APPENDIX II

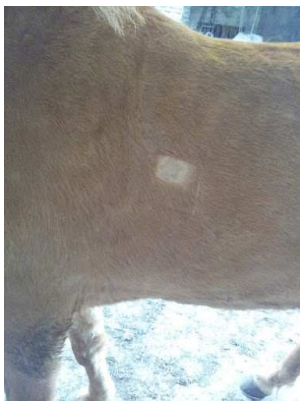
Project design/Forsøgsprotokol

EKG på islandske heste, speciale - forår 2012.

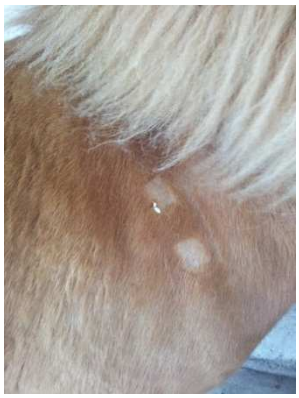
1. Ejer information + underskrive selvrisikoskema.
2. Opret patient i Excel + Televet.
    a. Stald
    b. Ejer
    c. Hestens navn
    d. Alder
    e. Køn (m/f/mc)
    f. Huld + estimeret vægt (målebånd omkring gjordleje). Stangmål hvis muligt?
    g. Noter metode (longe eller løbe frit i ridehal).

3. Klinisk undersøgelse med specielt fokus på hjerte og lunger.
    a. TPR + CRT
    b. Hjerteauskultation, Hjerterytme: Normal sinus rytme (NSR), 2. grads AV blok (AVB II)… Anden arytmi tydelig auskulterbar?
    c. Lungeauskultation

4. Klippe 4 små områder



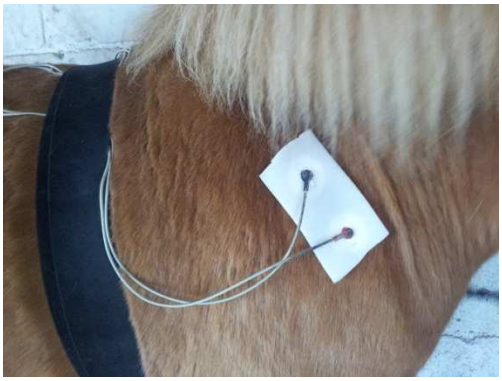
Figur 1 Venstre side



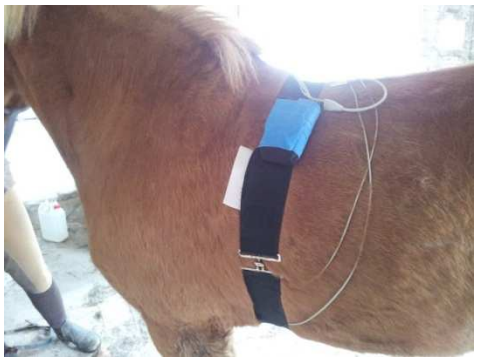
Figur 2 Højre side

5. Vaske/tørre af med vat+sprit

6. Hæfte fast elektroder + Snögg (rød over sort på højre skulder, gul i gjordlejet midt på maven på venstre side og grøn lidt til venstre for den ventrale midtlinje). Durapore hæftes over elektrode+ledning. Elastikgjord rundt mave, dækker elektroder. Televet-boks med SD kort kobles til. (Se at det blinker). Bagsiden af boksen skal væk fra hesten. Bindes fast med co-plus.



Figur 3 Højre side med EKG



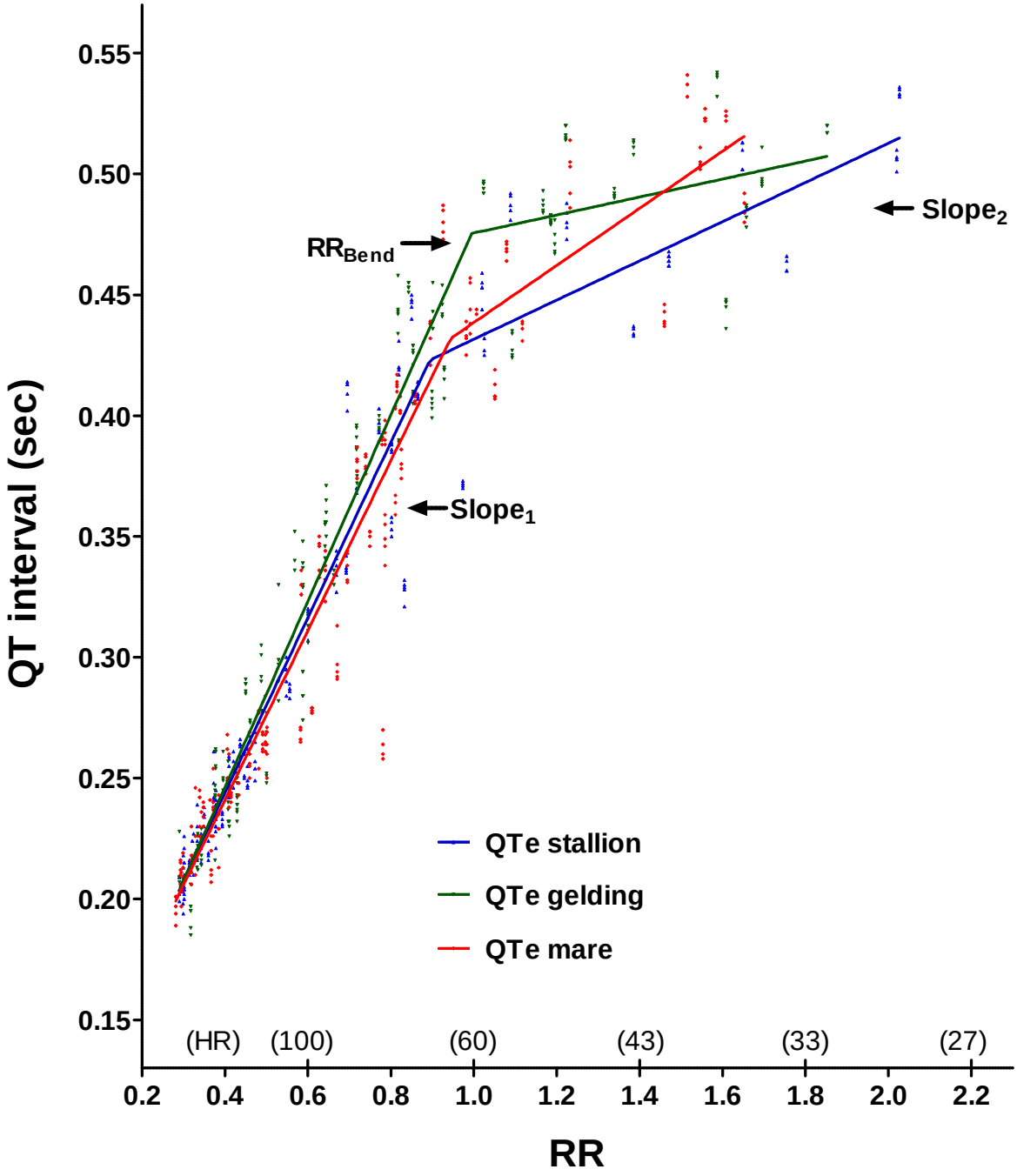
Figur 4 Venstre side med EKG

7. 10 min tilvænning. Hvilepuls (under 40 slag/min), la stå i fred på gang eller i boks.
8. 10-15 min arbejde (løbe løst i ridehal eller på longe)
   JÆVNT TEMPO og forsøge langsom øgning af puls!
   a. Leje ud af stald – skridt
   b. Skridte på bane
   c. Håndholdt trav evt. longere rolig trav
   d. Rolig galop
   e. Afslutte med frisk galop
9. 5 min med EKG på efter arbejde → puls ned.
10. Koble af udstyr. SD-kort i computer. Importer fra SD-kort til Televet. Gem fil på USB/PC.

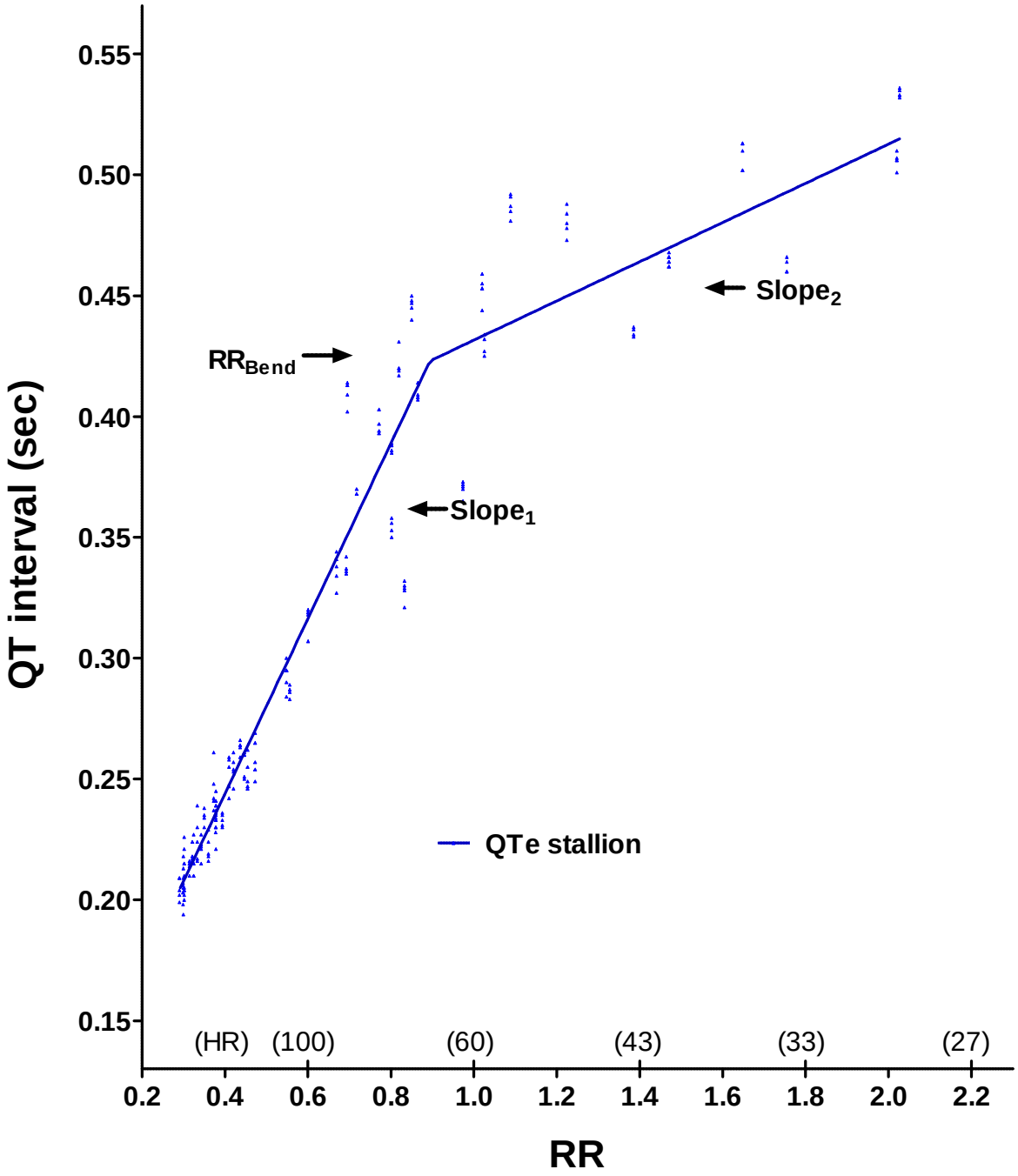
APPENDIX III

Results. QTe/RRplots with piecewise regression lines for each sex.

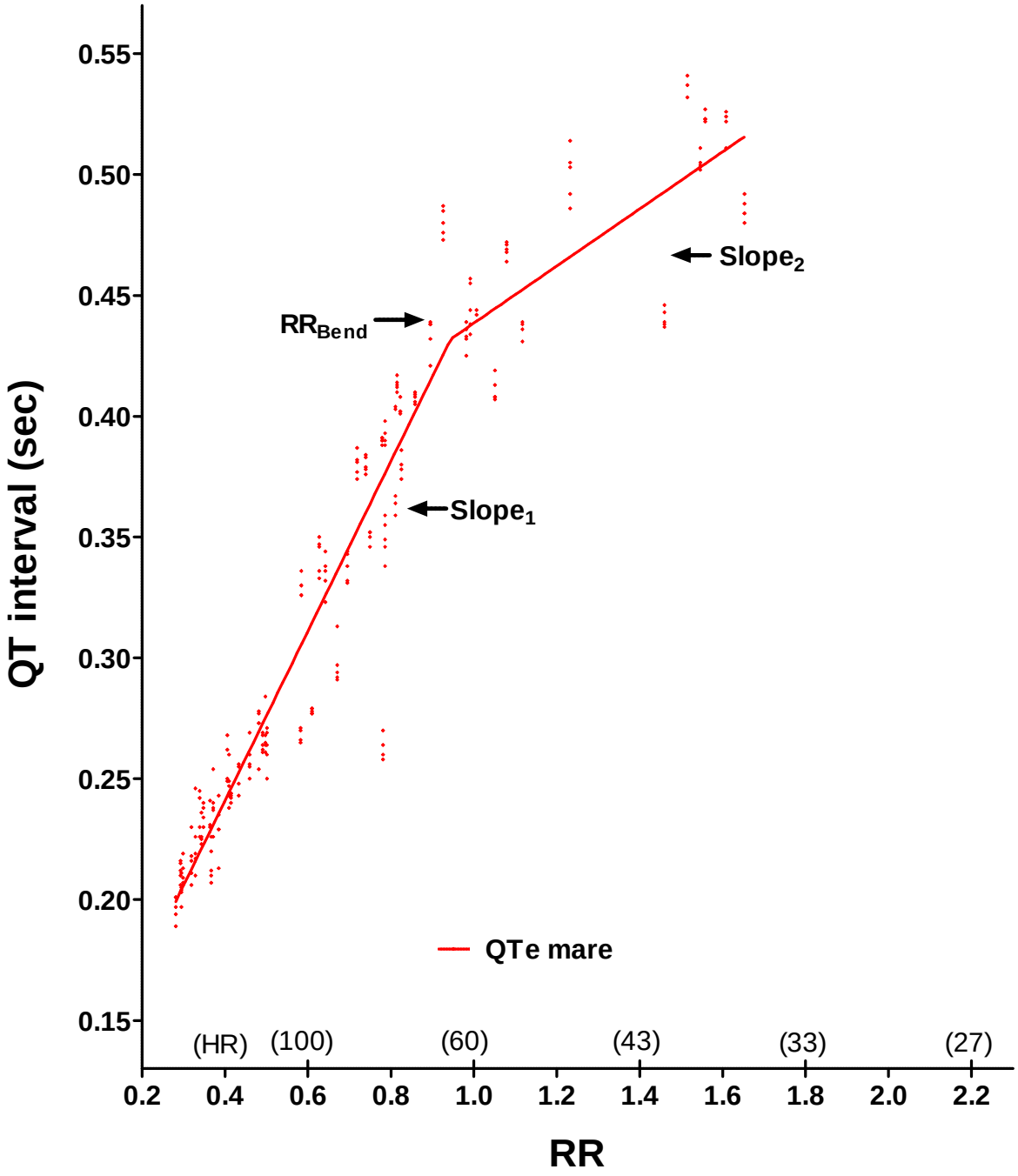
A) All sexes.



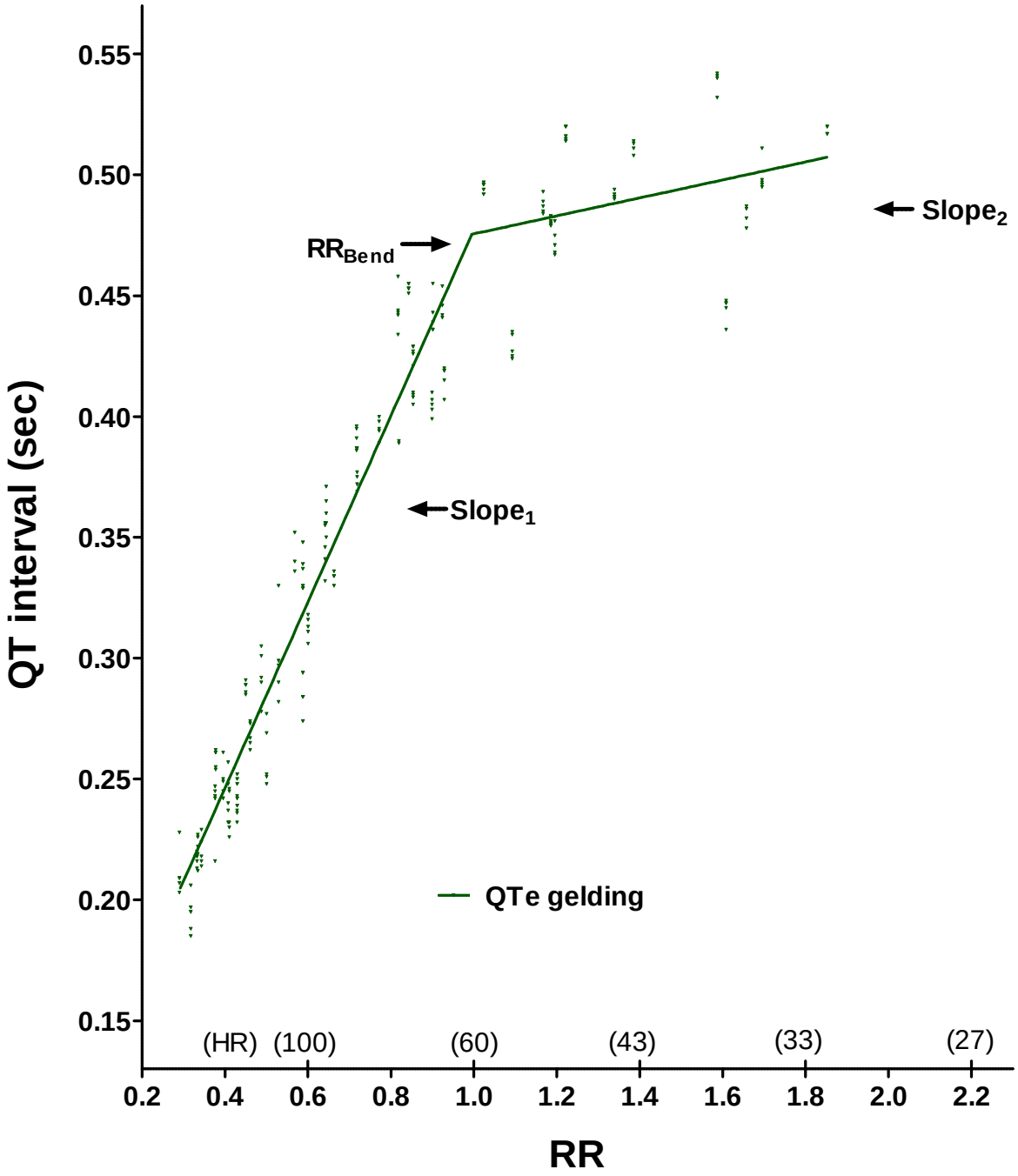
B) Stallions


QT interval (sec)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
RR$_{Bend}$
Slope$_2$
Slope$_1$
QTe stallion
(HR) (100) (60) (43) (33) (27)
0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2
RR

C) Mares


0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
QT interval (sec)
Slope2
RRBend
Slope1
QTe mare
(HR)
(100)
(60)
(43)
(33)
(27)
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
RR

D) Geldings


QT interval (sec)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
RR
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
(HR)
(100)
(60)
(43)
(33)
(27)
RRBend
Slope1
Slope2
QTe gelding

APPENDIX IV

Show the number of horses from each group which represents the measured QT-intervals.

(7 constant HR areas per horse and 5 QT-intervals measured per HR area).

	Mares	Stallions	Geldings	Total	
20 - 30		2		2	
31 - 40	5	4	5	14	
41 - 50	2	2	3	7	
51 - 60	4	3	5	12	
61 - 70	6	2	4	12	
71 - 80	7	6	6	19	
81 - 90	5	4	2	11	
91 - 100	3	1	4	8	
101 - 110	2	2	3	7	
110 - 120	2		2	4	No stallions
121 - 130	2	1	1	4	Few
131 - 140	2	3	3	8	
141 - 150	3	2	2	7	
151 - 160	1	4	3	8	
161 - 170	3	2		5	No geldings
171 - 180	3	3	2	8	
181 - 190	2	3	2	7	
191 - 200	1	3		4	Few, no geldings
201 - 210	3	2	1	6	

APPENDIX V

Sex-differentiated reference values for the QT-interval (sec) with prediction intervals at different HR/RR in Icelandic horses.

Heart rate	RR	Stallion	Gelding	Mare
30	2,0	0,51 (0,45; 0,57)	0,51 (0,45; 0,57)	0,56 (0,50; 0,61)
40	1,5	0,47 (0,41; 0,53)	0,49 (0,44; 0,55)	0,50 (0,44; 0,55)
50	1,2	0,45 (0,39; 0,51)	0,48 (0,43; 0,54)	0,45 (0,40; 0,51)
60	1,0	0,43 (0,37; 0,49)	0,48 (0,42; 0,53)	0,44 (0,38; 0,49)
70	0,86	0,41 (0,37; 0,45)	0,42 (0,38; 0,46)	0,40 (0,35; 0,45)
80	0,75	0,37 (0,33; 0,41)	0,38 (0,34; 0,42)	0,36 (0,31; 0,42)
90	0,67	0,34 (0,30; 0,38)	0,35 (0,31; 0,39)	0,33 (0,28; 0,39)
100	0,60	0,32 (0,28; 0,36)	0,32 (0,28; 0,36)	0,31 (0,26; 0,36)
110	0,55	0,30 (0,26; 0,34)	0,30 (0,26; 0,34)	0,29 (0,24; 0,35)
120	0,50	0,28 (0,24; 0,32)	0,29 (0,25; 0,32)	0,28 (0,23; 0,33)
130	0,46	0,27 (0,23; 0,31)	0,27 (0,23; 0,31)	0,26 (0,21; 0,31)
140	0,43	0,25 (0,21; 0,29)	0,26 (0,22; 0,30)	0,25 (0,20; 0,30)
150	0,40	0,24 (0,20; 0,28)	0,25 (0,21; 0,29)	0,24 (0,19; 0,29)
160	0,38	0,24 (0,20; 0,28)	0,24 (0,20; 0,28)	0,23 (0,18; 0,28)
170	0,35	0,23 (0,19; 0,27)	0,23 (0,19; 0,27)	0,22 (0,17; 0,27)
180	0,33	0,22 (0,18; 0,26)	0,22 (0,18; 0,26)	0,22 (0,17; 0,27)
190	0,32	0,22 (0,18; 0,26)	0,22 (0,18; 0,26)	0,21 (0,16; 0,26)
200	0,30	0,21 (0,17; 0,25)	0,21 (0,17; 0,25)	0,21 (0,15; 0,26)
210	0,29	0,20 (0,16; 0,24)	0,20 (0,16; 0,24)	0,20 (0,15; 0,25)
220	0,27	0,20 (0,16; 0,24)	0,20 (0,16; 0,24)	0,20 (0,15; 0,25)
230	0,26	0,19 (0,15; 0,23)	0,19 (0,15; 0,23)	0,19 (0,14; 0,24)
240	0,25	0,19 (0,15; 0,23)	0,19 (0,15; 0,23)	0,19 (0,14; 0,24)