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**SYMBOLIC DYNAMICS ANALYSIS: A NEW METHODOLOGY FOR
FOETAL HEART RATE VARIABILITY ANALYSIS**

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ABSTRACT – Italian

Introduzione:

La Cardiotocografia (CTG) risulta essere, nella pratica clinica, una tecnica di diagnostica fetale piuttosto diffusa sia per il monitoraggio “ante partum” che “intra partum”. Benché abbia valore legale in Italia ed altri paesi, presenta dei forti limiti nell’interpretazione dei tracciati registrati. In particolare, siccome il tracciato CTG viene sottoposto ad un’ispezione visiva, la sua interpretazione soffre di una forte variabilità intra- e inter- osservatore, rendendo di conseguenza la valutazione dello stato del feto fortemente soggettiva e difficilmente riproducibile. Al fine di superare i vincoli esposti, negli ultimi anni sono stati proposti numerosi metodi di interpretazione dei segnali di frequenza cardiaca fetale (FHR) e, più in generale, dei tracciati CTG. Particolare attenzione è stata rivolta alla stima della variabilità della frequenza cardiaca (FHRV), parametro legato allo stato del Sistema Nervoso Autonomo del feto.

Nel presente lavoro abbiamo suddiviso le tecniche adottate per l’analisi della FHRV in metodiche tradizionali, anche dette – per ragioni storiche – lineari, e metodiche meno convenzionali, altrimenti dette non-lineari. Delle prime fanno parte l’analisi nel dominio del tempo e della frequenza, mentre tra le seconde trovano posto tecniche originariamente utilizzate nell’analisi delle dinamiche dei sistemi non-lineari e caotici e successivamente estese allo studio dei sistemi biologici e della variabilità del ritmo cardiaco, spesso in combinazione con le metodologie classiche. Tutte le metodiche descritte nella presente tesi si sono rivelate particolarmente efficaci in casi specifici. Tuttavia, nessuna si è dimostrata di maggiore utilità o rilievo rispetto alle altre. Pertanto, abbiamo ritenuto necessario effettuare un’indagine dettagliata ed approfondita delle tecniche di analisi della FHRV presenti in letteratura.

In particolare, scopo della tesi è stato approfondire una specifica metodologia non-lineare, la Symbolic Dynamics Analysis (SDA), essendo già stata applicata con promettenti risultati all’analisi della variabilità cardiaca di soggetti adulti e, solo in pochi casi, nell’ambito fetale. Data la notevole semplicità di interpretazione che la caratterizza, questa tecnica potrebbe risultare un potenziale strumento di ausilio all’attività clinica e di efficace supporto al monitoraggio fetale.

Materiali e metodi:

Sono stati esaminati all’incirca 200 riferimenti bibliografici riguardanti l’analisi della variabilità del ritmo cardiaco sia nell’adulto che nel feto; di questi, circa 100 articoli specificamente incentrati sull’utilizzo di tecniche non-lineari.

A seguito dell’accurato esame della letteratura, sono state selezionate, revisionate, aggiornate ed implementate le seguenti metodologie di analisi:

- **Analisi nel dominio del tempo:** calcolo della Short Term Variability (indice della variabilità battito-battito del ritmo cardiaco);
- **Analisi nel dominio della frequenza:** calcolo delle potenze assolute e percentuali in ciascuna delle bande dello spettro della FHRV mediante Short Time Fourier Transform;
- **Analisi non-lineari:** calcolo dell’Entropia Campionaria, dei parametri forniti dalle mappe di Poincaré e dell’indice di variabilità ottenuto con la SDA.

E’ stata quindi condotta un’analisi multiparametrica allo scopo di realizzare un confronto fra le metodologie adottate ed individuarne i reciproci vantaggi e svantaggi nel monitoraggio fetale. Oggetto dello studio sono stati 580 tracciati CTG, registrati in ambiente clinico, di feti sani tra la 24^{ma} e la 42^{ma} settimana di gestazione.

Sono stati inoltre aggiornati gli algoritmi di elaborazione dei dati e dei segnali acquisiti e sono stati realizzati due software, uno per l'analisi dei segnali CTG reali ed un altro per la generazione di segnali CTG simulati a supporto dello studio condotto.

Infine, sono stati effettuati test statistici e prodotti grafici di regressione per esaminare le possibili correlazioni tra gli indici calcolati con l'analisi dei segnali ed alcuni parametri di interesse clinico quali il punteggio di Apgar, il tipo di parto (cesareo o spontaneo), le settimane di gestazione e lo stato fetale (attivo o a riposo).

Risultati e conclusioni:

Dai risultati ottenuti si evince che:

- Nessuno degli indici calcolati risulta più vantaggioso rispetto agli altri. L'uso combinato di più parametri potrebbe invece essere di maggiore utilità all'analisi e alla valutazione della FHRV.
- In accordo con la letteratura, lo stato del feto e le settimane di gestazione sono parametri di riferimento che dovrebbero essere sempre tenuti in considerazione per ogni tipologia di analisi effettuata.

Per quanto concerne la SDA, essa risulta uno strumento utile all'analisi della FHRV, riuscendo a distinguere – meglio o al pari di altre tecniche – lo stato del feto e la settimana di gestazione e, in alcuni casi, il tipo di parto. In aggiunta, essa consente di stimare correttamente e con maggiore semplicità rispetto ad altre tecniche (come l'analisi nel dominio del tempo) la variabilità complessiva di un segnale FHR. D'altra parte, sono necessari ulteriori studi per confermare queste evidenze. In particolare, tali studi dovrebbero includere anche casi di feti patologici al fine di valutare l'affidabilità dei parametri lineari e non nel discriminare feti in stato di buona o cattiva salute.

ABSTRACT – English

Introduction:

External Cardiotocography (CTG) is one of the most widespread diagnostic methods in clinical practice for checking foetal conditions both in the ante- and intra-partum period. However, even though it has legal value in Italy and in some other countries, it lacks of objectivity and reproducibility because of its dependence on observer's expertise and training.

In order to overcome these limitations, more objective methods for CTG interpretation have been proposed in recent years. In particular, many developed techniques aim to assess the foetal heart rate variability (FHRV), since its demonstrated relationship with the foetal Autonomous Nervous System functional state. Among them, some methodologies previously developed from nonlinear systems theory have been applied to the study of FHRV, often combined with more traditional analyses in time and frequency domain. All the techniques examined in this thesis have proved their validity and helpfulness in specific cases. Nevertheless, none of them seems to be more suitable or reliable than the others. Therefore, an in-depth study of these methods is necessary.

The aim of the present work is to deepen the FHRV analysis through the Symbolic Dynamics Analysis (SDA), a nonlinear technique - already applied with positive results to the adults and, in some cases, to the foetus - which allows a simple description of a system's dynamics by means of a limited amount of symbols and proper classification schemes. Thanks to its simplicity of interpretation, it could be a useful tool for clinicians in foetal monitoring.

Materials and methods:

We have performed an accurate literature study involving about 200 references on the heart rate variability analysis both in adults and fetuses; among them, approximately 100 works were focused on the application of non-linear techniques.

Then, we selected, reviewed, updated and implemented the following methods:

- **Time domain analysis:** Short Term Variability (beat to beat heart rate variability index) computation;
- **Frequency domain analysis:** absolute and percentage power computation for each of the FHRV spectral bands by means of Short Time Fourier Transform;
- **Non-linear analyses:** Sample Entropy, Poincarè maps and SDA parameters computation.

A multiparametric study has been carried out in order to compare the adopted methodologies and evaluate their strength and weakness points in supporting FHR monitoring. 580 antepartum recordings of healthy fetuses from the 24th to the 42th gestation week were examined. CTG traces were recorded by healthy patients during the clinical practice, using commercially available cardiotocographs.

Moreover, CTG signals were processed and analyzed using a developed and updated software for CTG analysis along with a new developed software for generating simulated CTG traces.

Finally, statistical tests and regression analyses were performed for estimating the relationships among indexes extracted from the adopted methodologies of FHRV analysis and other clinical data, such as Apgar score (low or normal), kind of delivery (cesarean or spontaneous), week of gestation (from the 24th and the 42th) and foetal status (active or at rest).

Results and conclusions:

The obtained results confirm that:

- None of the chosen indexes and employed techniques is more suitable or reliable than the others. Differently, each one should be used along with the others, complementing them in order to improve the FHRV evaluation.
- In agreement with the literature, each implemented analysis should take into account two relevant parameters, i.e. the foetal status (active or at rest) and the week of gestation.

As far as the Symbolic Dynamics is concerned, results confirm its usefulness and promising capabilities in the FHRV analysis. In fact, it allows recognizing foetal status and - in some cases - the kind of delivery and it is strongly correlated with the gestation week and, therefore, with the foetal development. In addition, it allows an accurate estimate of the global variability of foetal heart rate signals, even better than other methods such as the time domain analysis. Nevertheless, further studies are necessary to establish and definitively confirm the reliability of this parameter. In particular, they should involve pathological cases in order to compare the reliability of linear and non-linear parameters in distinguishing healthy from non-healthy fetuses.

INTRODUCTION

Background

In adults, experimental evidence for an association between a propensity for lethal arrhythmias and signs of either increased sympathetic or reduced vagal activity has encouraged the development of quantitative markers of autonomic activity. Heart rate variability (HRV) represents one of the most important such markers. However, the significance and meaning of the many different measures of HRV are more complex than generally appreciated and there is a potential for incorrect conclusions and for excessive or unfounded extrapolations [1].

Similarly, a significant relationship between the autonomic nervous system (ANS) and cardiovascular function was also found in foetuses. The analysis of foetal heart rate (FHR) signals represents a non-invasive, fundamental tool for checking foetal conditions in the ante-partum period. Among many techniques to provide information about foetal health, external Cardiotocography (CTG) is the most diffused indirect, diagnostic method in clinical practice, during last pregnancy stage and labour. CTG is based on the simultaneous recording of FHR and UC (Uterine Contractions) [2]. Important conditions such as foetal distress are evaluated from the cardiotocographic tracings, generally by means of clinicians' eye inspection, who evaluate specific clinical signs (FHR mean value, FHR variability, FHR accelerations, FHR decelerations, and foetal movements). Nevertheless, the efficiency of this method depends on observer's expertise and training, but obviously it lacks of objectivity and reproducibility and it is subject to human error [2]. For this reason, many researchers have attempt to make the recognition of some specific parameters more reliable, introducing the computerized analysis. Besides, to improve CTG analysis, more objective methods for CTG interpretation are of crucial importance; therefore, considerable efforts have been spent and several analysis methodologies have been proposed in recent years [90]. It has been demonstrated that also for the foetus, variability of the HR around its mean value, namely FHR variability (FHRV),

could be a valid support for a more objective analysis and for a better knowledge of ANS reactions and its functional state [101].

The traditional analysis of FHRV (time and frequency domain analysis) provide significant noninvasive parameters to investigate the cardiac autonomic modulation; however, many studies have shown some limitations in describing the nonlinear structure of the sympatho-vagal interactions. Therefore, in recent years, methods previously developed from nonlinear systems theory have been applied to biological system analysis and, in particular, to the study of heart rate variability.

Nevertheless, to date, no methods neither traditional or nonlinear has yet proved to be superior to the other or completely reliable in foetal health evaluation or in reducing the number of false positive in CTG interpretation.

This thesis focus the attention on the Symbolic Dynamic Analysis (SDA), a nonlinear technique which allows a simple description of a system's dynamics by means of a limited amount of symbols and appropriate classification schemes, which has already got some success both in HRV and FHRV studies [84, 155].

Research aims

The purpose of this research is to deepen the analysis of the signal FHRV, and, therefore, knowledge of the autonomic nervous system that can result, through a method called "Symbolic Dynamics Analysis" (SDA), already applied with positive results to the analysis of HRV of the adult, and in some cases to the foetus. We also propose to verify the contribution and the advantages that it can offer compared to traditional linear and nonlinear FHRV. All these methodologies aim to expand the range of information available to the physician, therefore improving the specificity of CTG, currently very low, in spite of its legal value.

Thus, this work mainly aims to carry out a multiparametric study by means of traditional (in time and frequency domain) and nonlinear techniques to compare different FHRV analysis methodologies and evaluate their strength and weakness points in supporting the FHR monitoring. A special attention is given to the Symbolic

Dynamics Analysis, a non-linear method that has been quite recently employed in literature to analyse foetal heart rate variability.

In carrying out a study so wide, more goals have been also achieved, listed below:

- development of a software for the analysis of the CTG traces, in order to reach a better signal pre-processing and processing, improving FHRV analysis performances and enhance data elaboration and visualization of the obtained information;
- development of a software for simulating FHR signals, as a useful tool to support the validation of the software for real CTG traces elaboration and the evaluation of the different adopted techniques for FHRV analysis;
- study of literature concerning linear and nonlinear methods in order to identify those most used and more effective for FHRV analysis;
- comparison of the results obtained by linear and nonlinear analysis, assessing usefulness and reliability of the computed indexes by means of statistical tests and regression graphs;
- proposal of a well defined methodology for evaluation of FHRV both in time and frequency domain, not yet available in literature despite to the wide use of this signal.

With regards to the developed software for analyze real CTG traces, it includes a simple interface, so it can be used even by non-experts, and provides estimates of:

- The series real FHR not evenly sampled;
- Percentage of beats lost and interpolated;
- The mean value and the oscillation of the baseline of the FHR signal;
- The number and kind of accelerations and decelerations;
- The presence of uterine contractions;
- Indices of short term variability;
- Indices from FHR signal spectrum;
- The variability indices calculated according to the SDA.

As far as the simulated CTG signals, they were used to test the performance of the above described software. The developed software for CTG simulation will generate

INTRODUCTION

artificial signals that simulate the actual CTG recordings. It will facilitate the comparison between different processing methods of the signals to determine their performance and also could be used also as a tool teaching for medical students.

Besides, over 100 articles published between 1996 and 2014 were analyzed in order to verify the state of the art about the application of nonlinear methods to the study of the heart rhythm. They cover both the foetus and the adult, from which usually part when studying the heart rhythm. For each technique considered to have been highlighted advantages and disadvantages, also in relation to the experimental results obtained.

It was then made a review of the nonlinear methods and examined their applicability for analysis of foetal heart rate and adult patients. The main advantage of the indexes of HRV is that they can be calculated in real time in a non-invasive way, while all biomarkers currently used in clinical practice involve the taking of blood samples for analysis and in no case could be used for the fetus that is not directly accessible.

The foetal heart rate is a signal not evenly sampled and contains artifacts and noise, the study carried out on nonlinear techniques has shown that nonlinear methods are also useful for the classification of foetal heart segments of short duration and that is promising for further research.

In order to compare the results obtained with the linear and non-linear analysis and hence to individuate the best methodology, were then calculated different indices. Following the bibliographic study, to evaluate the reliability of the different linear and non-linear FHRV indices calculated, were carried out statistical tests and regression analysis to examine FHR signals recorded in a clinical setting in order to classify the foetuses in relation to:

- Apgar score;
- Kind of delivery (cesarean or spontaneous).
- Week of gestation;
- Active or resting status;

Thesis structure

The presented thesis is organized in seven chapters as follows:

- Chapter 1: description of the physiological and anatomical principles related to the cardiovascular system of adult subjects along with an explanation of the role of ECG diagnostic methods and heart rate variability characteristics;
- Chapter 2: description of the foetal cardiovascular system development and principles of uterine physiology;
- Chapter 3: presentation of the main foetal heart rate diagnostic techniques, parameters and features;
- Chapter 4: overview of the main linear and non-linear methodologies for HRV and FHRV analysis, with an in-depth description dedicated to the Symbolic Dynamics Analysis and a literature report of relevant studies and works on the HRV and FHRV analysis;
- Chapter 5: description of the analyzed data (real and simulated CTG traces) along with processing and pre-processing techniques characterization;
- Chapter 6: presentations and discussion of the employed analysis methods;
- Chapter 7: presentations and discussion of the main obtained results.

CHAPTER 1

Physiology of the cardiovascular system and Heart Rate Variability

1.1 Anatomy and physiology

1.1.1 Physiology of the cardiovascular system

The cardiovascular system is a closed and continually active system. It can be considered as a machine consisting of defined components with their own functional roles and mechanics. This system ensures the transport and distribution to tissues of essential substances, such as respiratory gases (oxygen, carbon dioxide) and nutrient materials (aminoacids, glucose and fatty acids), and the elimination of metabolic waste products (degradation products of nutrients).

Moreover, the cardiovascular system is involved in the control of metabolic mechanisms, such as body temperature regulation, chemical messages transport for the communication between different points of the organism and oxygen and nutrients inflow regulation under various physiological conditions.

This apparatus consists of a central engine, the heart, a muscular organ that ensures the circulation of the blood (a fluid consisting of a suspension of cells in an aqueous medium that contributes to the oxygen and nutrients transport) through its rhythmic contractile activity, and a closed system of elastic tubes with different structures, which are:

- the arteries, which convey the blood from the heart to the periphery;
- the capillaries, the lightest and microscopic vessels whose walls are constituted by a single layer of cells exchanging oxygen and nutrients with surrounding tissues and collecting carbon dioxide and metabolic waste;

- the veins, which convey the blood from the periphery to the heart, thereby closing the circle.

The heart is formed by two pumps in series: the first pushes the blood into the lungs to exchange oxygen and carbon dioxide (pulmonary circulation) while the second pushes the blood into all tissues of the organism (systemic circulation). In order to fulfill its function, the heart is divided from a vertical median septum into a right and a left section and it is composed of four chambers, two atria and two ventricles. Four heart valves allow blood to flow either from one chamber to another or out of the heart in a forward direction by generating adequate intracavitary pressures to overcome resistance and permit blood ejection. The right and the left section of the heart are divided into an upper part, thin-walled chamber called atrium, which acts as a collection chamber, and a lower part, larger and thick-walled chamber called ventricle, which acts as a chamber of expulsion with greater contractile energy than the atrium. The right atrium communicates with the underlying right ventricle through a valve which consists of three flaps and therefore called the tricuspid; the left atrium, similarly, communicates with the left ventricle through a valve consisting of two flaps and called mitral. Under physiological conditions, these two valves allow the blood to flow in one direction only, from the atrium to the ventricle, thereby preventing the backflow from the ventricles to the atria. The two atria are separated by a wall of tissue called interatrial septum while the two ventricles are separated by the interventricular septum. The right atrium receives blood from the superior vena cava, which collects all the blood of the upper half of the body (head, brain, neck and arms), the inferior vena cava, which drains all the blood of the lower half of the body (venous blood, therefore deoxygenated) and the coronary sinus, which conveys the effluent from the heart. Blood flows from the right atrium into the right ventricle through the tricuspid valve and - from here - into the pulmonary artery through the pulmonary valve. Through this artery and its branches, the blood is pumped into the pulmonary circulation and, then, to the pulmonary capillaries that exchange oxygen with the atmospheric air. The oxygenated blood is then collected by the venules and, later, flows from pulmonary veins into the left atrium. Here, the blood flows into the left ventricle through the mitral valve and into the

aorta through the aortic valve. Through this artery and its ramifications, the blood is pushed into the systemic circulation up to the capillaries supplying nutrition to the tissues. The blood is then collected in the venous vascular system and - through the superior vena cava and the inferior vena cava - comes back to the right atrium. The role of the pulmonary and aortic semilunar valves is crucial because they prevent the backflow of the blood from the great arteries (pulmonary and aorta) to the ventricles [2].

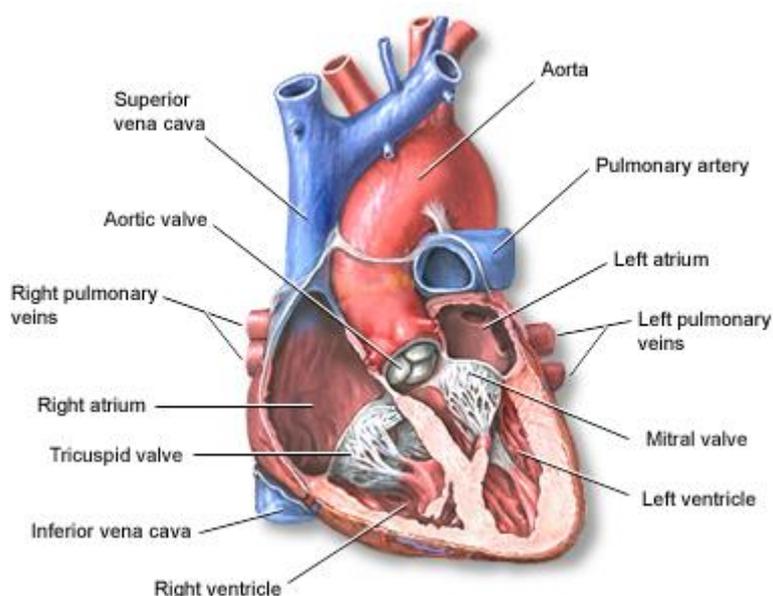


Figure 1.1 - Longitudinal section of heart. Source: Medline Plus. (2013). Heart, section through the middle [Online]. Available: <http://www.nlm.nih.gov/medlineplus/ency/imagepages/18093.htm> (last access January 2015)

The cardiac cycle is divided into a systolic phase, coinciding with the ventricular contraction, and a diastolic phase coinciding with the release ventricular. In particular, whereas the left ventricle when the latter begins to contract the ventricular pressure increases rapidly, as the ventricular volume does not vary since the atrioventricular valve closes to prevent reflux of blood from the ventricle to the atrium while the aortic valve is not yet open. When the ventricular pressure exceeds the aortic one, the valve opens and starts the ejection of blood. At this point, the pressure decreases until the gradient between the ventricle and the aorta is reversed, but the aortic valve is still open closes due to the energy accumulated during the first part of systole. At the closing of the aortic valve, ventricular pressure

decays rapidly, but the volume remains constant because the atrioventricular valve is not yet open. The opening of the atrioventricular valve, there is a rapid ventricular filling and, subsequently, when the atrium has emptied the accumulated blood in the ventricle, ventricular filling continues a slower, controlled directly by the venous return. This phase ends with the atrial systole which, however, is only partially effective, given the lack of valves from the venous side [2].

1.1.2 The conduction system

The electrical activity of the heart is the basis of the functioning of the cardiovascular system, for this is very often subjected to diagnostic and therapeutic monitoring. The cardiac electrical activity originates in the sinoatrial node, located in the upper zone of the right atrium, which acts as a pacemaker, and then propagates through a preferential way, indicated by the term “beam Bachmann” to the lobby spooky. Through further preferential ways the pulse is also conducted to the atrioventricular node located between the atria and ventricles at the lower front part of the atrial septum. Here, the signal is subject to a specific delay, such as to allow the filling of the ventricles with blood during the contraction of the atria. From here, the electric pulse propagates through a specific conduction beam called “bundle of His” and which is divided successively into two branches, the right and the left one, until then Purkinje fibers that form a real network of fibers in close contact with the muscle tissue of the ventricles. This procedure run is illustrated in the following figure [2].

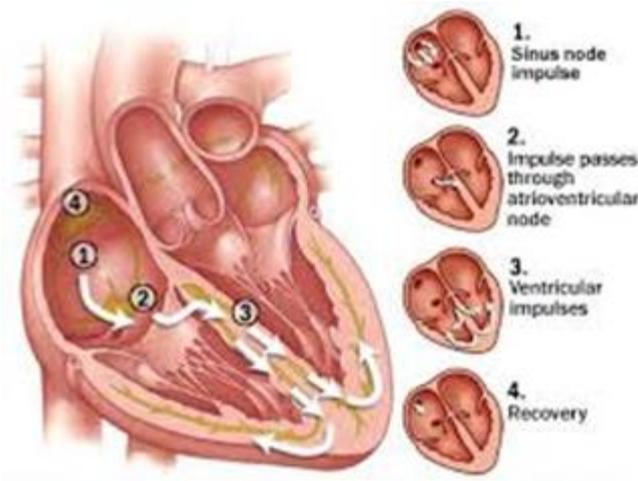


Figure 1.2 - Conduction of the action potential in physiological conditions. Source: Mayo Foundation for Medical Education and Research. MayoClinic.com Health Library [Online]. Available: http://www.riversideonline.com/health_reference/disease-conditions/ds00947.cfm (last access January 2015)

1.1.3 Cardiovascular control mechanisms

There is a difference in the rate of reaction to stimuli produced by the two branches of the vagus and sympathetic system. The physiological mechanisms of feedback control regulate the magnitudes considering loops with different propagation delays of the useful signal that travels along them, generating fluctuations in the rhythm at different frequencies and contributing to the spectrum of HRV in a manner significantly different depending on the specific range of frequencies associated with them.

In adults, the three main loops which are based on physiological processes homeostatic regulation and involved in the definition of the total variability of HR are:

Loop of breathing: breathing movements are triggered by pulses sent by the centers in the brain respiration. With the inspiration there is an increase in intrathoracic pressure, a decrease in the pulsatory volume, then a decrease in cardiac output, and a decrease in blood pressure. This reduction in pressure is detected by baroreceptors that send the corresponding information relating to the brain centers, which generate a signal of inhibition of vagal tone, signal which of course has the

effect of unbalancing the sympatho-vagal balance more on the side of the sympathetic causing an increase in heart rate, then the range and therefore of the pressure. The reverse mechanism occurs during the exhalation phase. In this way there is an oscillatory component of the HRV signal, defined by the name of respiratory sinus arrhythmia, synchronous with the respiratory rate, which corresponds to a spectral lobe in the range 0.15 to 0.40 Hz, centered around 0.3 Hz, which goes under the name component of HF (High Frequency) signal HRV. This component reflects vagal activity, as is confirmed by the significant reduction of the relative power following the administration of drugs blocking the vagus (such as atropine) [1, 2].

Loop of baroreceptor reflexes: baroreceptors detect pressure changes and send the corresponding information to the afferent brain centers from which impulses start and this situation causes an increase in heart rate, to compensate a decreasing in blood pressure, or a reduction of the same otherwise. There is the creation of a rhythmic component of HRV signal, called "Rhythm of 10 s", synchronous with fluctuations of blood pressure, known as "waves Mayer", which corresponds to a lobe in the spectral range 0.04 to 0.15 Hz, centered around 0.12 Hz, which goes under the name of component LF (Low Frequency) signal HRV. The frequency of fluctuations is determined by the time of delay of the system and increases with the increase in sympathetic tone. The LF component of HRV signal is mainly linked to the activity of the sympathetic nerve [1, 2].

Loop thermoregulatory and slow control mechanisms: the thermodynamic phenomena are characterized by very long transient. Consequently, the loop of thermoregulation, which reduces or increases thermogenesis irradiation in case of temperature above the threshold of wellbeing, and vice versa if the temperature drops below the setting threshold, is responsible for fluctuations in heart rate that develop in long periods and, therefore, the so-called component VLF (Very Low Frequency) signal HRV, ranging from DC to approximately 0.04 Hz. The phenomenon is linked, as a result of temperature changes, to changes in peripheral vascular resistance and blood pressure, which, by means of baroreceptor reflexes, results in slow fluctuations HR. The VLF component, such as LF, is mediated by both

the sympathetic and the vagal nerve, but noted a prevalence of incidence of the first [145].

It should be noted that other factors are also responsible of changes in sympatho-vagal balance and heart rate variability. These include the circadian rhythm, posture, the behavioral state and age. For example, the HR variability increases in the last months of gestation of the foetus and in the early months of the infant's life (which corresponds to the completion of the maturation of the nervous system and, therefore, the differentiation of its two sections of the vagus and sympathetic) while, in adults, decreases with age (and this reduction in non-pathological conditions, is likely to be of interest in the same way all the spectral bands, while leaving unchanged the sympatho-vagal balance) [132].

1.2 Heart Rate Variability

Although cardiac automaticity is intrinsic to various pacemaker tissues, heart rate and rhythm are largely under the control of the autonomic nervous system. There is a two-way communication system between the heart and the brain that regulates heart rate and blood pressure and it is the interaction of signals flowing between the two that causes the heart rate to vary with each beat. The sympathetic branch increases heart rate and the secretion of adrenal hormones, etc., whereas the parasympathetic slows heart rate and has a relaxing, protective role. Proper function and balance between the two branches of the ANS is important for good health. The parasympathetic influence on heart rate is mediated via release of acetylcholine by the vagus nerve. The sympathetic influence on heart rate is mediated by release of epinephrine and norepinephrine. Under resting conditions, vagal tone prevails and variations in heart period are largely dependent on vagal modulation. The vagal and sympathetic activity constantly interact. The RR interval variations present during resting conditions represent a fine tuning of the beat-to-beat control mechanisms. Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity. The opposite reflex effects are mediated by the stimulation of sympathetic afferent activity.

Efferent vagal activity also appears to be under 'tonic' restraint by cardiac afferent sympathetic activity. Efferent sympathetic and vagal activities directed to the sinus node are characterized by discharge largely synchronous with each cardiac cycle which can be modulated by central (e.g. vasomotor and respiratory centres) and peripheral (e.g. oscillation in arterial pressure and respiratory movements) oscillators. These oscillators generate rhythmic fluctuation in efferent neural discharge which manifest as short and long-term oscillation in the heart period. Analysis of these rhythms may permit inferences on the state and function of the central oscillators; the sympathetic and vagal efferent activity; humoral factors and the sinus node [2].

An understanding of the modulator effects of neural mechanisms on the sinus node has been enhanced by spectral analysis of HRV. The efferent vagal activity is a major contributor to the HF component. More controversial is the interpretation of the LF component which is considered by some as a marker of sympathetic modulation (especially when expressing it in normalized units) and by others as a parameter that includes both sympathetic and vagal influences. Spectral analysis of 24-h recordings shows that in normal subjects LF and HF expressed in normalized units exhibit a circadian pattern and reciprocal fluctuations, with higher values of LF in the daytime and of HF at night. These patterns become undetectable when a single spectrum of the entire 24-h period is used or when spectra of subsequent shorter segments are averaged. In long-term recordings, the HF and LF components account for approximately 5% of total power. Although the ULF and VLF components account for the remaining 95% of total power, their physiological correlates are still unknown. LF and HF can increase under different conditions. In studies researching HRV, the duration of recording is dictated by the nature of each investigation. Standardization is needed, particularly in studies investigating the physiological and clinical potential of HRV. Recording of approximately 1 min is needed to assess the HF components of HRV while approximately 2 min are needed to address the LF component. In order to standardize different studies investigating short-term HRV, 5 min recordings of a stationary system are preferred unless the nature of the study dictates another design. Averaging of spectral components obtained from sequential

periods of time is able to minimize the error imposed by the analysis of very short segments. Nevertheless, if the nature and degree of physiological heart period modulations changes from one short segment of the recording to another, the physiological interpretation of such averaged spectral components suffers from the same intrinsic problems as that of the spectral analysis of long-term recordings and warrants further elucidation.

Although the time-domain methods, especially the SDNN and RMSSD methods, can be used to investigate recordings of short durations, the frequency methods are usually able to provide more easily interpretable results in terms of physiological regulations. In general, the time-domain methods are ideal for the analysis of long-term recordings (the lower stability of heart rate modulations during long-term recordings makes the results of frequency methods less easily interpretable). The experience shows that a substantial part of the long-term HRV value is contributed by the day-night differences. Thus the long-term recording analysed by the time domain methods should contain at least 18 h of analysable ECG data that includes the whole night [1].

1.3 Heart Rate diagnostic methods

1.3.1 The ECG signal

The term ECG indicates the diagnostic technique that allows to explain the electrical activity of the heart via a recording of the time series of potential differences detected in one or more pairs of electrodes whose locations are called “lead”. The 12-lead electrocardiography traditional plans. The first three, called 'standard', are those obtained by projecting the electric dipole on the sides of an equilateral triangle called Einthoven triangle. In fact we apply the cardiac vector in the center of this triangle whose vertices are the pads connected to the right arm, left arm and left leg (for convenience usually the electrodes are allocated to the wrists and ankles) and the center is the heart [70].

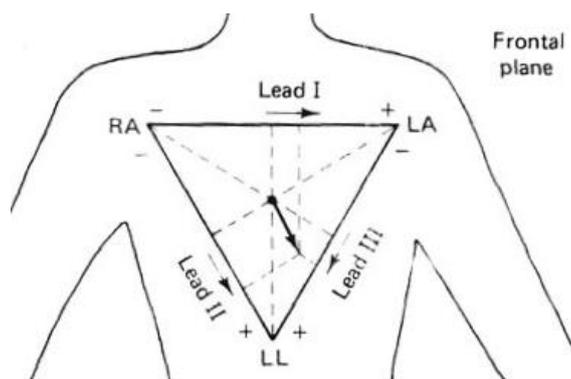


Figure 1.3 - Einthoven Triangle. Source: Biomedical Engineering Lab 355 [Online]. Available: <http://noodle.med.yale.edu/staib/bme355/ecg/prep.htm> (last access January 2015)

In this configuration, we have that the potential difference between any pair of electrodes (or vertices) of the triangle is proportional to the projection of the electric dipole on the section joining the two vertices of interest. From the need to identify local values of potential and no differences occurred the idea to refer to a point in which the electrical activity was the average of points very distant from the source rate. Therefore the other nine leads, in particular the six precordial leads and the three increased, devolve the Wilson central terminal defined as the common electrical point or node of three equal resistors connected to the electrodes of the three limbs [70].

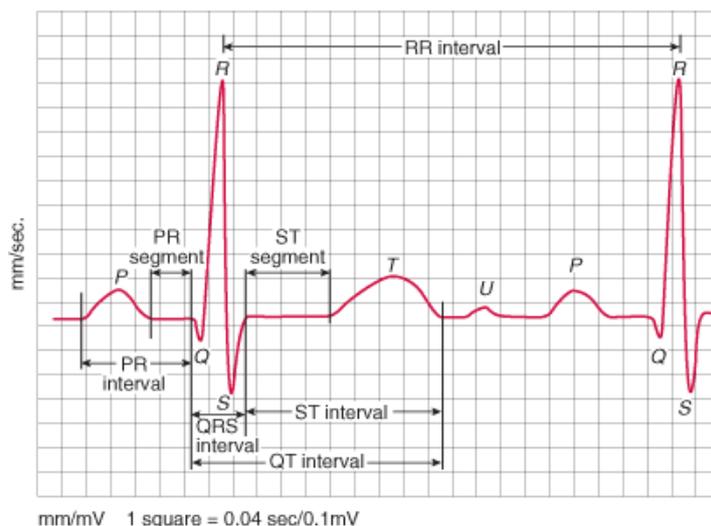


Figure 1.4 - A normal ECG Waveform. Source: Merck Manuals - Cardiovascular disorders. Standard ECG components [Online]. Available: http://www.merckmanuals.com/professional/cardiovascular_disorders/cardiovascular_tests_and_procedures/electrocardiography_ecg.html#v931664 (last access January 2015)

The following is a summary of the ECG wave morphologies and parameters that users can use as a guide to understand more about their ECG recordings.

P wave: The P wave results from atria contraction. P wave is generally about 1 box wide or 1 box tall. P wave that exceeds these might indicate atria hypertrophy, i.e., enlargement.

PR Interval: The PR interval is measured from the start of the P wave to the start of Q wave. It represents the duration of atria depolarization. Regular duration is from 0.12 to 0.20 seconds, about 3 to 5 box wide. If the PR interval is greater than 0.20 seconds, then an AV block might be present.

QRS Complex: The QRS complex is measured from the start of Q wave to the end of S wave. It represents the duration of ventricle depolarization. Regular duration is from 0.08 – 0.12 seconds, about 2 to 3 box wide. If duration is longer, it might indicate presence of bundle branch blocks.

QT/QTc: The Q T/QTc is measured from the start of the Q wave to the end of T wave. QT interval represents the duration of activation and recovery of the ventricular muscle. This duration varies inversely with the heart rate. The regular QTc is approximately 0.41 seconds or an accurate measurement, it is corrected with the heart rate with the following formula to get QTc:

$$QTc = QT + 1.75 (HR - 60)$$

ST Segment: The ST segment is measured from end of S wave, J point, to the start of T wave. This segment is important in identifying pathology such as myocardial infarctions (elevations) and ischemia (depressions).

CHAPTER 2

Foetal cardiovascular system and uterine physiology

2.1 Cardiovascular system

2.1.1 Physiology of the foetal cardiovascular system

The foetal circulation is one of the first systems to need to be able to function properly in order to sustain the foetus. Before a circulatory system has developed, nutrients and oxygen diffuse through the extraembryonic coelom and yolk sac from the placenta. As the embryo increases in size, its nutrient needs increase and the amount of tissue easily reached by diffusion decreases. Hence the circulation must develop quickly and accurately.

However, throughout the foetal stage of development, the maternal blood supplies the foetus with O₂ and nutrients and carries away its wastes. These substances diffuse between the maternal and foetal blood through the placental membrane.

In the foetal circulatory system, the umbilical vein transports blood rich in O₂ and nutrients from the placenta to the foetal body. The umbilical vein enters the body through the umbilical ring and travels along the anterior abdominal wall to the liver. About 1/2 of the blood passes into the liver. The other 1/2 of the blood enters a vessel called the ductus venosus of Aranzio which bypasses the liver. The ductus venosus travels a short distance and joins the inferior vena cava. There, the oxygenated blood from the placenta is mixed with the deoxygenated blood from the lower parts of the body. This mixture continues through the vena cava to the right atrium. In the adult heart, blood flows from the right atrium to the right ventricle then through the pulmonary arteries to the lungs. In the foetus the lungs are non-

functional and the blood largely bypasses them. As the blood from the inferior vena cava enters the right atrium, a large proportion of it is shunted directly into the left atrium through an opening called the foramen ovale. A small valve, septum primum is located on the left side of the atrial septum overlies the foramen ovale and helps prevent blood from moving in the reverse direction. The rest of the foetal blood entering the right atrium, including a large proportion of the deoxygenated blood entering from the superior vena cava, passes into the right ventricle and out through the pulmonary trunk. Only a small volume of blood enters the pulmonary circuit, because the lungs are collapsed, and their blood vessels have a high resistance to flow. Enough blood reaches the lung tissue to sustain them. Most of the blood in the pulmonary trunk bypasses the lungs by entering a foetal vessel called the ductus arteriosus of Botallo which connects the pulmonary trunk to the descending portion of the aortic arch. The more highly oxygenated blood that enters the left atrium through the foramen ovale is mixed with a small amount of deoxygenated blood returning from the pulmonary veins. This mixture moves into the left ventricle and is pumped into the aorta. Some of it reaches the myocardium through the coronary arteries and some reaches the brain through the carotid arteries. The blood carried by the descending aorta is partially oxygenated and partially deoxygenated. Some of it is carried into the branches of the aorta that lead to various parts of the lower regions of the body. The rest passes into the umbilical arteries, which branch from the internal iliac arteries and lead to the placenta. There the blood is reoxygenated. Both ductus venosus of Aranzio and ductus arteriosus of Botallo are completely closed after birth.

It is worth mentioning that the concentration of haemoglobin in foetal blood is about 50 % greater than in maternal blood. Foetal haemoglobin is slightly different chemically and has a greater affinity for O₂ than maternal haemoglobin. This is a sort of safety mechanism; in fact because of this characteristic, foetus can overcome relatively short lacks of oxygen.

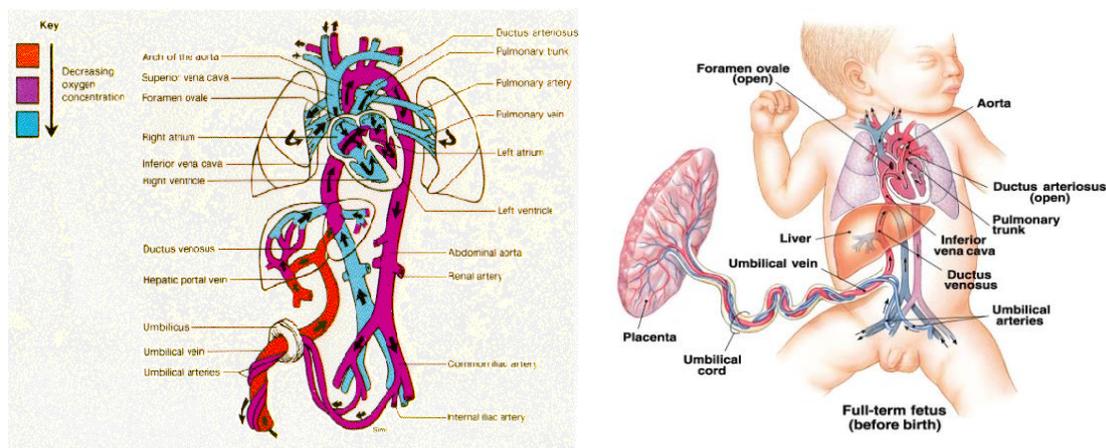


Figure 2.1 - On the right and on the left, different pictures of foetal blood circulation [2]

2.1.2 Conduction system and control mechanisms

Throughout the heart are clumps of specialized cardiac muscle tissue whose fibres contain only a few myofibrils. Electrical impulse originates in the Sinoatrial (S-A) Node (in the foetus, it is completely developed at 6th week of gestation), which consists of a small elongated mass of specialized muscle tissue just beneath the epicardium. Fibres are continuous with those of the atrial muscle fibres. Membranes of the nodal cells are in contact with each other and have the ability to excite themselves. Without being stimulated by nerve fibres or any other outside agents, the nodal cells initiate impulses that spread into the surrounding myocardium and stimulate the cardiac muscle fibres to contract; this activity is rhythmic.

The cardiac cycle refers to the repetitive pumping process that begins with the onset of cardiac muscle contraction and ends with the beginning of the next contraction. The duration of the cardiac cycle varies among people and also varies during an individual's lifetime. In an adult subject, the normal cardiac cycle (0.7-0.8 sec.) depends on two factors: capability of cardiac muscle to contract and functional integrity of the conducting system. Abnormalities of cardiac muscle, the valves, or the conducting system of the heart may alter the cardiac cycle and compromise the pumping effectiveness of the heart.

In a foetus, through labour and delivery, we can invasively record foetal heart electrical activity by means of direct scalp foetal ECG, attaching electrodes to the presenting part of the foetus after membrane rupture, and obtain an ECG signal very similar to those described for adults, even if with a lower amplitude. Otherwise, after 16th week's gestation, we can adopt the external abdominal ECG, putting electrodes on the maternal abdomen.

However, foetal heart rate variability is also intimately related to foetal central nervous system; particularly, the most important mechanism immediately involved in producing heart rate variability is the autonomic innervations of the heart. The cardioregulatory centre in the medulla oblongata regulates the parasympathetic and sympathetic nervous control of the heart.

Parasympathetic stimulation is supplied by the cardiac branches of the vagus nerve. It is of primary importance in producing beat-to-beat variability. It decreases heart rate and can cause a small decrease in the force of contraction (stroke volume). This component of cardiac innervations is well suited to a role of fine tuning the heart rate on a beat-to-beat basis because of the very rapid decrease in heart rate which occurs with vagal nerve stimulation, and the nearly equally rapid recovery after the end of a series of impulses. Moreover, postganglionic neurones secrete acetylcholine which increases membrane permeability to K^+ , producing hyperpolarization of the membrane.

Sympathetic stimulation is supplied by the cardiac nerves which are projections of the cervical sympathetic chain ganglia (spinal nerve). Sympathetic stimulation increases heart rate and force of contraction (stroke volume). Changes in heart rate with stimulation of cardiac sympathetic innervation are slower compared to stimulation of cardiac vagal innervations. Moreover, it dilates vessels in skeletal and cardiac muscle.

2.2 Uterine physiology

The human uterus is a massive, hollow, pear-shaped organ with a thick wall, situated deeply in the pelvic cavity between bladder and rectum. It is composed of two

distinct anatomic regions: the cervix and the corpus. The corpus is further divided into the lower uterine segment and the fundus. The cervix is a narrow cylindrical passage which connects at its lower end with the vagina. At its upper end, the cervix widens to form the lower uterine segment (isthmus); the lower uterine segment in turn widens into the uterine fundus. The corpus is the body of the uterus which changes in size and structure during pregnancy to accommodate itself to the needs of the growing embryo. Extending from the top of the uterus on either side are the fallopian tubes (oviducts); these tubes are continuous with the uterine cavity and allow the passage of an ovum (egg) from the ovaries to the uterus where the egg may implant if fertilized. Spatial organisation of the smooth muscle fibres in the uterine wall is complicated and still remains the matter of debate. The thick wall of the uterus is formed of three layers: endometrium, myometrium, and serosa or perimetrium. The endometrium (uterine mucosa) is the innermost layer that lines the cavity of the uterus. Throughout the menstrual cycle, the endometrium grows progressively thicker with a rich blood supply to prepare the uterus for potential implantation of an embryo. In the absence of implantation, a portion of this layer is shed during menstruation. The myometrium is the middle and thickest layer of the uterus and is composed of smooth (involuntary) muscle. The myometrium contracts during menstruation to help expel the sloughed endometrial lining and during childbirth to propel the foetus out of the uterus. The outermost layer, or serosa, is a thin fibrous layer contiguous with extrauterine connective tissue structures such as ligaments that give mechanical support to the uterus within the pelvic cavity. Non-pregnant uterine size and position varies with age and number of pregnancies.

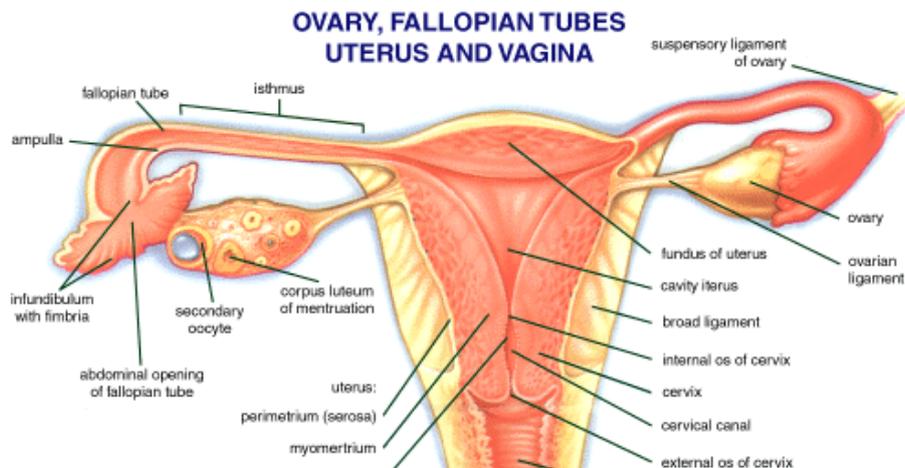


Figure 2.2 - Uterus or womb [2]

Uterine wall structure is aimed to effective expulsion of foetus if pregnancy is about to terminate. Although biological mechanisms prevent massive contractions of uterus during pregnancy, the uterine wall never remains quiet. Every single muscle fibre possesses the possibility to change its membrane potential slowly, which results in depolarisation. Working potential that it generates may be transmitted to other cells in a close neighbourhood, but the area it can spread on strongly depends on local properties of signal propagation. In course of a physiological pregnancy the intercellular communication is poorly developed, which seems to be a mechanism of a foetus' safety. This leads to the lack of coordination between muscle fibres which produces a kind of fibrillation of uterine wall with almost no significance rise of pressure inside its cavity. In a full term pregnancy, or in some pathological circumstances even sooner, uterine wall becomes well coordinated and uterine contractions frequent, intense, persistent and painful. Low resistance intercellular connections – gap junctions appear in a smooth muscle tissue enhancing trigger wave propagation. Even though there is no specialised trigger wave conducting system in uterus, gap junctions enable it to contract as a whole, presenting a specific pattern of contraction. The certain degree of synchronisation of smooth muscle cells amplifies uterine working potentials, since their appearance results from spatial and temporal summation of electrical activity of single fibres [3].

In spite of the fact that the uterine contractility is predominantly commanded by hormonal and biochemical factors (estrogens, oxytocin, prostaglandins), there are

indications that the sympathetic and parasympathetic innervations of the uterus may also have a considerable influence upon it. Independently of the majority of the uterine contractions being endocrinally and biochemically triggered, the myometrial contractile activity exhibits a very peculiar characteristic that seems to demonstrate the existence of a precise nervous coordination: it is the "triple descending gradient." This gradient gives to the uterine contractility its typical expulsive pattern.

Concerning their characteristics, uterine contractions become very rhythmic and regular in shape during labour, when the hypophysis releases a large dose of oxytocin. The contraction length ranges between 15 and 20 seconds at the begin of the labour and between 60 and 70 seconds at the end (expulsive period) [57]. Approximately at 20th week of gestation irregular contractions with very small amplitude, called Alvarez's waves, are present. They represent a located muscular contraction. In physiological conditions, their frequency decreases and their amplitude increases with gestation progress. In the second period of gestation, a large part of the uterus contracts itself giving rise to Braxton-Hicks' contractions, also slang called "preparations contractions". After 30th week, these contractions gradually become more frequent and strong [4].

CHAPTER 3

Diagnostic methods

3.1 Foetal Heart Rate diagnostic methods

3.1.1 Foetal electrocardiography

Foetal electrocardiography (FECG) has been deeply studied, but its recording through multiple electrodes placed on the maternal abdomen makes difficult to obtain high quality signals; moreover, the automated evaluation of FECG is less accurate than CTG [5 - 7].

ECG is a graphical recording of the electrical potentials generated in association with heart activity. Aristotle first noted electrical phenomena associated with living tissues and Einthoven was able to measure the electrical activity of the heart in 1901 that resulted in the birth of electrocardiography [8]. In adults, as the heart is not directly accessible, cardiac electrical activity is usually inferred from measurements recorded at the surface of the body, e.g., at the arms, legs, and chest.

For foetuses, electronic foetal monitoring by means of ECG can be external (outside), internal (inside), or both.

Internal methods for acquiring the FECG are invasive because internal monitoring involves placement of a small plastic device about the size of a pencil eraser through the cervix. A spiral wire called the foetal scalp electrode is placed just beneath the skin of the foetal scalp. The foetal scalp electrode then transmits direct information about the FHR through a wire to the foetal monitor that prints out this information. Because the internal foetal monitor is attached directly to the baby, the FECG signal is sometimes much clearer and more consistent than with an external monitoring device. However, there may be a slight risk of infection with internal monitoring.

Obviously, a foetal scalp electrode cannot be used in antepartum period as there is a significant risk of causing a mark or small cut on the foetal head [9].

In contrast, methods utilizing the abdominal FECG have a greater prospect for long-term monitoring of FHR (e.g., 24 h) in foetal well-being assessment using complex signal-processing techniques.

In fact, the FECG is an electrical signal that can be obtained non-invasively by applying multi-channel electrodes placed on the abdomen of a pregnant woman; therefore the three main characteristics that need to be obtained from the FECG extraction for useful diagnosis include [10]: FHR, waveform amplitudes and waveform duration.

The detection of FECG signals with powerful and advanced methodologies is becoming a very important requirement in biomedical engineering with the increasing in FECG signal analysis in clinical diagnosis and biomedical applications. The FECG contains potentially valuable information that could assist clinicians in making more appropriate and timely decisions during labour, but the FECG signal is vulnerable to noise, and difficulty of processing it accurately without significant distortion has impeded its use.

3.1.2 Phonocardiography

The preliminary evaluation done by Baskaran and Sivalingam [11] has shown that there are three significant differences in the characteristics of foetal heart sounds between intrauterine growth retarded and normal foetuses in the antenatal period. Although it was just a preliminary study, it has further inspired the possibility to employ FPCG to identify foetuses at risk. This could be a significant contribution to the pressing clinical problem faced by some unborn and newly born babies. FPCG performs a recording of UC by means of a usual pressure transducer and a passive (no energy beam is transmitted to the foetus), fully non-invasive and low cost acoustic recording of foetal heart sounds [7, 12, 13]. This signal can be captured by placing a small acoustic sensor on mother's abdomen without the use of gel and, if

appropriately recorded, it is a sensitive signal very useful in providing clinical indication.

The foetal heart is basically divided into two pairs of chambers and has four valves: the mitral and tricuspid valves. In the foetal cardiac cycle, when the ventricles begin to contract, the blood attempt to flow back into the lower pressure atrial chambers: this reverse flow of blood is arrested by the shutting of the mitral and tricuspid valves, which produces the first heart sound (S1). Whenever the pressure in the ventricular chambers becomes too high for the pulmonary valves to withstand, they open, and the pressurized blood is rapidly ejected into the arteries. While the ventricles are being evacuated, the pressure of the remaining blood decreases with respect to that in the arteries. This pressure gradient causes the arterial blood to flow back into the ventricles. The pulmonary valves, arrest this reverse flow by shutting, which gives rise to the second heart sound (S2) [14]. The intensity of S1 is generally increased by greater pressure within the left ventricle as the resistance within the pulmonary artery increases and as the blood passes from the left atrium. This greater pressure results in the closure of the mitral valve with greater force, thus producing a more intense sound [15]. On the other hand, S2 is considered to be particularly more useful when diagnosing cardiac disease [15] and is produced by the ejection of blood from the ventricles out through the aorta and pulmonary artery [16].

In conclusion FPCG provides valuable information concerning the physical state of unborn in the womb and has the potential for detection of cardiac functionality anomalies, such as murmur, split effect, extra systole, bigeminal/trigeminal atrial. Such phenomena are difficult to obtain with the traditional CTG technique or other methods [11, 17, 18, 57].

3.1.3 Magnetocardiography

The foetal magnetography (FMCG) is based on the measurement of the magnetic fields produced in association with cardiac electrical activity [8]. The recording uses the SQUID (Superconducting Quantum Interference Device) biomagnetometry

technique. The FMCG contains morphological and temporal similarity to the FECG even though they are based on very different types of measurements, i.e., the electrical field and the magnetic field.

The disadvantages of the FMCG are size, cost and complexity of the required instrumentation. It can be recorded reliably from the 20th week and onward. Moreover, it is mainly unaffected by the insulating effects of the vernix caseosa and the existence of preferred conduction pathways. The FMCG remains reliable for measuring the foetal electrocardiological activity throughout the second and third trimesters of pregnancy.

Thus far reported, the FMCG is generally of a higher quality than the FECG as it has the advantage of exhibiting virtually no interference from the maternal ECG. The FMCG can be used to classify arrhythmias such as heart blocks and atrial flutter, and to diagnose a prolonged QT-syndrome. Using FMCG, there are studies of detecting fetuses with congenital heart diseases.

Finally, it does still remain a research tool and is currently little used in clinical routine because of its size, cost, complexity of the required instrumentation and cumbersome sensors [19 - 21, 57].

3.1.4 Ultrasound techniques

The ultrasonographic technique does not take a directly foetal biophysical signal, but it derives the information from the changes that an ultrasound beam undergoes when it arrives on the foetus. Recall that an ultrasound is a mechanical wave of high frequency in the range that goes from 20 kHz to 1 GHz. In medicine essentially using ultrasonic waves to longitudinal propagation (motion of particles in the same direction of propagation wave) and frequencies used vary between 0.5 MHz and 10 MHz. The applications in diagnostic foetal are different. The most common are the echo-Doppler and ultrasound. In the first case the Doppler effect is used, in accordance with the optical properties of the ultrasonic beam. The term Doppler effect indicates alterations in frequency that undergoes a sound wave at the time when it is reflected from a moving surface. The Doppler effect, which occurs for all

types of motion wave and of which you have knowledge also in daily life, can be easily explained by considering a sound wave that bounces off a reflective surface.

Ultrasonography is a technique considered harmless to the mother and the embryo, simple, quick and painless, it is implemented at the beginning of every pregnancy to determine the exact number of embryos present and to locate them in the uterus, in order to prevent complications arising from the location of the placenta, and then to follow the regular unfolding of the pregnancy.

Malformations, skeletal, digestive, urogenital, limbs and their ends, the nervous system can be diagnosed by ultrasonography; the foetal health can be rated through the study of amniotic fluid, foetal movement and velocimetry of the uterine and foetal vessels.

As anticipated, the wave that propagates in the medium is, however, subject to an attenuation which follows an exponential law, the amount of attenuation depends on the absorption coefficient of the crossed tissues and it is directly proportional to the frequency of the ultrasound. Another feature related to the frequency is the beam width, which increases with decreasing of the lateral resolution, then the frequency will be chosen also according to the degree of resolution needed. In the case of wide beam, the reflection point is not uniquely determined and might appear in the signal of the pseudo-FHR fluctuations, called JITTER, which do not allow reliable estimates of the track.

Finally we observe that ultrasonography could actually be considered an invasive technique, because the ultrasound may make changes to the biological tissue exposed. The current state of knowledge allows to state that does not appreciate biological effects for beams of intensity below 0.1 W/cm^2 , and then, to believe secure cardiocographs already designed in order to respect this limit [22].

3.1.5 Cardiotocography

In the third trimester of pregnancy, for the monitoring of the foetus, the use of cardiocograph is helpful to the clinical use. This technique, especially after the

26th week, is an important control for the monitoring of the foetus, for the assessment of its state of health and for the prognosis perinatal.

The cardiotocograph is a device that detects and records, simultaneously, the FHR (Foetal Heart Rate - FHR), through the use of a Doppler probe, and uterine activity (Uterine Contractions - UC), by means of a transducer pressure; both probes are applied on the abdomen breast [22].

Then the tool provides two signals: the foetal heart rate, which is inversely proportional to the period of time between two beats, and tocogramma expression of the uterine activity.

The track, consisting of the two signals, is called cardiotocogramma. It is also very important to monitor uterine activity, for two reasons:

- a) The contractions cause an increase of intrauterine pressure and, therefore, a pressure on the head of the foetus (up to four times higher than the normal value), therefore, representing one very strong stimulus, it is interesting to evaluate the reactions of the foetus.
- b) By monitoring uterine activity you can check the "progression level" of labor and thus avoid excessively premature parts that could endanger the child [22].

In a CTG track the following aspects are to be evaluated:

- 1) the baseline foetal heart rate (FHR the baseline). The frequency is normal when it is between 120 and 160 beats per minute;
- 2) the development of FHR variability, which should always be more than 5 beats/min and that, otherwise, may indicate foetal distress;
- 3) the presence of foetal movements reported by short and low elevations of the tocogramma and uterine contractions;
- 4) the FHR response to foetal movements, which in the health foetus results in acceleration;
- 5) the response of the FHR signal to the uterine contractions.

In the external cardiotocography the quality of the measurement is strongly affected by possible movements of the transducer. Significant disturbances registration you

have to be in the presence of foetal movements or maternal ones; the latter are obviously not avoidable, therefore it is necessary to hold as much as possible the transducer on the abdomen of the mother.

In view of the problems described above, the external tocografia offers the added advantage of allowing an evaluation of the shape of the contractions and their frequency, eliminating the risk of infection to the foetus and the mother. The cardiac activity is recorded using a Doppler probe: the heart rate is obtained by considering the reflection of an ultrasonic wave generated by the cardiac movements. Obviously, the signal taken has a shape quite different from the cardiac signal recorded with the electrocardiogram on the body surface, but large utility in the assessment of heart rate.

Cardiotocography gives results really trusted if evaluated together with other surveys that may indicate the use or fill any uncertainties of interpretation; furthermore, if it is true that a situation of foetal distress has always reflected in a path characterized as pathological, the opposite is not always true, that does not mean that a path from the characteristics seemingly pathological corresponds to a situation of actual foetal distress [22]. In fact, the CTG has been accused of providing too many false positives (unjustified alarms) but undoubtedly contributed greatly to improving perinatal and obstetric care in recent decades.

The CTG has proven particularly useful in assessing foetal conditions in pregnancies at risk for disease (gestosis, underdevelopment) and in response to tocolytic therapy during threat of premature birth. It is also used in the course of labor in order to have an objective assessment of uterine contractions and foetal well-being of the state. Cardiotocography, in fact, is sensitive to hypoxia, for example it is able to highlight the mechanisms of compensation that the foetus carries the moment is hypoxic, even if what actually harms the foetus is metabolic acidosis, expression of the fact that the foetus has exhausted its ability to respond to hypoxia.

Finally, it should also provide a description of the internal cardiotocography, much less used than the previous technique because of some inherent limitations. It can only be done after the rupture of amniochorial membranes. The pressure variations are detected and measured through a catheter containing physiological fluid,

catheter coupled with a pressure-voltage transducer. Since the catheter is introduced directly into the uterus, the risks of infection are high. In consequence of this, although the internal tocografia allows an accurate measurement of pressure and basal tone, must be done only in exceptional cases. A further limitation of this examination is the impossibility of a good recording of foetal movements.

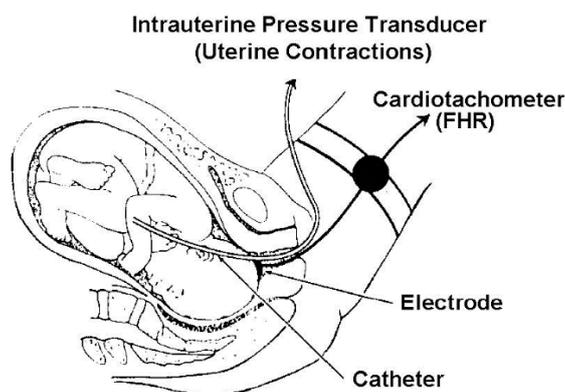


Figure 3.1 - Insertion of a catheter through intrauterine applicator. Source: The Brookside Associates [Online]. Available: http://www.brooksidepress.org/Products/Obstetric_and_Newborn_Care_II/images/MD0922_img_12.jpg (last access January 2015)

3.2 FHR parameters and characteristics

Some of the FHR signal features and their variation are very important in FHR analysis and recording, in order to monitor the foetal well-being.

According to the FIGO (International Federation of Gynaecology and Obstetrics) guidelines, the baseline of a FHR recording is defined as the mean level of the FHR, when this is stable, accelerations and decelerations being absent, determined over a period of 5 to 10 min [23, 25]. The FHR is under constant variation from the baseline. This variability reflects a healthy nervous system, chemoreceptors, baroreceptors and cardiac responsiveness.

Foetal hypoxia, congenital heart anomalies and foetal tachycardia cause decreased variability. However, reduced baseline variability is common also during foetal sleep cycles.

The minor fluctuations in baseline FHR occurring at 3 to 5 cycles per minute. It is measured by estimating the difference in beats per minute between the highest peak and lowest trough of fluctuation in a one-minute segment of the trace [24].

Beat-to-beat or short term variability is the oscillation of the FHR around the baseline in amplitude of 5 to 10 bpm [25, 26].

Long term variability is a somewhat slower oscillation in heart rate and has a frequency of 3 to 10 cycles per minute and amplitude of 10 to 25 bpm [25, 26]. Clinically, loss of beat-to-beat variability is more significant than loss of long-term variability [25]. Statistically, variability is commonly expressed by the width of the distribution of either RR intervals or heart rates [27].

Other authors proposed a modification to FIGO's ambiguous interdependence of definitions: baseline should be defined as the line that corresponds to the mean FHR level in the absence of foetal movements and uterine contractions rather than in the absence of accelerations and decelerations, as it is necessary to define a baseline rate before identification of an acceleration or deceleration is possible [23]. Also this definition is ambiguous and difficult to apply, mainly in automated analysis software. Some authors adopted another definition and consider the baseline as the running average of HR in the absence of accelerations and decelerations [24, 28], without specifying a time interval (for the average).

However, regardless of the way of calculating it, normal range of baseline is 120-160 bpm [25, 26]. Prematurity, maternal anxiety and maternal fever may increase the baseline rate, while foetal maturity decreases the baseline rate, since progressive vagal dominance occurs as the foetus approaches term [25] (figure 3.2). A baseline between 110 and 100 bpm is considered to be suspicious and one below 100 bpm as pathological [27].

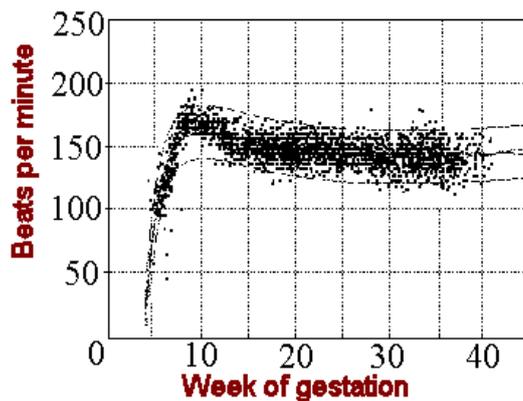


Figure 3.2 - Mean FHR versus week of gestation [2]

Foetal tachycardia is defined as a baseline HR greater than 160 bpm [25, 26] for more than 10 min [6]. Tachycardia is considered mild when the HR is 160 to 180 bpm and severe when greater than 180 bpm [25, 28]. Some of the possible causes of foetal tachycardia are foetal hypoxia, maternal fever, parasympatholytic drugs, sympathomimetic drugs and prematurity [25]. On the other hand, foetal bradycardia is defined as a baseline HR less than 120 bpm [25, 26] for more than 3 min [28]. Bradycardia is severe if FHR is less than 100 bpm [28]. Some of the possible causes of foetal severe bradycardia are prolonged cord compression, cord prolapse, tetanic uterine contractions, epidural and spinal anaesthesia, maternal hypotension and post-maturity [25, 27]. However, it is possible to say that almost any stressful situation in the foetus evokes the baroreceptor reflex, which elicits selective peripheral vasoconstriction and hypertension with a resultant bradycardia [25]. Both these patterns (tachycardia and bradycardia) often are not associated with severe foetal distress unless decreased variability or another abnormality is present [25, 26].

FHR patterns present also periodic changes as accelerations and decelerations. Both are defined as deviations from baseline with a certain amplitude and duration and can be present also in conditions of tachycardia or bradycardia.

Accelerations are transient increases of the FHR from the baseline of at least 10 bpm for at least 15 bpm [28]. They are usually associated with foetal movements, vaginal examinations, uterine contractions, umbilical vein compression, foetal scalp

stimulation, external acoustic stimulation or transient hypoxia, which activates sympathetic system by means of chemoreceptors. The presence of accelerations is considered a reassuring sign of foetal well-being [25] and a good indicator of good perinatal outcome [24]. Vice versa, the significance of no accelerations on an otherwise normal CTG is unclear [24]. However, a series of accelerations may create confusion. If one acceleration immediately follows another during a series of gross body movements, there is insufficient time for the FHR to return to the baseline level and the accelerations may fuse into tachycardia, as can regularly be observed during the 4F state (see following paragraphs). The number of accelerations in associations with foetal movements increases with advancing gestational age and has been related to advancing maturity of the foetal nervous system [27]. Recapitulating, it is possible to say that some studies evaluated changes in FHR pattern with advancing gestation and found a gradual fall in baseline with advancing gestational age up to 30 weeks corresponding to the progressive vagal dominance [25]. Similarly, an increase in variability was seen and an increase in the number of accelerations [24], which become larger in amplitude and duration [27]. Transient decrease of the FHR below the baseline level of at least 10 bpm for at least 15 bpm [28].

Decelerations can be classified into early, variable, late and prolonged decelerations and each type can be connected to a specific pathophysiological phenomenon [27].

Early decelerations: they are caused by foetal head compression during uterine contractions, resulting in vagal stimulation and slowing of the HR. They represent uniform, repetitive, periodic slowing of FHR corresponding to the contractions. This type of deceleration has a uniform shape, with a slow onset that coincides with the start of the contraction and a slow return to the baseline that coincides with the end of the contraction. Thus, it has a characteristic mirror image of the contraction. Although these decelerations are not associated with foetal distress and thus are reassuring, especially during the second stage of labour, they must be carefully differentiated from the other, non-reassuring decelerations [25].

Late decelerations: they are associated with uteroplacental insufficiency and are provoked by uterine contractions. Any decrease in uterine blood flow or placental dysfunction can cause late decelerations. A late deceleration is a symmetric fall in the foetal heart rate, beginning at or after the peak of the uterine contraction and returning to baseline only after the contraction has ended. The descent and return are gradual and smooth. Regardless of the depth of the deceleration, all late decelerations are considered potentially ominous [25]. They are particularly found in association with severe intrauterine growth retardation, a reduction in the amount of amniotic fluid and abnormal flow-velocity waveforms in foetal or umbilical vessels [27]. Moreover, in some studies, a marked increase in the number of cerebral palsy was found in association with multiple late decelerations. This risk was further increased if both late decelerations and reduced baseline variability were present [24].

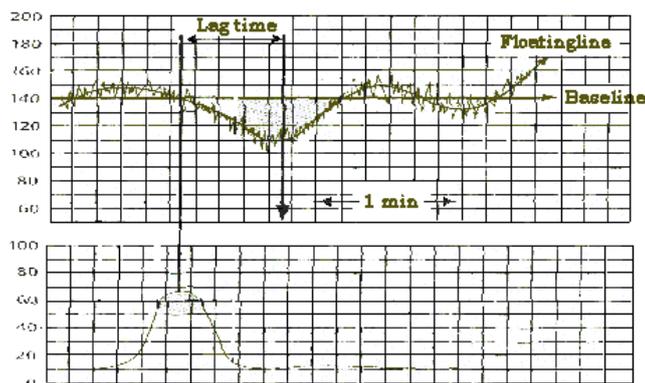


Figure 3.3 - On the top example of late deceleration, on the bottom relative UC [2]

Variable decelerations: they are shown by an acute fall in the FHR with a rapid downslope and a variable recovery phase. They are characteristically variable in duration and intensity. Time relationships with contraction cycle are variable and may occur in isolation. Variable decelerations are baroreceptor mediated and reflect changes in the blood pressure of the foetus due to compression of the umbilical cord [27]. Pressure on the cord initially occludes the umbilical vein, which results in an acceleration and indicates a healthy response. This is followed by occlusion of the umbilical artery, which results in the sharp downslope. Finally, the recovery phase is due to the relief of the compression and the sharp return to the baseline, which

may be followed by another healthy brief acceleration or shoulder. Variable decelerations may be classified according to their depth and duration as mild, moderate and severe (depth below 70 bpm and duration longer than 60 s) [25]. Uncomplicated variable decelerations were not consistently shown to be associated with poor neonatal outcome [24].

Prolonged decelerations: they are abrupt decreases in FHR values to levels below the baseline that lasts at least 60-90 seconds [24]. These decelerations become pathological if they cross two contractions.

3.3 Foetal Heart Rate Variability

According to some authors, the variability of the baseline foetal heart rate is defined as the fluctuation of the baseline of the FHR signal of two or more cycles per minute [4]. These fluctuations, being random, are quite irregular in amplitude and in frequency. Statistically variability is commonly expressed by the standard deviation, the square root of the variance, distribution or duration values of RR intervals or instantaneous values of heart rate. The presence of beat to beat variability reflects the normal functioning of the autonomic nervous system and in general is a good indicator of foetal well-being. The degree of fluctuation observed in a path, depending on the width from the peak to the dip, are classified as follows: "No Variability", if you cannot locate a margin width, "Reduced Variability", if the margin of amplitude is less than 5 bpm, "Normal Variability", if the amplitude margin is between 5 and 25 bpm, "increased Variability", if the amplitude margin is greater than 25 bpm [22].

The variability, therefore, is considered normal if the signal oscillates around the FHR baseline between 5 and 25 bpm [157]. The frequency of changes in the long term (of the oscillations in the low frequency) is usually between 2 and 6 cycles / minute, or between 0.03 and 0.1 Hz. An increase in the level of the baseline is accompanied by a concomitant decrease of variability.

The following figure shows examples of FHR tracks with different variability.

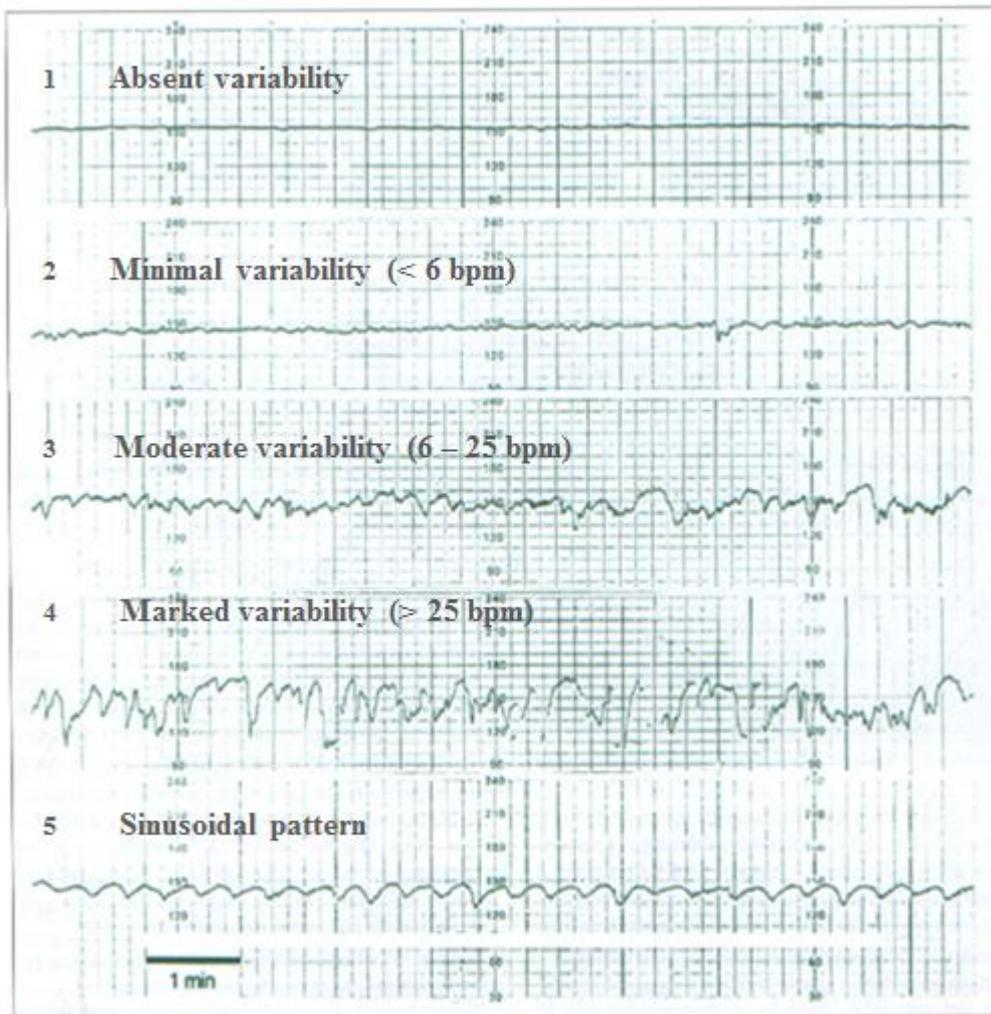


Figure 3.4 - Types of variability. Source: Medical Quick Review of Basics. Obstetrics [Online]. Available: <https://drkamaldeep.files.wordpress.com/2011/01/jpg.png> (last access January 2015)

The track in which it is present an increased variability is also called "saltatory" and is usually caused by acute hypoxia or by mechanical compression of the umbilical cord. This is considered not reassuring, but there must not lead to an immediate delivery. The loss or reduction of variability are not always synonyms of serious complications for the foetus; in fact, they could be due to the quiet foetal, although in this case the variability should increase spontaneously within 30 or 40 minutes. It is also important to evaluate how this reduction takes place that is if slowly or abruptly so as to be better able to track down the cause of trying to remove it

without resorting to an emergency childbirth. The loss of beat to beat variability is a most worrying factor compared to the loss of long-term variability.

Many factors can influence the FHRV. They are summarized in Table below.

Table 3.1 - Conditions that increase or reduce Variability

<i>Reduced Variability</i>	<i>Increased Variability</i>
<u>Dressings maternal</u> (<i>anesthetics, tranquilizers, narcotics, barbiturates</i>)	Dressings maternal (<i>ephedrine</i>)
<u>Marked prematurity</u>	<u>Increase in foetal activity</u>
<u>Foetal sleep</u>	<u>Umbilical cord compression</u>
<u>Chronic Hypoxia</u>	<u>Excessive uterine contractions</u>
<u>Brain damage</u>	<u>Post-date pregnancy</u>
<u>Foetal Cardiac arrhythmias</u> (<i>tachycardia</i>)	<u>Meconium aspiration</u>
<u>Late decelerations</u>	<u>Reduction of baseline level</u> (<i>bradycardia</i>)
<u>Passage of variable decelerations from moderate to severe</u>	<u>Variable decelerations</u>
<u>Depression of the sinus node</u>	

From this table it is evident that the FHR variability is suppressed by all the factors that affect foetal brain function or the relative myocardial contractility and it is always reduced as a result of prolonged hypoxia and acidosis [158]. In other words, the reduced variability reflects a "depression" of the foetal central nervous system and its disappearance may be the inability to compensate for a system subject to prolonged stress and increased. In clinical practice foetal asphyxia is often associated with the observation in the path of a decrease of FHR variability or lack thereof, and so it has acted as one of the indicators of the level of oxygenation and cerebral infarction, while its presence differs decelerations and events bradycardic of physiological character from those of pathological character [22]. A flat baseline (for example, with a variability superimposed 0-2 bpm) is one of the most ominous

signs for foetal health but remember that a foetus dying can keep your heart rate in a range of normal values.

CHAPTER 4

Heart Rate Variability Analysis

4.1 Introduction

In this chapter, some techniques to assess the heart rate variability, both in adult and foetal subjects, are described. They can be classified into more traditional methods, also called linear techniques, and less traditional methods, otherwise called non-linear techniques.

The former consist in time and frequency analyses of time series whereas the latter are methodologies that have been previously developed from nonlinear systems theory and then applied to the study of biological systems and, in particular, heart rate variability.

4.2 Time Domain Analysis

Variations in heart rate can be evaluated by a number of methods. Among them, the simplest to perform are perhaps the time domain measures. With these methods either the heart rate at any point in time or the intervals between successive normal QRS complexes are determined. In a continuous electrocardiographic (ECG) recording, each QRS complex is detected, and the so-called normal-to-normal (NN) intervals (intervals between adjacent QRS complexes resulting from sinus node depolarization), or the instantaneous heart rate is determined.

Simple time-domain variables that can be calculated include the mean NN interval, the mean heart rate, the difference between the longest and shortest NN interval, the difference between night and day heart rate, etc [1].

4.2.1 Statistical methods

From a series of instantaneous heart rates or cycle intervals, particularly those recorded over longer periods (traditionally 24 h), more complex statistical time-domain measures can be calculated. These can be divided into two classes:

- (a) those derived from direct measurements of the NN intervals or instantaneous heart rate;
- (b) those derived from the differences between NN intervals.

These variables may be derived from analysis of the total electrocardiographic recording or may be calculated using smaller segments of the recording period. The latter method allows comparison of HRV to be made during varying activities (e.g. rest, sleep, etc.). The simplest variable which can be calculated is the standard deviation of the NN intervals (SDNN), i.e. the square root of variance. Since variance is mathematically equal to total power of spectral analysis, SDNN reflects all the cyclic components responsible for variability in the period of recording. In many studies, SDNN is calculated over a 24-h period and thus encompasses both short-term high frequency variations, as well as the lowest frequency components seen in a 24-h period. As the period of monitoring decreases, SDNN estimates shorter and shorter cycle lengths. It should also be noted that the total variance of HRV increases with the length of analysed recording.

Thus, on arbitrarily selected ECGs, SDNN is not a well-defined statistical quantity because of its dependence on the length of recording period. Thus, in practice, it is inappropriate to compare SDNN measures obtained from recordings of different durations. However, durations of the recordings used to determine SDNN values (and similarly other HRV measures) should be standardized [1].

Other commonly used statistical variables calculated from segments of the total monitoring period include:

- SDANN, the standard deviation of the average NN intervals calculated over short periods, usually 5 min, which is an estimate of the changes in heart rate due to cycles longer than 5 min;

- SDNN index, the mean of the 5-min standard deviation of the NN intervals calculated over 24 h, which measures the variability due to cycles shorter than 5 min.

The most commonly used measures derived from interval differences include:

- RMSSD, the square root of the mean squared differences of successive NN intervals;
- NN50, the number of interval differences of successive NN intervals greater than 50 ms;
- pNN50 the proportion derived by dividing NN50 by the total number of NN intervals.

All these measurements of short-term variation estimate high frequency variations in heart rate and thus are highly correlated [1].

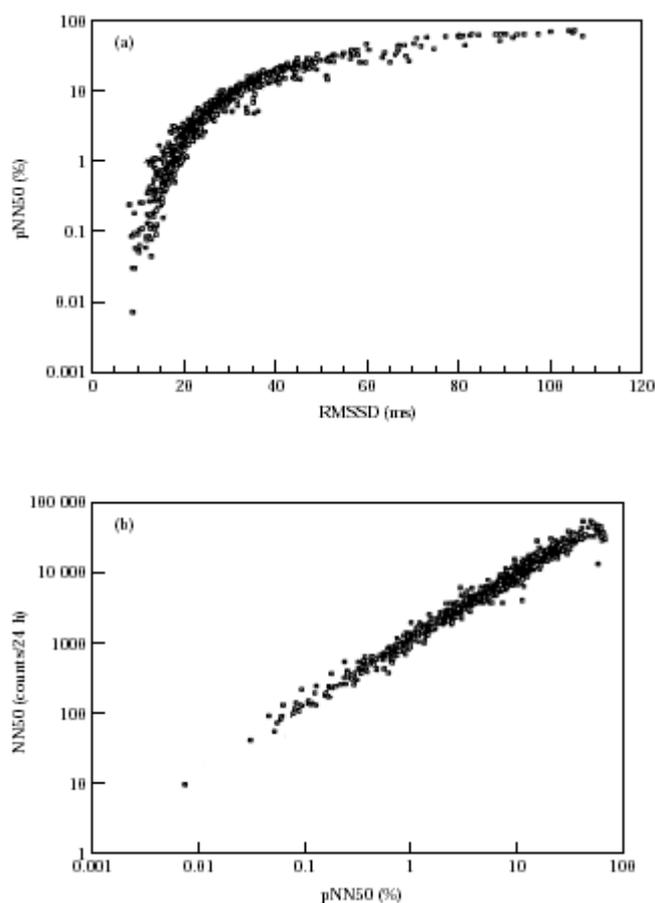


Figure 4.1 - Relationship between the RMSSD and pNN50 (a), and pNN50 and NN50 (b) measures of HRV assessed from 857 nominal 24-h Holter tapes recorded in survivors of acute myocardial infarction [1]

4.2.2 Geometrical methods

The series of NN intervals can also be converted into a geometric pattern, such as the sample density distribution of NN interval durations, sample density distribution of differences between adjacent NN intervals, Lorenz plot of NN or RR intervals, etc., and a simple formula is used which judges the variability based on the geometric and/or graphic properties of the resulting pattern. Three general approaches are used in geometric methods:

- (a) a basic measurement of the geometric pattern (e.g. the width of the distribution histogram at the specified level) is converted into the measure of HRV;
- (b) the geometric pattern is interpolated by a mathematically defined shape (e.g. approximation of the distribution histogram by a triangle, or approximation of the differential histogram by an exponential curve) and then the parameters of this mathematical shape are used;
- (c) the geometric shape is classified into several pattern-based categories which represent different classes of HRV (e.g. elliptic, linear and triangular shapes of Lorenz plots).

Most geometric methods require the RR (or NN) interval sequence to be measured or converted to a discrete scale which is not too fine or too coarse and which permits the construction of smoothed histograms. Most experience has been obtained with bins approximately 8 ms long (precisely 7.8125 ms=1/128 s) which corresponds to the precision of current commercial equipment [1].

The HRV triangular index measurement is the integral of the density distribution (i.e. the number of all NN intervals) divided by the maximum of the density distribution. Using a measurement of NN intervals on discrete scale, the measure is approximated by the value:

$$\text{(total number of NN intervals)} / \text{(number of NN intervals in the modal bin)}$$

which is dependent on the length of the bin, i.e. on the precision of the discrete scale of measurement. Thus, if the discrete approximation of the measure is used with NN

interval measurement on a scale different to the most frequent sampling of 128 Hz, the size of the bins should be quoted. The triangular interpolation of NN interval histogram (TINN) is the baseline width of the distribution measured as a base of a triangle, approximating the NN interval distribution (the minimum square difference is used to find such a triangle). Details of computing the HRV triangular index and TINN are shown in Fig. 4.2. Both these measures express overall HRV measured over 24 h and are more influenced by the lower than by the higher frequencies. Other geometric methods are still in the phase of exploration and explanation [1].

The major advantage of geometric methods lies in their relative insensitivity to the analytical quality the series of NN intervals. The major disadvantage the need for a reasonable number of NN intervals construct the geometric pattern. In practice, recordings of at least 20 min (but preferably 24 h) should be used to ensure the correct performance of the geometric methods, i.e. the current geometric methods are inappropriate to assess short-term changes in HRV [1].

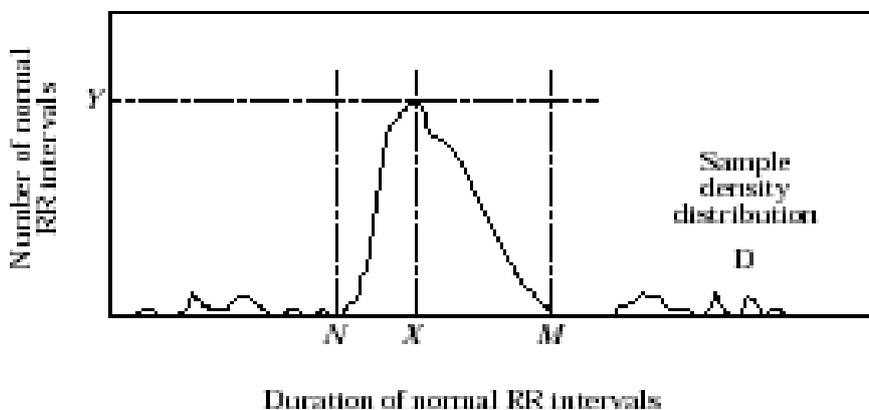


Figure 4.2 - To perform geometrical measures on the NN interval histogram, the sample density distribution D is constructed, which assigns the number of equally long NN intervals to each value of their lengths [1]

The most frequent NN interval length X is established, that is $Y=D(X)$ is the maximum of the sample density distribution D . The HRV triangular index is the value obtained by dividing the area integral of D by the maximum Y . When constructing the distribution D with a discrete scale on the horizontal axis, the value is obtained according to the formula:

$$\text{HRV index} = (\text{total number of all NN intervals}) / Y.$$

For the computation of the TINN measure, the values N and M are established on the time axis and a multilinear function q constructed such that $q(t)=0$ for $t < N$ and $t > M$ and $q(X)=Y$, and such that the integral:

$$\inf \int_0^X (D(t)-q(t))^2 dt$$

is the minimum among all selections of all values N and M . The TINN measure is expressed in ms and given by the formula:

$$\text{TINN} = M - N$$

4.2.3 Summary table of HRV time-domain measures

The variety of time-domain measures of HRV is summarized in Table 4.1. Since many of the measures correlate closely with others, the following four are recommended for time-domain HRV assessment: SDNN (estimate of overall HRV); HRV triangular index (estimate of overall HRV); SDANN (estimate of long-term components of HRV), and RMSSD (estimate of short-term components of HRV). Two estimates of the overall HRV are recommended because the HRV triangular index permits only casual pre-processing of the ECG signal. The RMSSD method is preferred to pNN50 and NN50 because it has better statistical properties [1].

The methods expressing overall HRV and its long- and short-term components cannot replace each other. The method selected should correspond to the aim of each study.

Distinction should be made between measures derived from direct measurements of NN intervals or instantaneous heart rate, and from the differences between NN intervals. It is inappropriate to compare time-domain measures, especially those expressing overall HRV, obtained from recordings of different durations [1].

Table 4.1 - Selected time-domain measures of HRV [1]

Variable	Units	Description
Statistical measures		
SDNN	ms	Standard deviation of all NN intervals.
SDANN	ms	Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording.
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.
SDNN index	ms	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording.
SDSD	ms	Standard deviation of differences between adjacent NN intervals.
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording. Three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer.
pNN50	%	NN50 count divided by the total number of all NN intervals.
Geometric measures		
HRV triangular index		Total number of all NN intervals divided by the height of the histogram of all NN intervals measured on a discrete scale with bins of 7.8125 ms (1/128 s). (Details in Fig. 2)
TINN	ms	Baseline width of the minimum square difference triangular interpolation of the highest peak of the histogram of all NN intervals (Details in Fig. 2.)
Differential index	ms	Difference between the widths of the histogram of differences between adjacent NN intervals measured at selected heights (e.g. at the levels of 1000 and 10 000 samples) ^[21] .
Logarithmic index		Coefficient ϕ of the negative exponential curve $k \cdot e^{-\phi t}$ which is the best approximation of the histogram of absolute differences between adjacent NN intervals ^[22] .

As previously mentioned, the measurement of HRV requires first detection of each heartbeat. In order to evaluate the effects of modulation of the sinus rhythm and then HR products from the regulation implemented by the control mechanisms independent, would make more sense to detect, from a physiological point of view, the activity of the sinoatrial node, corresponding to the waves P on ECG, matching to the "sinus nodal events".

In practice, however, this is technically difficult without an intracardiac electrogram; for this reason measurements of HRV are usually based on the sequence of the values of duration of RR intervals [1].

The analysis of HR variability in time domain provides for the calculation of appropriate indexes of Variability starting from the time sequence of RR intervals. They are divided into indexes of Short Term Variability (STV), or indexes Beat-to-Beat, indicating variations of the fast pace, and indexes of Long Term Variability (LTV), which indicate the presence of a slower pace.

Finally, it is important to underline that the types of analysis in the time domain used to estimate the signal HRV in adults are also extended to FHRV.

4.3 Frequency Domain Analysis

Various spectral methods for the analysis of the tachogram have been applied since the late 1960s. Power spectral density (PSD) analysis provides the basic information of how power (i.e. variance) distributes as a function of frequency. Independent of the method employed, only an estimate of the true PSD of the signals can be obtained by proper mathematical algorithms.

Methods for the calculation of PSD may be generally classified as non-parametric and parametric. In most instances, both methods provide comparable results.

The advantages of the non-parametric methods are:

- (a) the simplicity of the algorithm employed e.g. Fast Fourier Transform (FFT);
- (b) the high processing speed.

The advantages of the parametric methods are:

- (a) smoother spectral components which can be distinguished independently of preselected frequency bands;
- (b) easy post-processing of the spectrum with an automatic calculation of low and high frequency power components and easy identification of the central frequency of each component;
- (c) an accurate estimation of PSD even on a small number of samples on which the signal is supposed to maintain stationarity.

The basic disadvantage of parametric methods is the need to verify the suitability of the chosen model and its complexity (i.e. the order of the model).

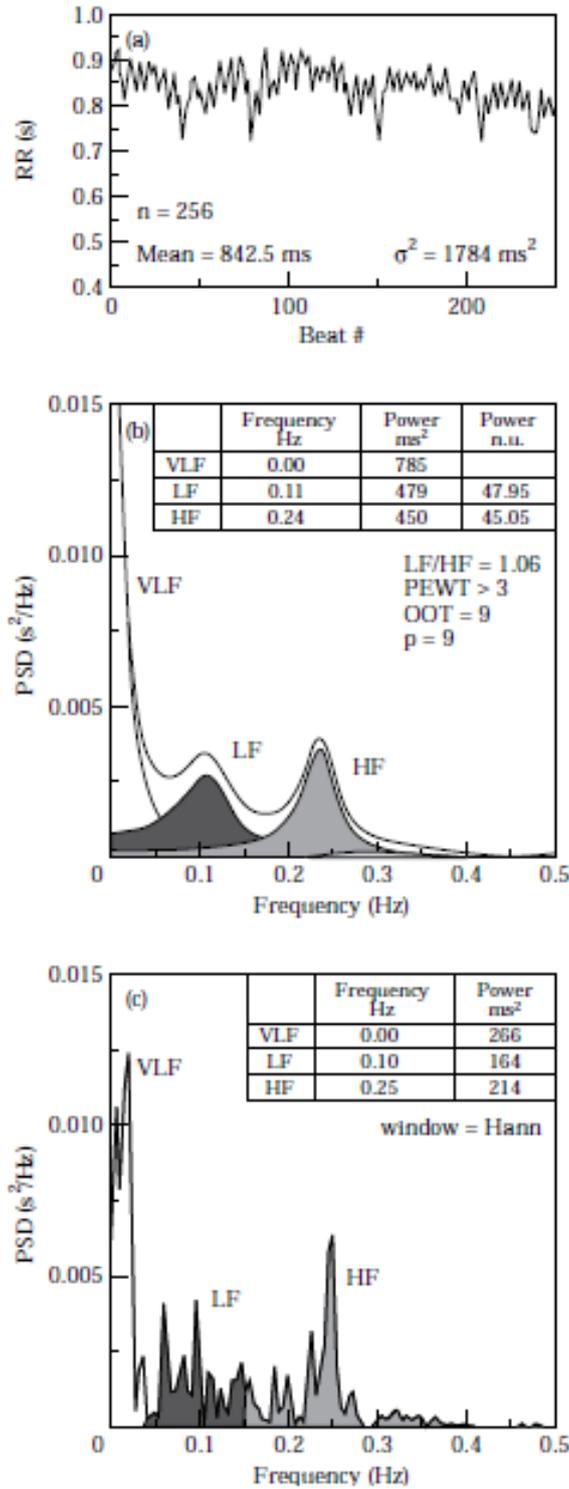


Figure 4.3 - Interval tachogram of 256 consecutive RR values in a normal subject at supine rest (a). The HRV spectra are shown, calculated by parametric autoregressive modelling (b) and by a FFT based non-parametric algorithm (c) [1].

4.3.1 Spectral components

a) Short-term recordings:

Three main spectral components are distinguished in a spectrum calculated from short term recordings of 2 to 5 min: very low frequency (VLF), low frequency (LF), and high frequency (HF) components.

The distribution of the power and the central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of the heart period. The physiological explanation of the VLF component is much less defined and the existence of a specific physiological process attributable to these heart period changes might even be questioned [1].

Measurement of VLF, LF and HF power components is usually made in absolute values of power (ms^2), but LF and HF may also be measured in normalized units (n.u.) which represent the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in n.u. emphasizes the controlled and balanced behaviour of the two branches of the autonomic nervous system. Moreover, normalization tends to minimize the effect on the values of LF and HF components of the changes in total power [1].

Nevertheless, n.u. should always be quoted with absolute values of LF and HF power in order to describe in total the distribution of power in spectral components [1].

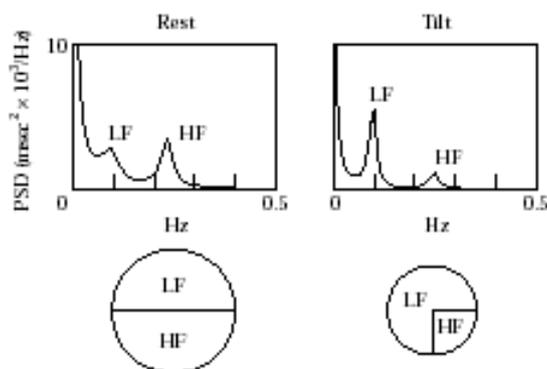


Figure 4.4 - Spectral analysis (autoregressive model, order 12) of RR interval variability in a healthy subject at rest and during 90° head-up tilt. The normalized representation makes clearer the relative change of the components of interest [1]

b) Long-term recordings

Spectral analysis may also be used to analyse the sequence of NN intervals in the entire 24-h period. The result then includes an ultra-low frequency component (ULF), in addition to VLF, LF and HF components. The slope of the 24-h spectrum can also be assessed on a log–log scale by linear fitting the spectral values.

The problem of ‘stationarity’ is frequently discussed with long-term recordings. If mechanisms responsible for heart period modulations of a certain frequency remain unchanged during the whole period of recording, the corresponding frequency component of HRV may be used as a measure of these modulations. If the modulations are not stable, interpretation of the results of frequency analysis is less well defined. In particular, physiological mechanisms of heart period modulations responsible for LF and HF power components cannot be considered stationary during the 24-h period. Thus, spectral analysis performed in the entire 24-h period as well as spectral results obtained from shorter segments (e.g. 5 min) averaged over the entire 24-h period (the LF and HF results of these two computations are not different) provide averages of the modulations attributable to the LF and HF components. Such averages obscure detailed information about autonomic modulation of RR intervals available in shorter recordings.

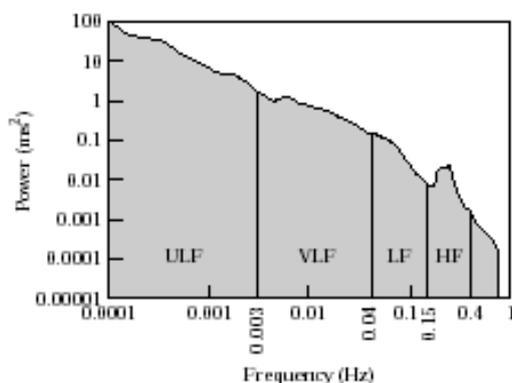


Figure 4.5 - Example of an estimate of power spectral density obtained from the entire 24-h interval of a long term Holter recording. Only the LF and HF component correspond to peaks of the spectrum while the VLF and ULF can be approximated by a line in this plot with logarithmic scales on both axes. The slope of such a line is a measure of HRV [1]

4.3.2 The spectrum of FHRV signal

The spectrum of FHRV of foetuses to term, is essentially two bands. A predominant band at low frequency (from 0 to about 0.2 Hz), typically divided into two lobes, which contains about 75% of the total power, and a high frequency band that contains little power and is not always present [2].

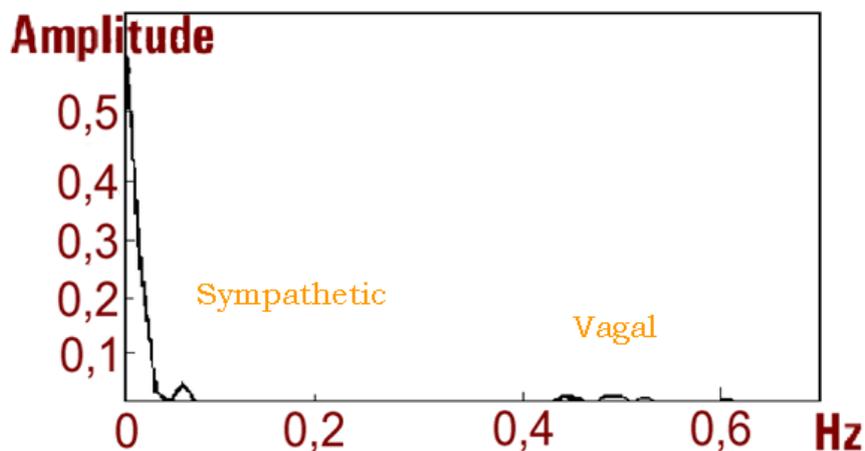
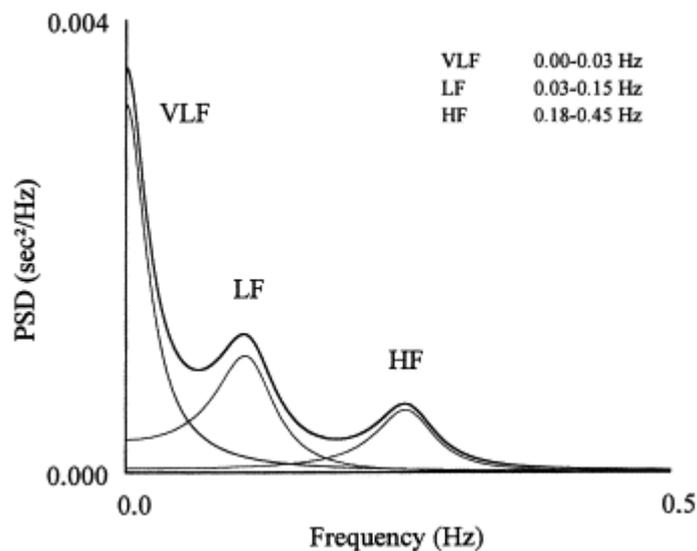


Figure 4.6 – Example of HRV spectrum (up) [53] and FHRV spectrum (bottom) [2]

To the foetus, as for the adult (figure 4.6) we can distinguish, in the power spectrum, three frequency components (corresponding to the mechanisms of regulation of the foetal heart rate):

- A dominant in the very low frequency (VLF), due to the dynamic control of the temperature, mediated by thermoreceptors chemical type (whose mechanism is not yet fully known), and other mechanisms of adjustment lenses;
- A low-frequency (LF), due to the control of blood pressure and, remember, regulated by the baroreceptors;
- The latest in high frequency (HF), linked respiratory activity (as explained later).

Let's see in detail some of the features:

- The power spectral density between 10^{-3} and 10^{-1} Hz (VLF) proceeds of the type $(1/f^\alpha)$ with $\alpha = 0.8 \div 0.9$ (figure 4.7);

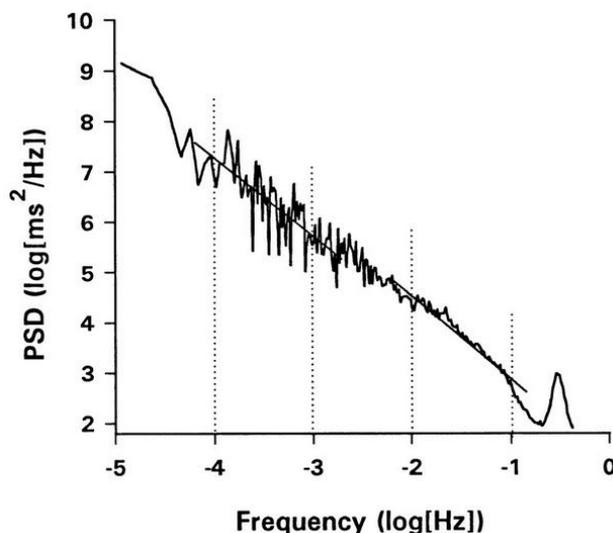


Figure 4.7 – HRV power spectrum between 10^{-5} and 10^{-1} Hz (VLF) [73]

- The LF component is related to the activity of the sympathetic and has a clear peak around 0.1 Hz;
- The last lobe (whose position ranges between 0.5 and 0.8 Hz) appears during the 36th week of gestation foetal breathing movements (FBM), has lower amplitude of 1÷2 orders of magnitude compared to previous and is absent, for example, growth-retarded foetuses.

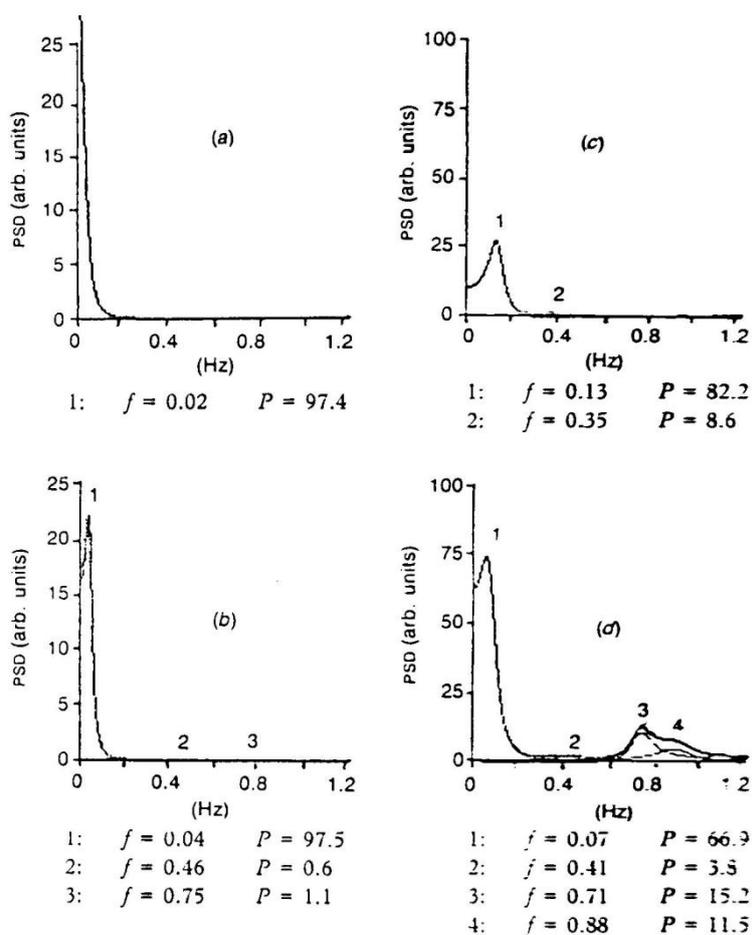


Figure 4.8 - Analysis of the spectral power density foetal. (a) and (b) refer to a foetus at 26 weeks gestation, (c) and (d) to one of 36 weeks. Furthermore, the higher figures are relative to analyzes carried out during periods of rest of the foetus and lower in the presence of respiratory movements. Under the figures, shows the frequencies investigated, in Hz, and the relative power density, P , in percent [125]

Regarding foetal breathing movements, it is important to reiterate that do not correspond to an active respiration, rather seems that they are only of preparation and training of the muscles thoracic (which otherwise would not grow) to the first breathing after birth. They remain irregular throughout the first half of pregnancy and become slower (the lobe corresponding moves, in fact, towards lower frequencies), more regular and more frequent during the last weeks before the birth. However, they disappear almost completely before and during delivery, probably because, during the passage through the pelvis, intrauterine pressure is such as to inhibit the expansion of the chest.

During the FBM, as already seen in the adult, the contraction of the thoracic musculature leads to an increase in intrathoracic pressure and a decrease in cardiac output and blood pressure. The pressure drop is indicated by the baroreceptors to the respiratory center which causes a suppression of the vagus. The result is an arrhythmia of the FHR with a frequency related to that of breaths [2].

However, there is some variability in the characteristics of the respiratory sinus arrhythmia (RSA) in the foetus; for example, in the spectrum, the corresponding peak in high frequency, decreases in the absence of respiratory activity, but not always disappears during periods of apnea; Moreover, since it is not yet possible to quantify precisely the breathing, it is not clear whether the differences in RSA reflect a real difference in the activity of the central nervous system or simply reflect individual differences in the sequence of breath.

For what concerns the two peaks in the low frequency, however, it can be said that increase their amplitude both with gestational age is, at the same age, in concomitance of foetal movements.

At 36 weeks gestation, the PSD shows frequency components similar to those of the adult [2].

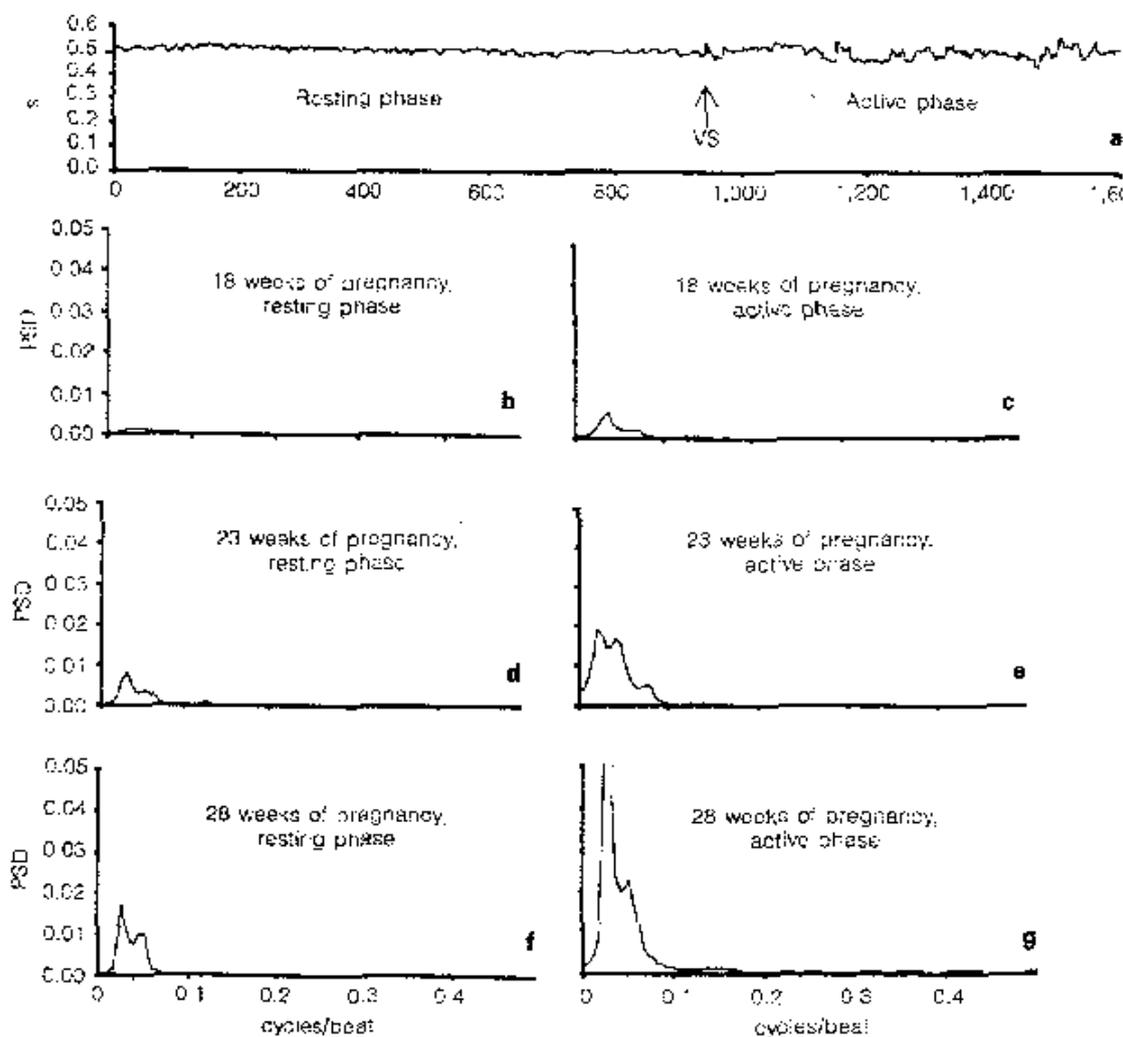


Figure 4.9 - Lobe abnormalities in low frequency with gestational age and, in the second column, the same age but in periods of activity. VS is the acoustic stimulation which causes the awakening of the foetus [2, 127]

It was also found that the characteristics of the spectrum of FHRV make possible to differentiate the various behavioral states of the foetus [2].

There are at least two criteria to study the spectrum of FHRV: the amplitude of the energy, that discriminates between paths corresponding to different behavioral states of the foetus, and the position of the peak of maximum energy in the high frequency band in which, to paths dishes, discriminates between a state of foetal stress and a wellness [2].

4.3.3 Short Time Fourier Transform

The short-time Fourier transform (STFT), or alternatively short-term Fourier transform, is a Fourier-related transform used to determine the sinusoidal frequency and phase content of local sections of a signal as it changes over time. Simply, in the continuous-time case, the function to be transformed is multiplied by a window function which is nonzero for only a short period of time. The Fourier transform (a one-dimensional function) of the resulting signal is taken as the window slides along the time axis, resulting in a two-dimensional representation of the signal. Mathematically, this is written as:

$$STFT\{x(t)\} = \int_{-\infty}^{+\infty} x(t) w(t - \tau) e^{-jt\omega} dt$$

where $w(t)$ is the window function, commonly a Hanning window or Gaussian window bell centered around zero, and $x(t)$ is the signal to be transformed [159]. $X(\tau, \omega)$ is essentially the Fourier Transform of $x(t)w(t-\tau)$, a complex function representing the phase and magnitude of the signal over time and frequency. Often phase unwrapping is employed along either or both the time axis, τ , and frequency axis, ω , to suppress any jump discontinuity of the phase result of the STFT. The time index τ is normally considered to be "slow" time and usually not expressed in as high resolution as time t [30].

4.3.4 Autoregressive Method

An autoregressive (AR) model is a parametric method used to model time series data. It provides better identification of discrete frequency oscillations for non-stationary records and it has been extensively applied to heart rate variability analysis as an alternative to the traditional Short Time Fourier Transform.

However, a crucial point is the appropriate model order selection. A too small model order could not represent all the properties of the signal, while a too high model order could also represent noise and so not to be a reliable representation of the true signal. An AR series can be expressed by:

$$x(n) = - \sum_{k=1}^p a_k * x(n) + e(n)$$

where $x(n)$ is the input signal, p is the model order, a_k are the AR model parameters and $e(n)$ is the error term, which can be considered like a white Gaussian noise with mean equal to zero and variance equal to σ [30].

To determine a_k , the Yule-Walker equations should be solved through the Levinson-Durbin recursive algorithm, which estimates the parameters of a model of order p from the parameters of a model of order $p-1$. Several criteria exist to estimate the model order. One of these is called Akaike's Information Criterion (AIC). The possible model order is selected when it minimizes the following function:

$$AIC(p) = N \cdot \ln \sigma_p^2 + 2 * p$$

where N is the number of data points and σ_p^2 is the mean squared error:

$$\sigma^2 = \frac{1}{N} \sum_{p=1}^{N-1} e^2(n)$$

Cesarelli et al. [103], in order to determine which the optimum model order is, analysed 50 real antepartum recordings by means of AR method. According to literature indications, the authors tested model orders in the range 4-14. Once obtained the minimum of the AIC function in that range, if it satisfies the whiteness test, then it is chosen like the optimum model order. Known the AR coefficients, the PSD ($S(f)$) of FHRV can be evaluated using the equation:

$$S(f) = \frac{\sigma^2 T}{|1 - \sum_{k=1}^p a_k \cdot e^{-j2\pi f k T}|^2}$$

where T represents the sampling period [30, 103].

This analysis indicated 6 and 8 as optimum model orders to estimate PSD of FHRV. Considering that the whiteness test was positive even using 8 for signals characterized by a model order equal to 6, the authors concluded that the 8 order could be used for all signals under study.

4.3.5 *Lomb Method*

The Lomb method is based on the minimization of the squared differences between the projection of the signal onto the basis function and the signal under study. Let $x(t)$ be the continuous signal under study and $b_i(t)$ an orthogonal basis set that defines the transform. The coefficients $c(i)$ that represent $x(t)$ in the transform domain are [29]:

$$c(i) = \int_{-\infty}^{+\infty} x(t)b_i(t) dt$$

and these coefficients $c(i)$ are those which minimize the squared error $e(c_i)$ defined as [29]:

$$e(c_i) = \int_{-\infty}^{+\infty} (x(t) - c(i)b_i(t))^2 dt$$

The Lomb method can be generalised to any transform estimation on unevenly sampled signals. When the signal is accessible only at unevenly spaced samples, the solution has generally been to reduce it to an evenly sampled signal through sampling interpolation. However, as stated above, this process introduces some distortion in the spectrum (or transform). To avoid this problem, Lomb proposed to estimate the Fourier spectra of an unevenly sampled signal by adjusting the model [29]:

$$x(t_n) + \varepsilon_n = a\cos(2\pi f_i t_n) + b\sin(2\pi f_i t_n)$$

in such a way that the mean squared error (ε_n) is minimized with the proper a and b parameters [29].

4.3.6 *Frequency analysis of FHRV: Matching Pursuits Method*

As for the HRV signal, also for the signal FHR the starting point for a procedure of PSA (Power Spectral Analysis) is the construction of the tachogram, i.e. the sequence of values of duration of the interbeat intervals RR as a function of the number of detected beats [71].

A new approach to the analysis of time-frequency variability of FHR is the "Matching Pursuits" (MP) analysis. It is proposed as a way to capture complex energy structures ranging from the type to burst activity continues in the time-frequency plane, rather than other methods such as analysis of the power spectrum or wavelet transform (WT) can't reveal. The MP method was chosen because the classical Fourier transform can't represent signals that don't have features stationary, and the wavelet transform can't represent signals whose FT has a frequency band narrow. Metin Akay et al. [72] have shown that MP is higher than both STFT and WT in identifying multiple periodicity in a highly non-stationary signal such as foetal breathing. In particular MP was used both in the time domain and frequency and FHR monitoring was performed at the 31-th and 38th week of gestation by means of a Doppler ultrasonic cardiocograph. It is seen that the signal FHR to 38th week was more variable during a peaceful sleep and then this signal contains values greater high frequency. But the most interesting discovery was to note the presence of activity in the sinusoidal distribution. This study has demonstrated so clearly the advantage of the method MP, it is very sensitive in detecting disturbances in dynamic models of normal FHR signal. The FHR is not random and may be governed by regulatory systems highly complex [72].

4.4 Main nonlinear techniques

The recent literature has shown that methods of estimating the complexity of the regulatory systems involved in the control of heart rate may contribute to the description of the variability of biological signals. Parameters such as entropy and regularity have already been used for the classification of the signal HR (Heart Rate) of the adult and foetus.

Recent results show that, in order to quantify the contributions "complex" of the FHR new methods and algorithms should be applied that perform nonlinear analysis, estimate the spectrum $1/f$, Approximate Entropy, the Detrended Fluctuation Analysis, the Multiscale Entropy [74].

Examples of non-linear techniques described in the literature are:

- Entropy;
- Poincaré Maps;
- Fractal analysis;
- Symbolic Dynamics.

4.4.1 Entropy measures

It is a measurement parameter that quantifies the regularity/irregularity or randomness of the fluctuations of heartbeats. One of its advantages is to be able to infer changes of complexity of the system by a relatively small time series of data. We must emphasize that there are various versions of calculation of entropy: Approximate Entropy (ApEn), Sample Entropy (SampEn), Multiscale Entropy (MSE). Approximate entropy (ApEn), introduced by Pincus in 1991, which defines it as a model that quantifies the independent statistical irregularities in the time series data [31]. The latter technique entropic easily applicable to the test series since the parameters can be estimated from a family of statistics. Are lower values of ApEn for time series more regular while higher values for those less predictable (more complex). The approximate entropy should not be understood as an approximate indication of the other, it is rather a statistical regularity [32]. ApEn can potentially distinguish a wide variety of systems: deterministic, stochastic, chaotic etc. [33, 75, 76, 93]. The advantages of this technique regards the applicability even in data sets of medium length and with the presence of noise [31]. ApEn is, however, a statistical part that lacks consistency in how the results are highly dependent on the length of the data: for this reason in the literature is introduced a new measure, entropy sampling SampEn [34]. The latter is able to decrease the dependence on the length of the series of data, and then the bias of the parameter, thanks to the development of a simplified algorithm [35 - 41]. It has been demonstrated that the statistics SampEn appears to be relatively constant on families of processes where statistics ApEn are not. SampEn is, in essence, a counting statistics of events, where the events are examples of vectors similar to each other, when these events are sparse, the statistics should be unstable and there is a lack of relative consistency. Statistics

SampEn therefore are valued as best as they retain a relative size them where statistics ApEn not show this aspect [34].

As far as the application of the entropy measures to the foetal heart rate variability, we can considered the study of P. Van Leeuwen et al [105], who combined the magnetocardiography with the FECG in the registration of foetal heart rate variability (in order to examine changes in foetal heart period variability measures in the course of pregnancy). They assessed the status of nonlinear dynamics in the analysis of heart rate variability, opening new diagnostic possibilities. They investigated 19 women with healthy pregnancies in the second trimester; eighty recordings were acquired between the 16th and 41st week of gestation. The mean time between recordings was 4 ± 2 weeks. In order to extract the foetal heart period, the foetal QRS complexes were detected by analysing with an appropriate template a row data channel characterized by a strong foetal signal and minimal interference of the maternal components. Complexes, not automatically recognized, were visually identified and added manually; similarly, falsely marked artifacts were removed. Heart period was determined with an accuracy of 1 ms, as the time between consecutive QRS complexes. In the following, heart period will be referred to as RR interval. In the time domain the mean RR interval (mRR), the standard deviation (SD) and the root mean square of successive differences (RMSSD) of each time series were calculated. In the reference [105] there is the construction of a series consisting of the moving averages of three successive squared differences; a threshold was visually determined on the basis of the variation in the undisturbed part of the series. All beats exceeding this threshold were marked as outliers and excluded from the calculation of time domain variables.

As far as the non-linear analysis is concerned, authors [105] measured four variables, to describe the heart rate dynamics of each foetus at the time of recording:

1. the approximate information dimension (ApD1)
2. the approximate entropy (ApEn)
3. the approximate maximal Lyapunov exponent (ApML)
4. the trajectory divergence rate "r".

They performed a statistical analysis of the values obtained by using means and standard deviation or medians. The dependence of mRR, SD, RMSSD, ApD1, ApEn, ApML and r on gestational age was examined using the Pearson correlation coefficient [105]. So, by applying this method, the authors measured clear changes in foetal heart rate behaviour in the course of the second and third trimester of pregnancy: in the time domain SD and RMSSD clearly increased with gestational age for the pooled data and this was confirmed by the correlation analysis; however, ApEn and r were the most consistent variables as confirmed by correlation analysis: they, in fact, did not display a sensitivity to outliers, so their application resulted more attractive; an increase of interindividual variance over gestational age could be found for ApEn and ApML, finally for r there was a decrease; furthermore, the increase of complexity as gestation progresses may be a further indication of the changes in parasympathetic influence as the autonomic nervous system develops.

4.4.2 Poincarè maps

The *Poincarè maps analysis* is a graphical technique. The maps' shapes are categorized into functional classes and provide detailed information about the heart's behaviour. These maps are designed as quantitative and qualitative two-dimensional representations, in particular as clouds of points obtained by plotting each value of the RR series as a function of the following value. This technique does not require data in time continuous series nor in a normal distribution of the samples of the signal as in the time or frequency analysis. Typically, such maps are drawn considering three indexes: the standard deviation of the variability of RR intervals in the short term (minor axis of the cloud, SD1), the standard deviation of the variability of RR intervals in long-term (major axis of the cloud, SD2) and the axis ratio (ratio between the two previous indices, SD1/SD2). We can recognize the shape of these plots, reducing them to specific patterns. In fact, a normal subject determines an image of type comet oriented with the points of highest frequency concentrated around the bisector of the axes and those with lower frequency more dispersed. The limitations of SD1 and SD2 estimation are depending on the measures performed in the time domain, so their accuracy is related to the used

methodology. In the literature [42] was done a study, using these maps, on 17 healthy subjects aged between 24 and 26 years and 12 between 62 and 66 years, through the use of sonography during spontaneous breathing in a average period of 4 min. All subjects had not had any cardiovascular problem or systemic historical, no medication and had a normal sinus rhythm and a normal ECG-color Doppler. The ventricular volume was measured in real time and along the ECG has been converted with an A/D converter. The signals were automatically processed to extract the beat to beat variability and peak ECG R were identified through a limit value of an algorithm. Through this analysis, the method of the Poincarè maps allows to identify the differences between the young subjects and those in advanced age evaluating the parameters of the variation in ventricular volume (VV) [43, 44]. Another study with the Poincaré maps was performed on 24 healthy subjects and 24 patients infarcted analysing Holter recording signals of 24 hours. In the control group the plot had a shape of comet while this is not true for infarcted patients. Were also identified three forms characteristics for infarcted patients each badge for a single pattern [45, 46].

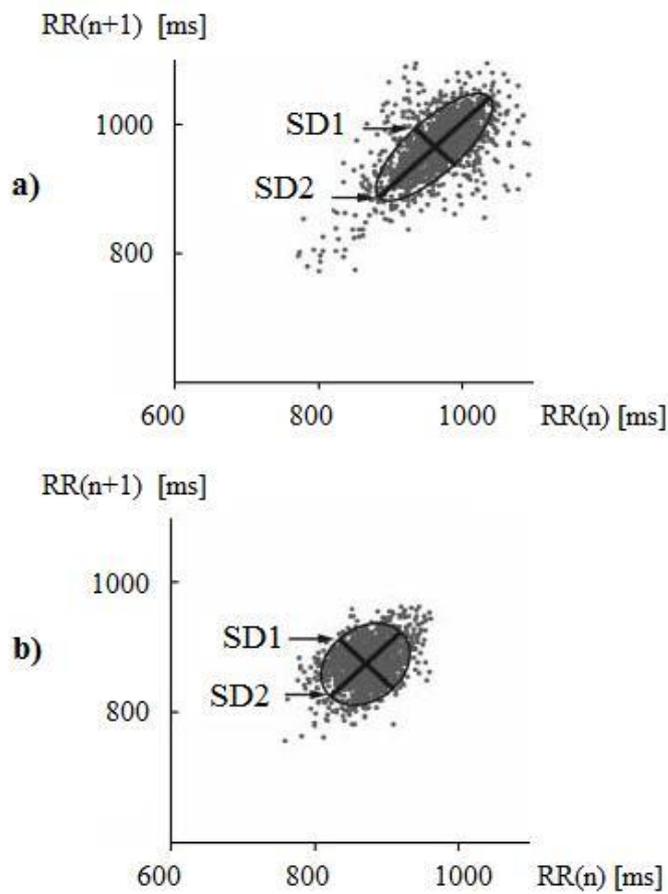


Figure 4.10 - Poincaré plots: a) healthy subjects; b) subjects with myocardial infarction [63]

The technique of the Poincaré maps has also been used to determine to what degree the chaotic structure in the FHR variability is maintained for different periods of observation. This was assessed by investigating the dispersion characteristics in Poincaré maps. It is observed that the FHR pattern is chaotic and that this chaotic structure is maintained in short intervals of 3-5 minutes. The variability in the dispersion is increased for shorter periods of observations. This method was used as a quantitative measure for the evaluation of dynamic changes in the activity electrocardiographic and dispersion of intervals between beats to the foetus. In detail a continuous segment of 30 min transabdominal foetal electrocardiography has been sampled at a rate of 1000 Hz during sleep for a foetus of 36-th week. RR intervals were extracted through an interactive graphical interface combined with a particular algorithm. Finally were then observed maps Poincaré in 30 minutes [77].

4.4.3 Fractal analysis

A method for evaluating foetal heart rate variability is the fractal analysis, as demonstrated by Di Rienzo et al [94]. The non-linear measures of complexity, there are measures of fractal properties that reflect the invariance to different time scales: many physiological signals do not have a characteristic time scale and it can happen that the signal $y(t)$ and its scaled version $y(at)$ have the same statistics (mean, variance, etc.) [47].

The term fractal is relative to a geometrical concept: in classical geometry forms are regular and have size, in contrast, in the fractal geometry are very irregular and have fractional dimensions. The analysis of the fractal dimension allows to calculate the degree of irregularity of the object fractal examined managing to subdivide the object itself in many fundamental units (fractal) that have the same shape in different proportions of the object to which they belong [48]. The use of fractal properties was used to classify sequences FHR physiologically relevant. The use is motivated by the difficulties exhibited by the other traditional methods in discriminating some classes of sequences FHR and because these signals exhibit characteristics of different scales of observation, just like the signals fractals [49].

4.5 Other nonlinear techniques

4.5.1 Detrended Fluctuation Analysis (DFA)

This method was evaluated as one of the most suitable methods for the evaluation of biomedical signals and for evaluating a positive correlation (or negative) of the signals themselves [78]. It is the time series of integrated blocks of size n , with n gradually increased. The fluctuation of each block is calculated as the root mean square of the difference between the integrated and his best linear fit in the block. These fluctuations follow a power law and the exponent is said parameter self similarity [79]. It has shown so far that the measures involving fractal properties can well to predict mortality in patients with recent myocardial infarction, heart transplant and chronic heart deficit. This method is capable of removing interference (trends) external and considers only the intrinsic characteristics that

are present in all of the signal. The DFA is typically applied by segmenting the signal into short windows. The objective of the DFA is to calculate two coefficients α_1 and α_2 which reflect the correlation in the short and long term, respectively, of a signal "detrended" which essentially consists in the elimination of the continuous component and linear from the signal itself. An important step of the DFA involves segmenting the signal into windows shorter. This, however, has two undesirable effects: i) if the signal length is not a multiple of the length of the window, and no sample must be discarded, at least one window will have less samples than the other; ii) if a window is considerably shorter than the others, then the energy of the detrended signal within that window will be much lower than the rest of the signal. Secondly, the discontinuities are observed on the signal "detrended" to the edges of each window. These two effects are observed especially when the coefficient α_2 is calculated, which requires the use of larger windows [79-83].

4.5.2 *Hidden Markov Models*

A further method of nonlinear analysis is that the theory of discrete hidden Markov models HMMs, introduced by Baum in 1960. It concerns a probabilistic model in which sequences are generated by two concurrent stochastic processes. The first is constituted by a Markov model in all generality we can consider the first order, and then graph as a set of states connected by arrows symbolizing the transition probability while the second process is represented by the issue by each state of a character of an alphabet according to a probability distribution that depends only on the state of those of the previous instants.

4.5.3 *The Lyapunov Exponent*

In the theory of nonlinear dynamic state space is reconstructed from the series of RR intervals (embedding) and the dynamic behavior of the dynamics reconstructed can be quantified by the maximum Lyapunov exponent and the correlation dimension. However, revealed the fundamental difficulties in the implementation of these methods because of: noisy nature of physiological signals, finite length of the

data series and their non stationarity. The spectrum of Lyapunov exponents is one of the most useful tools for the study of the dynamics of non-equilibrium systems. In fact, Lyapunov exponents can be considered as an estimate of the average speed of convergence or divergence exponential trajectory of a dynamical system in the vicinity of a whole attractor. They provide, therefore, a qualitative and quantitative characterization of the dynamic behavior of a system (for further information see Appendix A).

4.5.4 Hypothesis tests based on surrogate data

It is used to test the hypothesis that the dynamics that you are using is due to a non-linear mechanism, rather than a linear stochastic process [55]. Often nonlinear analysis of a series can lead to wrong conclusions about the process that generated it because there are simple stochastic processes that generate signals with apparent nonlinear properties. The analysis with the surrogate data allows us to test if the result of a nonlinear analysis depends on properties deterministic nonlinear or linear stochastic [55] (for further information see Appendix A).

In the following paragraphs we would review in detail another non-linear technique, which represents the subject of this thesis, the Symbolic Dynamics Analysis.

4.6 Symbolic Dynamic Analysis: theory and literature overview

The starting point of this method is to symbolize the data of the original time series. There are many approaches that identify different classification rules. The maximum number of symbols expected to associate with the time series RR (or differences) is 6.

The simplest association rule is instead one that provides only two symbols. In particular, given a time series of RR intervals indicated by t_i with $i = 1, 2, 3, \dots, n$ we can think to turn it into a sequence of symbols is indicated with $i = 1, 2, 3, \dots, n$ according to the law:

$$s_i = \begin{cases} 1 & \text{if } t_i \geq \text{value} \\ 0 & \text{in other cases} \end{cases} \quad (1)$$

Or can we expect to perform the transformation on the strength differences between consecutive intervals and thus:

$$s_i = \begin{cases} 1 & \text{if } t_{i+1} - t_i \geq \text{value} \\ 0 & \text{in other cases} \end{cases} \quad (2)$$

You can also think of transforming the time series according to a criterion with two symbols, but using the absolute difference between two consecutive RR:

$$s_i = \begin{cases} 1 & \text{if } |t_{i+1} - t_i| \geq \text{value} \\ 0 & \text{in other cases} \end{cases} \quad (3)$$

so as to highlight those cases in which it manifests a small difference between two consecutive intervals, beat to beat (symbol '0') and those in which this difference exceeds a given limit [58]. It should be noted that, from the theoretical point of view, the symbols that are associated to the original series should not have a fixed nature, in the sense that both alphanumeric and may be of any other type. For example, some authors in the transformation of the time series in two symbols refer to the symbols '+' and '-' [59]. Typically, when using these symbols, if two consecutive RR intervals are of increasing duration then joins them the symbol '+', otherwise, if they are of decreasing duration, joins them the negative symbol '-'. In this case, the positive sign denotes decelerations in heart rate, while a negative sign denotes accelerations [58, 59]. The transformation can be done in a more complex way by referring to a greater number of symbols, belonging to a determined alphabet. The choice of the number of symbols depends on a good compromise between some including the need for reliability in estimating the frequency and complexity of the algorithm which is to be determined.

According to a criterion with four symbols, using the average interval RR indicated with ' μ ' and a special parameter called 'a' parameter called weight-average interval

of the heartbeat that quantifies the standard deviation of the signal RR, the transformation is [58]:

$$s_i = \begin{cases} 0 & \text{if } t_i > (1 + a)\mu \\ 1 & \text{if } \mu < t_i \leq (1 + a)\mu \\ 2 & \text{if } (1 - a)\mu < t_i \leq \mu \\ 3 & \text{if } t_i \leq (1 - a)\mu \end{cases} \quad (4)$$

There is also another transformation, always based on a criterion with four symbols but which considers the differences between the values of two adjacent RR intervals [58]. The rule is the following:

$$s_i = \begin{cases} 0 & \text{if } \Delta t_i > 1.5\sigma_\Delta \\ 1 & \text{if } 0 < \Delta t_i \leq \sigma_\Delta \\ 2 & \text{if } -1.5\sigma_\Delta < \Delta t_i \leq 0 \\ 3 & \text{if } \Delta t_i \leq -1.5\sigma_\Delta \end{cases} \quad (5)$$

where $\Delta t_i = t_{i+1} - t_i$ and σ_Δ is the variance of Δt_i [58].

In analyzes based on a criterion of symbolization with six symbols, quoted at the beginning of the paragraph, the whole range of the sequences is uniformly segmented on six levels, from 0 to 5; par do what you set five thresholds, getting the six intervals in which they can fall back the values of individual RR intervals.

The next step is usually the organization of the symbols into strings, called 'words'. In this case, one must not do is to group together the symbols according to the length of each word. Fixed this length, the grouping process most commonly used is to proceed along the time series of symbols a step at a time, and each step reveals a new sequence typically through inclusion of all symbols except the first of the previous string [58]. This happens when, choosing a window of fixed length "L", it flows along the time series sample by sample, with an overlap of two consecutive windows of L-1 symbols. For example, if a fixed length of three symbols for each

string, the first word will be constituted by the first three symbols in temporal order, the second word will be constituted by the second, the third and fourth symbol, and so on.

If we denote by ξ the number of symbols chosen and L the number of symbols that form each word (or even the length of each word), the number of possible sequences will be equal to ξ^L . This number will grow exponentially with the length of the word. This shows that, as mentioned in the previous section, the choice of these parameters is based on a real compromise in obtaining the explanation of some of the dynamics and sufficient statistics to estimate the distribution of the frequency of occurrence without excessively increasing the computational complexity. For this reason many authors who have dealt with the criterion of symbolization two symbols ($\xi = 2$), with recordings of short duration, have limited the words resulting to a length equal to two or three [60 - 62].

For classifications two symbols (the positive and negative), based on the sign of the differences between the values of two adjacent RR intervals, with words of length L of two, you can highlight the increasing sequences (+, +), decreasing (-, -) or alternating (+, - or -, +). With an alphabet of two symbols and words of length two are obtained $2^2 = 4$ possible words. Authors such as A. and G. Porta D'Addio have also excluded all pairs with differences of less than 5 ms that can be attributed to noise, in order to study only the real physiological dynamics.

Another type of analysis according to the criterion of two symbols was conducted by Cammarota et al. [59] who have instead made words of length three, getting $2^3 = 8$ possible words. These can be incorporated into three groups, the first of which is formed by words with three accelerations or three decelerations (- - - + + or + respectively), the second is made up of strings in which the sequence of four consecutive beats presents accelerations or decelerations for at least two consecutive beats (words of the type - + + - - + + - - + + -) and finally the third is formed by strings where the accelerations and decelerations are alternating (words belong to this group the type + - + and - + -) [59]. With reference instead to the process of symbolization four symbols ($\xi = 4$), not to grow too much the number of possible combinations, most of the authors is to create a symbol strings of length

equal to three ($L = 3$). In this way the number of possible words will be equal to $4^3 = 64$. To realize how critical the choice of length of the word, just think that selecting a length for the words higher, for example equal to four, the number of words possible passes, in this example, from 64 to 256.

Realizing strings of symbols with these specifications ($\xi = 4$ and $L = 3$) you can highlight words consisting of alternating, constant, increasing or decreasing segments of symbols that reflect respectively vagal activity. Therefore, with the length of the strings equal to three are unable to satisfy on one hand the possibility of having a limited number of possible words and on the other the possibility of incorporating sufficient information on the dynamics involved. The situation is more complex for the encoding process to six symbols ($\xi = 6$). In this case in fact for strings of length equal to three ($L = 3$) we have to deal with a number of possible words significantly higher than in previous situations, equal to 216. In this case follow the changes in the frequency of these words could be complicated. Therefore, in these cases, is applied to a reduction of redundancy, by grouping all possible words in four 'families' without any loss of information and according to the number and types of variations from one symbol to the next. The 'family' of words are:

- strings of words with no variation (denoted with 0V): all the symbols are the same;
- strings of words with one variation (denoted with 1V): two consecutive symbols are equal and the available time is different;
- strings of words with two similar variations (denoted with 2LV): the three symbols form a ramp up or down;
- strings of words with two different variations (denoted with 2UV): the three symbols form a peak or a "valley".

Typically the frequency of occurrence of these strings is shown as % 0V, 1V%, 2LV%, 2UV% [63].

The main purpose of the above steps is to get the tools to carry out an analysis with which to extrapolate information on the dynamics involved in the regulation of the cardiovascular system. The end result of these procedures is a series of 'words' of

symbols that at this point only need to be studied. From the series of encoding obtained, in fact, we can make the right analysis using different parameters. The first step is usually in the graphic display of the dynamic behavior involved, as described below.

Typically, when the symbolization is based on the criterion with two symbols and when the word length is equal to three, and all the possible words obtainable are grouped according to three groups (one with 3 identical symbols, the one with two consecutive symbols equal and one with 3 consecutive symbols different), the graphical display of the results is to simply bring in a table the values of the frequency of occurrence of individual groups, such as comparing the results between patients and healthy subjects in various pathological conditions [59]. When, instead, the length of each word built is equal to two, all sequences decreasing, increasing or alternating which occur can be easily displayed in a diagram in four quadrants. For the processes of quantization levels higher (with the number of possible symbols greater than two) the graphical representation most appropriate and used is definitely the histogram, which shows the probability distribution of each type of word. By displaying these frequencies with which they appear the individual symbols or rather the words formed by the latter, you can distinguish uniform distributions from the most complex. In fact, some studies indicate that about a greater complexity, and therefore irregularities can be manifested in systems health. The common hypothesis is that the body is a highly complex adaptive system, and that the complexity of his behavior takes into account the broader scope of adaptive responses due to different input levels within a physiological range [63].

Subsequently, starting from histograms it is possible to calculate the parameters that can quantize the complexity of the systems of interest. A parameter is the classical Shannon entropy. The probability $p(s^L)$ of words of length L , you can define the Shannon entropy of L -th order:

$$H_L = - \sum_{s^L, p(s^L) > 0} p(s^L) \log_2 p(s^L)$$

This parameter can be seen in turn as a particularization of a more general definition, the Renyi entropy, defined as:

$$H_L^{(q)} = (1 - q)^{-1} \log_2 \left(\sum_{s^L} p(s^L)^q \right)$$

where 'q' is a real number other than -1. In particular, this parameter includes different medium probability and Shannon entropy converges to the value of 'q' tending to unity [64]. Both, as previously specified, are measures of complexity and function of the latter assume certain values:

- H = 0 for constant sequences;
- H = log₂ (m) in the case of the first period periodicity with 'm', with m < L;
- H_L = log₂ (a) in which 'a' is the number of symbols. This is the maximum value of complexity associated to uniform distributions;
- If q > 1 the words of length L with high probability of occurrence largely determine the value of Entropy;
- otherwise if 0 < q < 1 are the words with low probability to determine in large Apart from its value.

In this way, the first case will allow for highlight the complexity due to words with high probability, the second is those with low probability. In reality, some changes were made in order to improve the reliability of the Shannon entropy for example has been made the correction to be systematic and random errors in entropy through the 'Correct Shannon Entropy' (CSE). Was also introduced also the evaluation of the so-called 'Corrected Normalized Entropy Shannon' (NCSE), which allows for example to compare the two values of the CSE for different lengths of words at the same threshold level, which was not possible only with the 'correct Shannon Entropy.

Another measure of complexity is the count for 'bad words'. This parameter indicates the number of words, among those possible, which never occur. A high number of 'bad words' is a rather regular behavior in time series. Contrary if the

time series are highly complex in the sense of Shannon, then you will present only a few 'bad words' [65].

Ultimately, with the Symbolic Dynamics has been proposed a non-linear method of HRV analysis to quantify the prevalence of cardiac sympathetic or parasympathetic modulation in those conditions in which the use of the classical methods for the study of HRV is restricted. In this type of study has been shown that the number of 'words' stable or constant tends to increase when the sympathetic nervous system is more active and tend to modulate the duration of the heart period, while decreased unstable periods characterized by rapid change of heart period found in 'words' relevant [66]. Through the symbolic analysis can identify indexes that can characterize the differences between subjects in rhythmic health and those in pathological conditions, and characteristics circadian rhythm for the same category of patients or between different categories.

Regarding to the application of the symbolic dynamics in order to study the HRV in adult subjects, we can consider also the study performed by D'Addio et Al [67]. They investigated the changes induced - in post-myocardial infarction patients and normal subjects - by tilting on quantitative indexes derived from Poincarè plots and symbolic dynamics patterns and compared these changes to those observed using classical spectral parameters. They found that Poincarè indexes provide more sensitive information than spectral indexes in assessment of changes of cardiac autonomic outflow; they also found that the symbolic dynamics indexes were not able to detect these changes, but they clearly indicated a structurally different pattern of HRV between normal and pathological subjects.

In a further study, Porta, D'Addio et Al [68] performed the symbolic analysis to beat-to-beat heart rate variability data derived from 24 h Holter recordings both in healthy and heart failure populations. They found that this method allowed to distinguish two physiological conditions characterized by a different status of the autonomic nervous system (i.e. day-time and night-time) in healthy subjects and to discriminate between healthy and pathological populations. Indexes derived from symbolic analysis deserve to be added to traditional time and frequency domain parameters in standard analysis of heart rate variability.

Finally, as shown by the work of Voss et Al. who already in 1996 [69] purposed the use of the symbolic dynamics in order to find a method for the prognosis of the risk of cardiac arrest. Hao et Al, in a paper published in 2009, [70] in which techniques based on mono- and multifractal analyses and symbolic dynamics have been successfully applied to clinical studies, we can conclude that, today, the question is no longer about whether or not methods from Non Linear Dynamic should be applied, but it is relevant to assess which of the methods should be selected and under which basic and standardized conditions should be applied.

To summarize the above extensively discussed methodology, a synthetic scheme of the main steps of the HRV Symbolic Dynamics Analysis is shown below:

Table 4.2 - Main steps of Symbolic Dynamics Analysis

Main steps of SDA	Description
<i>Series computation</i>	Measuring, computing or deriving the RR (inter-beat intervals) or the ΔRR series (difference between consecutive inter-beat intervals).
<i>Alphabet choice</i>	Selecting meaningful and appropriate symbols according to the aim of the analysis.
<i>Series encoding</i>	Symbolizing the data of the original time series by associating each value of the time series to a specific symbol from the defined alphabet according to determined classification rules.
<i>Words generation</i>	Organizing the symbols into strings, called 'words' by grouping together the symbols according to a fixed length for each word.
<i>Words classification and/or complexity measurement</i>	Grouping all possible words in 'families' according to defined criteria; calculating parameters that can quantize the complexity of the systems, such as the Shannon entropy.

4.7 HRV non-linear analysis: a brief literature report

In the study [151] of Hu 2010 introduces a new complexity measure multiscale: Lyapunov exponents scale-dependent (SDLE), to characterize the relative importance of non-linear dynamics, chaotic, and stochastic dynamics of HRV, subjects in health, patients with congestive heart failure (CHF), and patients with atrial fibrillation (AF). It is shown that while the HRV data of all these three types are mostly stochastic, the stochasticity is different among the three groups. Furthermore, in order to distinguish healthy persons from patients with CHF, it is seen that the characteristics derived from SDLE are more effective than other measures of complexity as the Hurst parameter, sample entropy, and entropy multiscale.

SDLE is defined in a phase space by consideration of a set of trajectories. In the case of a scalar time series $x(1), x(2), \dots, x(n)$, a space of suitable phase can be obtained using a time delay embedding to construct vectors of the form:

$$V_i = [x(i), x(i + L), \dots, x(i + (m - 1)L)]$$

where m and L are called embedding dimension and delay time, respectively. For chaotic systems, L must be chosen according to a given optimization criterion. None of the three types of HRV is low dimensionality, the size increases from normal to CHF and AF. It is seen that both the entropy of sampling is the Hurst parameter are very ineffective. Although MSE has a great improvement compared to the latter, it is still much less effective than two characteristics derived from the SDLE. MSE improves the entropy sample, as it contains an additional parameter, the bs scale for the smoothing, and hence, provides a better characterization of the dynamics of HRV. Also in the same article was also addressed the study of entropy and the Kolmogorov-Sinai Entropy rates. As noted in several articles in the literature, increasing age is associated with a reduction of the total variability of the heart rate as well as to changes in the complexity of the physiological dynamics. Study objective of Takahashi et al. [152] was to determine whether alterations in the autonomic modulation of heart rate caused by the aging process could be detected

Shannon entropy (SE), the conditional entropy (CE) and symbolic analysis (SA). The complexity analysis was carried out in 44 healthy subjects divided into two groups: elderly group ($n = 23$, 63 ± 3 years) and young group ($n = 21$, 23 ± 2 years). The indices above were analyzed during a brief heart period (200 heartbeats) derived from ECG recordings during 15 minutes of rest in the supine position. The sequences characterized by three periods heart that do not show significant changes (0 V), and that with two different significant changes (2ULV) reflect changes in sympathetic and vagal modulation, respectively. For statistical analysis we used the unpaired t test. In the aging process, the distributions of the models (SE) remain similar to those of young subjects. However, the regularity is significantly different; models are more repetitive in the elderly group. The sum of the model types are different: 0V is increased and 2LV and 2ULV are reduced in the elderly group. These differences indicate a marked change of autonomic regulation. The EC and the SA are possible techniques to detect altered autonomic control of heart rate in the older group. The methods used in this study can quantify the complexity of cardiovascular regulation, and may be useful for screening and monitoring of vulnerabilities in clinical patients older adults. It is interesting to note that, since the complexity of cardiovascular regulation can be assessed by variables that are estimated in the invasive and non-invasive manner during common tests, this evaluation does not require additional procedures or devices, and can be performed in short recordings few minutes. The evaluation of the complexity HRV may facilitate the understanding of cardiovascular function in health and disease, and can provide clinical information not redundant with no need for further analysis extremely sophisticated.

Also Guillen et al [98], in 2000, focused on the non-linear dynamics. They investigated tachograms (R-R intervals) of two groups, obtained from high resolution ECG: the first consisting of 15 chagasic patients and the second of 18 healthy persons as a control group. They performed a statistical analysis of the first Lyapunov exponent and assessed the complexity changes through the symbolic dynamics method.

4.8 FHRV non-linear analysis: a brief literature report

To understand what the fractal analysis is, we can consider the study of Goldenberger et al [95], who - basing on the phase-space reconstruction - obtained graphs, also named as attractors, suggesting that the fluctuations in the normal heart rate are typical of a chaotic nonlinear system. They had in fact shown that the technique of fractal analysis can be successfully and reliably applied to the study of FHR variability, demonstrating that its application in case of fatal arrhythmogenesis following a coronary artery occlusion allows to obtain a decrease of the correlation dimension.

In 1996, Di Rienzo et al [94] used the fractal analysis with the aim to identify foetal pathologic cases. They used cardiogram traces of approximately 20 minutes duration obtained by two groups of healthy normal or severely distressed/preterminal foetuses, even in the presence of high levels of noise. Foetal heart rate data were obtained by processing a total of at least 600 heart beat intervals from each tracing. Routine tracings were evaluated as belonging to one of two groups; the normality - technically indicated the foetal "reactivity" - of the traces was established using conventional CTG criteria, by following the Dawes' criteria [94, 95]. Phase-space attractors were created by using a time delay technique to produce a three dimensional vector from a one-dimensional time series. Dimensional analysis was performed by means of the method of Grassberger and Procaccia [97] who introduced in the chaos theory the idea of correlation dimension, a means of measuring a type of fractal dimension of the strange attractor. By applying this method, Di Rienzo [94] observed that the healthy term foetus showed a large central attractor with lots of random projection of energy; when the tracing appeared questionable, the attractor became smaller; with further deterioration, the attractors become further small. This result indicated an impending distressing situation of distress, so they were able to demonstrate that, using fractal analysis of foetal heart rate variability, we may clearly distinguish normal FHR tracings from the abnormal ones.

Kurths et al. have carried out a study on 41 women subject to hypertension due to pregnancy, 34 women with preeclampsia and 15 women with chronic hypertension.

The mathematical model was used to describe the physiopathological states of patients and blood pressure was measured noninvasively, and finally all measurements had a duration of about 30 minutes. In the same study the method of HMMs distinguishes optimally pregnancy under normal conditions from a condition of hypertension due to a model based on time series interval beat to beat with 15 hidden states. The analysis revealed a sufficient classification using a higher number of stages that seems hidden influence more complex variations in heart variability compared to the signals of blood pressure. The disorders in pregnancy and PE PHI seem to have a different pathophysiological regulation of blood pressure [90].

The identification of antepartum foetal distress was performed by G.Magenes in 2004 through multiparametric analysis using approximate entropy (ApEn) and a model to support vector machines (SVM), which receives a set of parameters linear and nonlinear extracted from FHR. SVM is a set of methods of supervised learning for regression and classification of patterns, developed in the 90s by Vladimir Vapnik. Belongs to the category of generalized linear classifiers also known as maximum margin classifiers, because at the same time minimize the empirical classification error and maximize the geometric margin. The study was performed on 70 cases: 35 normal and 35 with IUGR. A test based on 30 cases unknown showed good values of measurement accuracy, specificity and sensitivity [89].

The approximate entropy method ApEn was used in the analysis of foetal health by MG Signorini in 2003 using monitoring through cardiotocograph. This method is based on 'multi-parameter analysis of FHR, which includes spectral parameters of autoregressive models and nonlinear algorithms. The study was performed on 14 normal fetuses, 8 cases of gestational diabetes and 13 fetuses with intrauterine pregnancy. This analysis showed a significant difference between the different groups of subjects and the spectral parameters also establish an important difference between the normal and the pathological ones [85].

In 2013, Van Leeuwen has carried out a study using the method ApEn of 11 fetuses who underwent mcg to 2135 minutes during the second and third trimesters of pregnancy. For each subject were assessed HRV and regression analyzes were carried out in combination with RR intervals. The coefficient was used to evaluate

the data fitting. The dispersion coefficient quartile CQD was used instead to compare the regression parameters for each measure HRV. The foetuses were then observed for 20 weeks with a weekly recording of f HRV. All data have found a positive relationship between measures of HRV in relation to age. Considering not only gestational age but also the mean values of RR intervals of each recording, the consistency of regression models improves and finally it was noted that factors such as the behavior and maternal conditions may play an important role in the 'increase' accuracy of the models [85].

Lim Jongil used ApEn to assess foetal stress and lack of oxygen, but also the metabolic and respiratory acidosis in women at the end of their pregnancy. Foetuses, analyzed at 37-th week of gestation, were recorded FHR for an hour and a half before the birth. Were monitored at about 450 shares consecutive and all recordings were divided into 3 groups: recordings in the second stage of pregnancy prior to vaginal delivery, registrations in the first stage of labor before using for emergency Caesarean section and finally in the previous recordings the cesarean delivery itself. It was found that the indices ApEn and Sampen are significantly different in relation to the stage of labor during which these are measured [86]. ApEn and Sampen have been used to analyze the variability of the parameters in the time domain of 46 mcg foetal recorded in the range that goes from 21-38 weeks by Moraes et al. [87]. The advantage found in the art Sampen than ApEn is overcoming the limitations related to the length of the recordings and the possible lack of consistency in the quantification of the regularity of the signals.

With the term intrauterine growth restriction IUGR describes a foetus that can't reach their genetic potential for growth. It is a subclass of births in which the foetus is smaller than it should be compared to the state of gestation. The intrauterine growth restriction concerns those with a birth weight in this range, due to underlying diseases. Apart from a malfunction of the placenta can also be caused by a number of environmental factors such as an infection transmitted from the mother, multiple gestations, typical toxins in cigarette smoke, or genetic factors such as chromosomal abnormalities. Unfortunately, it is difficult, if not impossible, to know the expected size. Biometrics foetal ultrasound is a noninvasive technique that

includes the assessment of gestational age, foetal weight and measure abdominal. In the first gestational period is useful to define the size of the foetus for gestational age SGA. SGA is defined in relation to the standards of the population and the health of the foetus [88]. In the study by M. Ferrario were compared traditional parameters that are derived from the FHR signals with ApEn and with a new parameter, the complexity of Lempel Ziv (LZ). The latter is an algorithm used for data compression without loss of information. The input of the algorithm of compression and decompression is constituted by a finite sequence of symbols (string) belonging to a set said alphabet. Sometimes the input string contains some parts (sub-strings), which are repeated several times within the same. The idea of the algorithm is that of exploiting these repetitions for compression. In this study [88] it is shown how this algorithm and the index of the standard deviation STV are able to discriminate IUGR from healthy foetuses. In contrast, the introduction of regularity parameters as the ApEn Sampen and does not provide for an increase of the ability of classification of the FHR. In conclusion, the algorithm LZ was the only one to provide significant results every time for such discrimination [88].

4.8.1 Symbolic Dynamics Analysis: applications to FHRV

In mathematics, symbolic dynamics is the practice of modelling a topological or smooth dynamical system by a discrete space consisting of infinite sequences of abstract symbols, each of which corresponds to a particular state of the system, with the related dynamics that represents its evolution.

In the context of the HRV analysis, this technique involves the transformation of the original time series of RR intervals in a series of symbols, in order to extract useful information about the state of the control of Heart Rate system. Briefly, the Symbolic Dynamics represents a signal as a patterns expression, so that the signal's samples are reduced to a few possible symbols, simplifying the study of the dynamic behaviour of the signal itself. The series of differences, through the definition of some classification rules - such as values that are less than or greater than a threshold - can be re-encoded in accordance with the corresponding combination of

symbols: in this way it is possible to obtain a series of such symbols which can furthermore be structured in 'words' defined as strings of consecutive symbols.

There are many approaches to identify different classification's rules that are already treated in the previous chapters, which generally allow to obtain up to six symbols. Particularly, we have many options to make the symbolization: we can associate to a single sample of the signal a sample with two symbols; we can consider the differences between two consecutive intervals or between the RR-interval modulus.

At this point, it is useful to clarify that the symbols associated to the tachogram can be either alphanumeric or of any other type, according to the analysis criteria. The symbols obtained are then organized into strings called "words", after the definition of the length fixed for each string: so that, the assembling process is moving step by step, and each step reveals a new sequence composed by all symbols remaining, except those included in the previous string. Words can then be grouped in such "families", according to the variations which they are presenting (i.e., no variations, one, two or three), and the frequency of the strings presenting each variation is considered to extrapolate the necessary information about the HRV. We can observe that the number of symbols and the string length are fixed considering their effects on both the correct definition of the dynamics which are object of the study, and the statistical significance to estimate the HRV. It must be observed that with the increasing of these two parameters, it will increase the computational complexity.

Many authors agree on the fact that the use of this analysis should be used with the aid of traditional methods of HRV in the time domain and frequency in order to improve the reliability of the risk stratification of sudden cardiac. In fact, through the use of Symbolic Dynamics we can describe the complex rhythm fluctuations and separate structures of non-linear behaviour in the time series of heart rate more successfully than by means of traditional linear methods. We can consider these studies performed to assess the results obtained by using the symbolic dynamics and other non-linear methods in the context of the HRV analysis [91].

CHAPTER 5

CTG signals

5.1 Real CTG traces

5.1.1 Database

For this PhD thesis, it was analyzed a set of 580 cardiotocographic signals (please note that all the patients gave their informed consent to participate in the research about foetal monitoring) recorded between the 24th and 42nd week of gestation.

The database was completed with other clinical information of pregnant women and infants, including: Apgar score, used to assess the health of infants, at 1 and 5 minutes after birth (APG1, APG5); the type of delivery (spontaneous or cesarean); the week of gestation and others.

5.1.2 CTG signal pre-processing

The CTG signals are recorded with the Doppler technique along with the use of the autocorrelation function. To have good quality (that is, correlation, or more simply similarity between the current beat and the previous, assessed by means of the autocorrelation function) there should always be a good alignment between the probe and the foetal heart, as this is obviously not always possible, signals are noisy by their nature. The signal quality of the FHR recorded is provided by the device and stored in a variable (called q) which can assume a value of 0 (poor quality), 1 (average quality) or two (good quality). Besides having sections of not good quality, there may be samples set to zero (HP) or not registered at all (in these cases the variable quality will assume the value 0). For the Sonicaid system, even in case of poor quality of the signal, it is unlikely that the q variable assumes a value of 0.

Instead, there may be signal losses due to the fact that, in those cases, the signal is not generated at all. The FHR signal can be corrupted even by artifacts (due to sudden movements of the foetus or the mother) or by cardiac arrhythmias (which are not the subject of study by the CTG). Both of these phenomena occur on the signal as outliers that can alter the subsequent signal analysis.

In addition to these characteristics, common to all cardiocographic recordings, the paths output from cardiocographs HP have the peculiarity of being stored with a technique of zero-order interpolation. This means that the cardiocograph provides FHR output samples at fixed instants (4 samples per second) and being in reality the series FHR by its nature non-uniform, to obtain this result, some samples are delayed and other duplicates (figure 5.1).

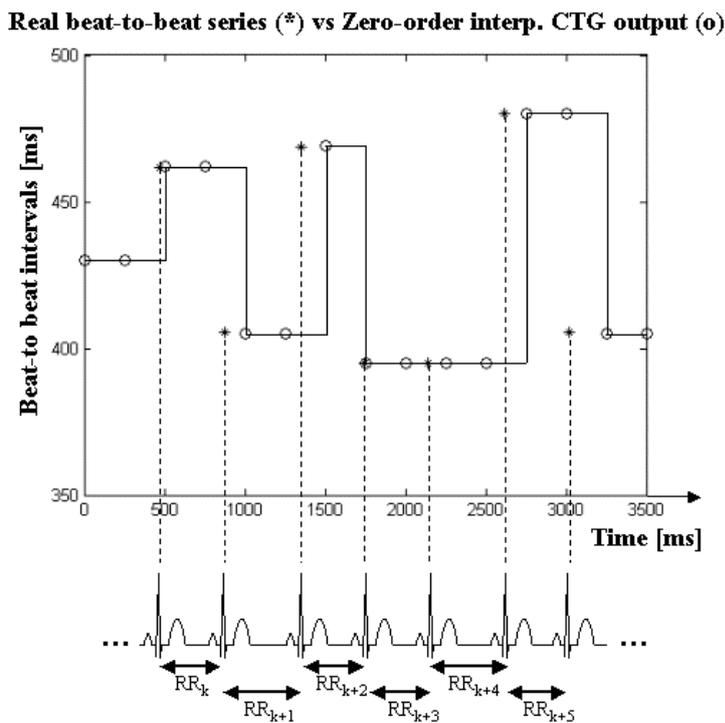


Figure 5.1 - Zero order effect

This technique, which practically does not alter the time course of the signal in any significant way to the naked eye, generates FHR signal alterations that affect some analyzes (for example attenuate the high frequencies in the power spectrum) [99].

Another operation performed by cardiotocographs is to round off the values of the output signal FHR providing them with a resolution of 0.25 bpm. Although the HP manual does not specify in detail this, it is assumed that the cardiotocographs run after interpolation, rounding the value recorded at the lower module .25 (i.e., If the recorded value is 140.31 bpm, cardiotocograph outputs the value 140.25 bpm).

This rounding results in a lengthening fictitious of RR intervals. Given the structure of the data, with alternating segments of good and bad quality, then indicated by the instrument itself as reliable, to speed up the computation time, the pre-processing processes only segments with not low quality.

The main steps that will have to perform the pre-processing are therefore:

- reading of cardiotocographic signal and extraction of variables FHR, Toco (uterine contractions) and quality;
- recognition of the traits of good and bad quality of the recording (the latter are not analyzed); for simplicity we call "good", segments with q equal to 1 or 2 and "bad", ones with q equal to 0.

For each signal tract of good quality:

- Only for cardiotocographs HP, recovery of real FHR series (with its time axis);
- Recognition and replace of outliers.

For detecting outliers, literature provides rather complex procedures [100] that distinguish between local outliers (isolates) and global outliers.

Experiments on real signals, however, highlighted that these procedures do not give satisfactory results; so the procedure for global outliers recognition was replaced with a simplified one in which each sample is compared with respect to a threshold set equal to the FHR mean value; concerning the recognition of local outliers other rules were added [138].

- After each cycle, the signals are reconstructed by alternating good segments processed in previous steps and original bad segments;
- linear interpolation of the traits of poor quality (or signal loss for cardiotocographs Sonicaid) up to 3 s (to avoid excessive segmentation of the signal).

Since, as mentioned, the logic signal recording FHR changes as a function of cardiocographs adopted, the functions that operationally implement the theoretical operations described above can be different.

As far as the outliers, the literature about HRV proposes to define a temporal trend in which are located the inter-beat intervals in presence of a normal cardiac rhythm. The samples which do not belong to the temporal trend are considered outliers [120, 121]. Other approaches involve the use of thresholds set on the basis of a mean value computed with different rules [120, 122].

The correction step can implement two different approaches: elimination of the outliers or their substitution with a sample that fits better to the series trend [120, 121]. The most widely used solutions are median filters and linear and non-linear (cubic spline) interpolations.

The developed and updated software consisted into two main steps: outliers detection and correction. It can be divided in other two steps: detection and correction of local outliers (cardiac arrhythmias and short time artifacts); detection and correction of global outliers (signal losses) [118, 119].

In detection step, global outliers are now defined as all the samples for which the absolute difference respect to the FHR mean is out of a range fixed by a threshold, set as percentage of the FHR mean; in particular, the threshold is 30% for samples of good quality and 20% for samples of medium quality.

The detection of local outliers is carried out analyzing only the samples that are not global outliers. A candidate local outlier is a sample for which the difference between its amplitude and the median value calculated on five samples positioned before and after the sample in analysis exceeds a threshold set according to the sample quality. The threshold is increased of 30 % of the set value to analyze the samples positioned at the extreme limits of each FHR signal tract. To avoid that samples belonging to slow baseline variations are detected as outliers, another check has been added. The candidate outlier is elected local outlier only if it likely does not belongs to the line estimated as regression line of the neighbour samples.

The correction step uses for the substitution the median value calculated on 8 samples. The median is computed centring the vector on the outlier and excluding other eventual outliers already detected.

The software allows setting number of consecutive outliers (NOC) to be substituted with the median procedure and length of bad quality segment to be interpolated (LI); however, in default setting, the *correction* procedure substitutes only isolated outliers.

Moreover, when consecutive outliers are not substituted their quality is set equal to zero so that in a following step of pre-processing can be interpolated. The software was tested on different simulated and real signals. As example, in figure 5.2 it is possible to observe that local outliers (PVD and isolated outliers), gradual signal loss (red ovals in figure 5.2) and the abrupt signal losses (blue ovals) were recognised (marked as green stars in figure 5.2).

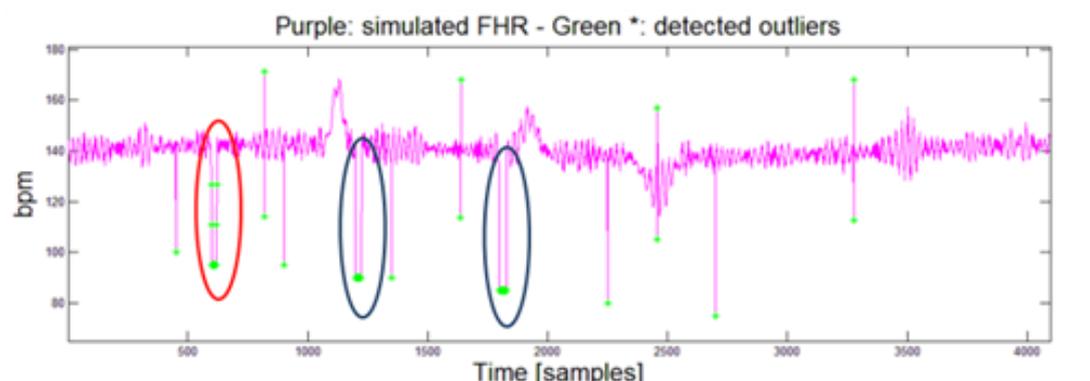


Figure 5.2 - Green stars: detected outliers

The developed algorithm for outliers detection and substitution provided very satisfying performances on 450 simulated and 100 real FHR signals.

Obtained results prove that outliers detection is fundamental to perform accurate analysis on FHR signals.

5.1.3 CTG signal processing for classical analysis

The classical analysis is based on a software which aims to integrate and complement some important characteristics of other automatic systems. It allows recording of CTG signals both from HP and Sonicaid systems and is accompanied by a user interface which prints on the screen analysis results and gives the possibility to choose between a compact print and a detailed print of the analysis.

It provides the estimation of the real uneven FHR series, the baseline and FHR mean value, the floatingline (median line of the FHR), the number of accelerations (and their classification in big, small and very small), the number of decelerations (and their classification in big, small and very small), the number of contractions (total, big and small), the short term variability, frequency parameters (both from STFT and Lomb spectrum), the indexes by symbolic dynamics, the percentage of lost and interpolated signal and the track length.

Following Mantel criteria, the baseline is defined as current average of the heart rate in absence of accelerations and decelerations [101].

For floatingline estimation, a non-linear filtering is implemented. The filter band is different in presence or absence of accelerations (which enlarge the frequency band of the floatingline) [102].

The algorithm responsible for the identification of the accelerations is divided into three main phases:

- Preliminary identification of the accelerations (based on criteria concerning amplitude and duration)
- Control of the gap (lowering of the heart rate below a certain threshold within the same acceleration) and therefore eventually division or elimination of the acceleration previously identified
- Eventual elimination of accelerations containing interpolated values.

For the decelerations, the algorithm is similar to for accelerations detection but, of course, adopted criteria are different in all the phases.

With regards the detection of uterine contractions a reference is calculated (a kind of basal tone) with an algorithm very similar to that developed for the calculation of

the baseline; then, uterine contractions are detected by evaluating their duration and amplitude with respect to the computed basal tone.

Short term variability of FHR is computed by means of standard deviation after subtraction of the floatingline, as previously proposed [103].

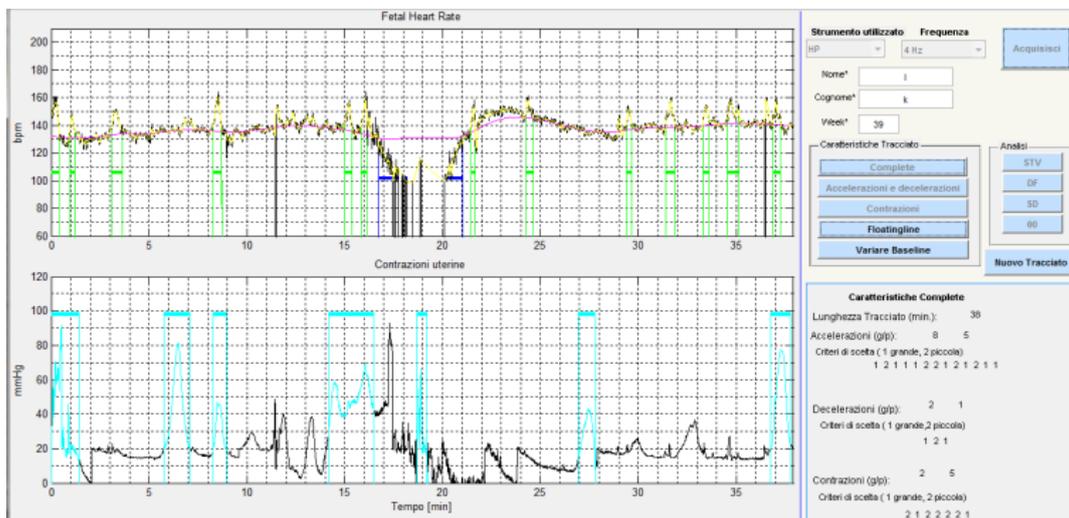


Figure 5.3 - Screen shot of an example of analysis results

The analysis provided by the software was compared with evaluation carried out from a team of 18 expert obstetriuacians. Results have been satisfactory in eighty-five percent of the cases and, in some cases, the software appears to provide better results than those provided by commercial systems available in clinical environments. There remains a fifteen percent of cases in which the results are not entirely satisfactory, but in most of these cases doctors also have some difficulty in expressing an opinion given a non-complete agreement about the identification of various parameters, due to the very low signal-to-noise ratio.

5.2 Simulated CTG traces

Part from our analysis has had as its object a database of simulated signals, i.e. generated by a suitable software developed in Matlab. The simulated signals can cover a wide range of physiological conditions and recording situations, simulating the main features and characteristics of a real cardiotocographic trace (baseline fluctuation, accelerations, decelerations and contractions). Moreover, also the

anomalies due to the acquisition system and/or cardiac arrhythmia (signal loss and outliers) can be generated artificially by the software.

In the main code, called "simulaFHR", you can fix the following parameters:

- Mean FHR;
- Amplitude variability of FHR;
- Band LF;
- HF band;
- Power ratio LF / HF;
- Number and kind of Accelerations;
- Number and kind of Decelerations;
- Number and kind of Arrhythmias;
- Number and kind of Signal losses.

We therefore have the possibility to know a priori all the features of the signal that will be examined, and then to assess the effectiveness of some analysis.

In order to resemble real physiological situations, baseline fluctuation, accelerations and decelerations were simulated, adopting classical definitions [26]. In particular, we used a slowly varying sinusoid to simulate the FHR baseline that can be modified in amplitude and frequency, and, with regards to accelerations and decelerations, we used Gaussian-like signal tracts [100] with waveform features and parameters (amplitude, position, duration) that can be directly set by the user. As far as the basal UC signal, it was simulated by low-pass filtering a white noise. The software here proposed is able also to simulate the contraction events with known features (amplitude, frequency, position, duration). The algorithm for the simulation of these events is similar to that of the accelerations, using Gaussian-like signal tracts with features chosen at user discretion.

Moreover, the software allows the simulation of these outliers. In particular, typical arrhythmias, such as Premature Ventricular Depolarization (PVC), simulated by the succession of two FHR samples, respectively with very high and very low value; losses of signal, for example due to relative movements between the Doppler probe and the foetus; or simply isolated outliers, resembling CTG spikes due to erroneous

recording of some FHR sample can be simulated. The signal loss can be simulated as abrupt, groups of samples with values very different from FHR mean, or gradual, in this case the change in FHR value is gradual, i.e. there are sample with intermediate value.

The software provides two different graphical representations of the simulated signals, the default one employed by Matlab, and one that reproduces the clinical standard. In the latter case, the CTG trace is shown according to the following requirements: CTG paper running speed equal to 1 centimetre (cm) per minute, 25 cm of recording for each page, FHR range equal to 50-210 bmp and UC range equal to 0-100 a.u..

The following figures show an example of a simulated CTG signal with two accelerations, one deceleration and two uterine contractions [156] and an example of a synthetic FHR signal which shows arrhythmia and outliers. In particular, 3 tracts of signal loss, 4 PVC and 5 isolated outliers were simulated in this case [156].

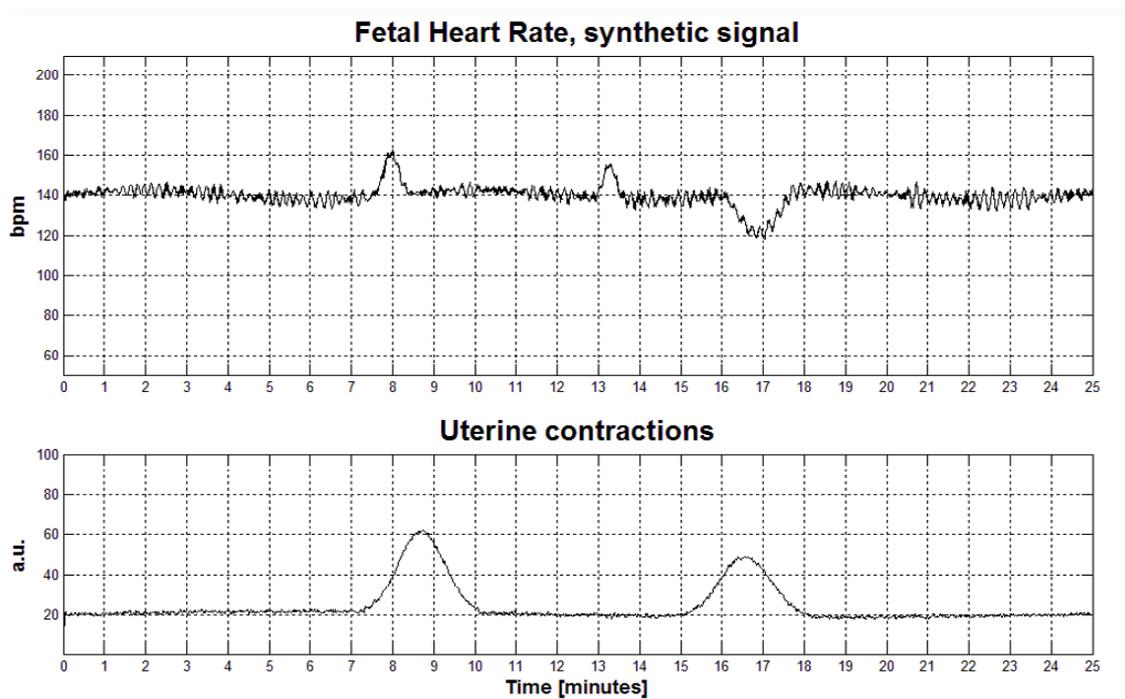


Figure 5.4 - Simulated CTG signal with two accelerations, one deceleration and two uterine contractions

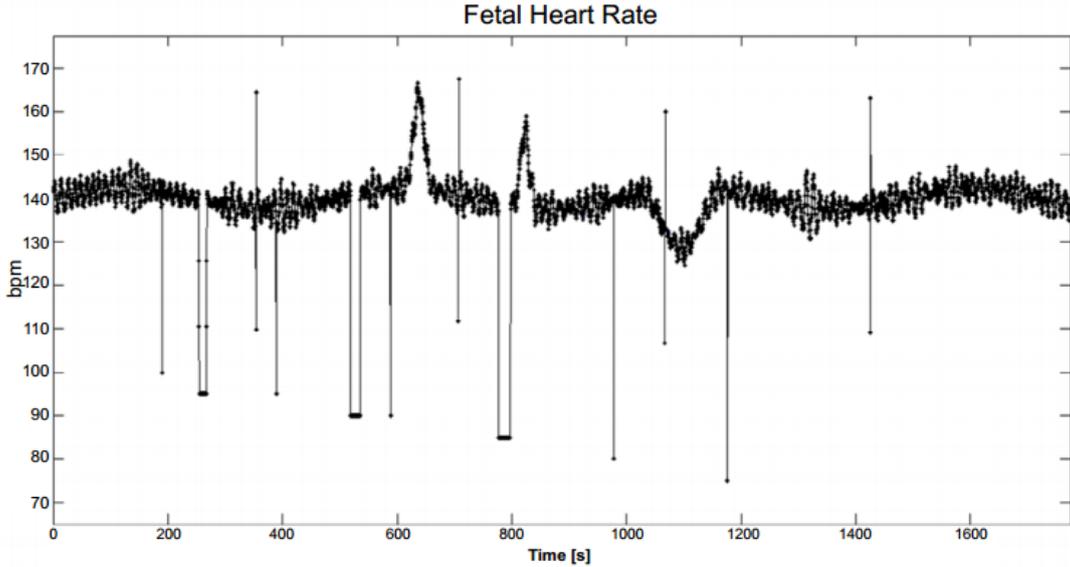


Figure 5.5 - FHR simulated with signal loss (gradual the first, between 250 and 270 s, and abrupt the others, around at 520 and 780 s), arrhythmia (PVC) and isolated outliers.

CHAPTER 6

Methodologies employed for FHRV analysis

6.1 Introduction

In this work, different methods have been employed to analyze FHRV. In the following paragraphs, procedures adopted to estimate the FHRV are illustrated and the linear and non-linear techniques chosen and carried out are presented. They range from the more traditional analyses like Short Term Variability measurement (as a time domain analysis) and Short Time Fourier Transform (as a frequency domain analysis) to the less common non-linear parameters like Entropy, Poincarè and Symbolic Dynamics indexes.

Each main paragraph of the chapter is dedicated to one specific methodology or parameter and gives a brief description of its importance according to the literature, in order to reinforce the taken choice, along with an explanation of the practical or theoretical steps undertaken to make it suitable and useful to the aim of this study. A special attention will be given to the Symbolic Dynamics Analysis.

In conclusion, adopted statistical and regression analyses are also introduced but will be extensively discussed in the next chapter.

6.2 FHRV definition and estimation

The FHRV, an important parameter for the evaluation of foetal well-being, it can be analyzed both in time domain and in frequency domain. In particular, the FHRV estimated with the spectral analysis is known to have an important and significant relationship with the autonomic nervous system (ANS) and changes in FHRV are also correlated with foetal development [125, 126]. In spite of this, not many

literature works inspect this issue. Besides, the great majority of them are old [127, 128] or use foetal magnetocardiography to record foetal heart activity [19, 130]. Finally, they often disagree about FHRV definition and indexes to employ, both in time and frequency domain. Our estimation of FHRV is based on the evaluation of the floatingline, median line of the FHR, and the FHR signal, as we can see in the following equation.

$$\mathbf{FHR - floatingline = FHRV}$$

To estimate the floatingline was developed and then updated a new algorithm implemented in several steps, which computes the floatingline through a non-linear filtering of the segments of good quality and subsequently the various sections of the floatingline are linearly interpolated. The filter band is different in presence or absence of accelerations (which enlarge the frequency band of the floatingline) [141].

6.3 Time Domain: STV

Among the time domain measures of FHRV, Short term variability (STV) and long term variability (LTV) are usually distinguished. STV refers to the continuous variation in difference between successive inter-beat intervals and it is difficult to interpret reliably with the naked eye. LTV refers to fluctuations in the FHR over seconds [106, 107].

Short Term Variability could vary in correspondence of critical events and characteristics, such as FHR decelerations during intra partum period. Labor course reflects a long and stressful process that involves uterine contractions, placental function, and changes in birth canal width [108]. Actually, many studies analyze occurrence of decelerations and/or their characteristics and compare them with other measurements in order to establish the effective capability to predict neonatal outcome. For example, patterns showing much reduced variability associated with persistent late decelerations, severe variable decelerations, and prolonged decelerations are generally believed to be ominous and may correlate with hypoxia of such severity that foetal central nervous system damage may already have

occurred [109]. However, no accordant results have been obtained yet and, in particular, there is no agreement about the significance of some FHR patterns [109, 110]. Indeed, STV, or beat-to-beat variability, is the index that concisely takes into account short term oscillations of the FHR around baseline (generally variations of 5–10 bpm). This variability permits to investigate foetal reactions to internal or external stimuli (such as UC). In general, large variability reflects a healthy autonomic nervous system (ANS) and also chemoreceptors, baroreceptors and cardiac responsiveness; while foetal hypoxia, congenital heart anomalies and stress cause decreased variability [85, 112 - 114]. For this reason, STV can represent a valid support to diagnose foetal health [123-125].

In a previous study of Cesarelli et al. [124] nine different STV indexes, cited in literature and/or used in clinical applications, were compared. Arduini, Dalton, Organ, Sonicaid8000, Van Geijn, Yeh, Zugaib, a modified version of Arduini index and Standard Deviation were considered and then compared by means of simulated FHR signals. In particular, their accuracy, repeatability and their dependence on CTG “storage rate” and FHR mean value were evaluated. To test the STV indexes performances, synthetic FHR signals were artificially generated, via software, using a slightly modified version of a method proposed for adults by other authors [29, 115, 116] and already employed in a previous work [117]. A large set of synthetic foetal heart rate series with known features was used to compare indexes performances. Results indicate that although the indexes are able to recognize STV variation, they show substantial differences in magnitude and some in sensibility. Results depend on the frequency used to acquire and store FHR data (depending on devices); in general, the lower is data rate the more degraded are the results. Furthermore, results differently depend on FHR mean, some for their intrinsic definition; differences arise also in correspondences of accelerations and decelerations. Moreover, results demonstrate that only indexes which refer directly to differences in FHR values, such as Organ and SD indexes, not show dependence on FHR mean, therefore, the use of the Standard Deviation index may provide efficient information while showing independence from the considered variables.

Another important issue related to the STV evaluation is the presence of outliers in FHR signals due to the acquisition system and recording process [138]. The authors [138] estimated STV by means of the standard deviation (SD) after floatingline subtraction, as previously proposed [9, 10]. SD was computed on FHR time-windows of 30 s with an overlap of n-1 samples obtaining a vector of SD values (one for each time-window) aligned with the FHR signal. Since it was not possible to compute SD of the first and last 15 s of each good segment of FHR signal, these samples were set to zero.

Mean value of the obtained vector, without null samples, is the STV index. The following figure show an example of STV computation for a simulated FHR signal.

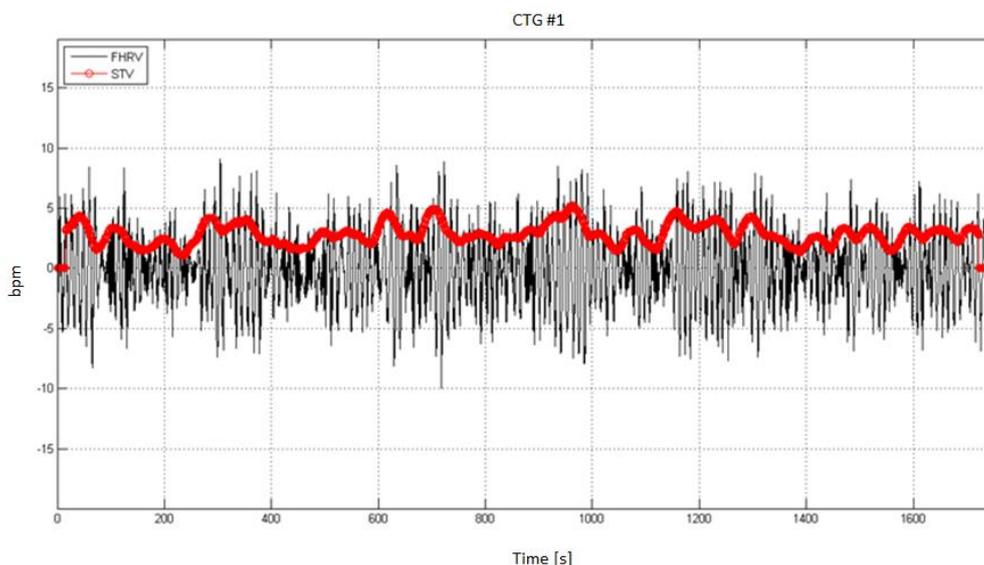


Figure 6.1 - In red STV time trend superimposed on FHRV in back (STV is equal to 2.87)

6.4 Frequency Domain

The variability of the foetal heart rate around its baseline provides extremely significant information concerning the cardiac and autonomous nervous systems during pregnancy up to labour. Our aim is to study power content in all bands of FHR power spectrum during the course of pregnancy. Even if the FHRV analysis is widely used for assessment of foetal well-being and development [125 - 128, 133], there is a lot of confusion about the definition of frequency bands. So, the study

started with an extensive analysis of the works published in literature about the frequency analysis of FHR. This study revealed that today there is still a disagreement about the definition of frequency ranges of FHRV power spectrum. Then, according to the most values found in literature, the authors defined the ranges of the three main bands of PSD. In these bands the power content was computed using a software properly developed [135, 141].

6.4.1 Literature Overview

Most of literature agrees that three bands can be detected in the FHR power spectrum, a very low frequency band (VLF), a low frequency band (LF) and a high frequency band (HF), but there is no agreement in definition of the exact extremes of frequency bands. Unfortunately, despite the large amount of research works about the FHR frequency analysis, only a small number provide precise information about the bands and are often incomplete [125, 127, 128, 133, 134].

In order to clarify the definition of frequencies bands, a study of literature was conducted, involving about eight hundred literature works concerning foetal monitoring, published between 1983 and 2013. Among these only a hundred are directly related to frequency analysis and only about thirty works gave details about the three bands.

On the basis of this study, the authors set the three bands of FHR power spectrum as follows: from 0 Hz up to 0.03 Hz (VLF); from 0.03 Hz up to 0.2 Hz (LF); from 0.2 Hz up to 1 Hz (HF).

The table below shows the different values reported in literature about the bands of FHRV power spectrum.

Table 6.1 - Values reported in literature of bands of FHRV power spectrum (Lower, "l", and upper, "u", boundaries)

Ref	First Author	Year	VLFl	VLFu	LFl	LFu	MFl	MFu	HFl	HFu
133	<i>Oppenheimer</i>	1994			0,00	0,20			0,70	0,95
101	<i>Sibony</i>	1994	0,02	0,05	0,05	0,15			0,15	0,50
160	<i>Sibony</i>	1995	0,02	0,04	0,04	0,16			0,16	0,30
161	<i>Sibony</i>	1995							0,20	0,50
128	<i>Ohta</i>	1999			0,06	0,31			0,30	0,50
175	<i>Van Geijn</i>	1994	0,017	0,04	0,04	0,2			< 0,2	< 0,2
162	<i>Jezewski</i>	1998							0,6	1
125	<i>Ferrazzi</i>	1989							0,70	0,90
163	<i>Cerutti</i>	1989	0,00	0,03	0,04	0,15			0,20	0,40
165	<i>Karin</i>	1992							0,60	0,80
104	<i>Divon</i>	1985							0,7	0,95
85	<i>Signorini</i>	2003	0,00	0,30	0,03	0,15	0,15	0,50	0,50	1,00
167	<i>Siira</i>	2005			0,04	0,15			0,15	1,00
147	<i>Van Leeuwen</i>	2003			0,04	0,15			0,15	0,40
20	<i>Zhuravlev</i>	2002		0,05	0,05	0,20			< 0,2	
168	<i>Yum</i>	2004			0,04	0,15			0,15	0,40
181	<i>Richards</i>	1995							0,333	1,25
169	<i>David</i>	2006	0,01	0,08	0,08	0,20			0,40	1,50
170	<i>Schneider</i>	2009	0,02	0,08	0,08	0,20			0,40	1,70
129	<i>Van Laar</i>	2009			0,04	0,15			0,40	1,50
171	<i>Rassi</i>	1995			0,07	0,12			0,56	1,10
176	<i>Reinhard</i>	2012			0,04	0,15			0,15	0,4
177	<i>Kwon</i>	2012			0,04	0,15	0,15	0,5	0,5	1
178	<i>Goncalves</i>	2013	0	0,03	0,03	0,15	0,15	0,5	0,5	1
166	<i>Warrick</i>	2012			0,03	0,15	0,15	0,50		
164	<i>David</i>	2007	0,02	0,08	0,08	0,20	0,20	0,40	0,40	1,70
179	<i>Warrick</i>	2010	0	0,03	0,03	0,15	0,15	0,5	0,5	1
130	<i>Padhye</i>	2004			0,05	0,25			0,25	1,00
180	<i>Tsoulos</i>	2006	0	0,03	0,03	0,15	0,15	0,5	0,5	1,5

The literature study involved also the analysis of power values, mean and/or percentage, reported by the different authors.

Table 6.2 - Values reported in literature of mean and percentage power

Ref	First Author	VLF	LF	MF	HF	Tot	Meas. Unit
133	<i>Oppenheimer</i>	2.95/22,4%	2.26/17,1%	1.43/10,9%	4.08/30,9%	13.18	ms ² / %
134	<i>Groome</i>	35.6±15.3	28.6±10.7		35.8±13.2		%
125	<i>Ferrazzi</i>	66.9	3.8	15.2	11.5		%
163	<i>Cerutti</i>		8,1/27,3%	2.8/9,3%	10.2/44,2%		ms ² /%
85	<i>Signorini</i>		324±174	28±26		614±394	ms ²
85	<i>Signorini</i>	31,1	56,84	8,37	0,18		%
168	<i>Yum</i>		100.5±6.3		15.5±0.9		ms ²
129	<i>Van Laar</i>		429±410		21,3±41,3		ms ²
130	<i>Padhye</i>		17,38+1,98t		11,54+1,96t	22,33+1,48t	dB
182	<i>Signorini</i>	274,82±234,41	136,76±84,21	19,13±10,93	4,81±3,61		ms ²
182	<i>Signorini</i>		83,82±4,79	12,26±2,51	3,93±2,55		%

6.4.2 Definition of PSD and bandwidths by means of simulation

In the simulated signals, although there was a floatingline constituted by very slow oscillations (baseline sine wave, accelerations and decelerations), there is the lack of other physiological components that are part of VLF, which, consequently, has a smaller amplitude. Except for this consideration, the obtained results (figures 6.2-6.5) seem to confirm what obtained analysing the real signals. In particular, comparing figures 6.2, 6.3 and 6.4 it is noted that the detrending operation leaves a residual VLF component superimposed on the LF whereas removing floatingline allows obtaining a spectrum with only the LF and HF components. This result is confirmed by a comparison of figures 6.3, 6.4 and 6.5. In fact, thanks to the simulation, it is shown that floatingline subtraction allows a better estimate of both the LF and HF components rather than the detrending method. The floatingline itself could be used to measure the VLF components.

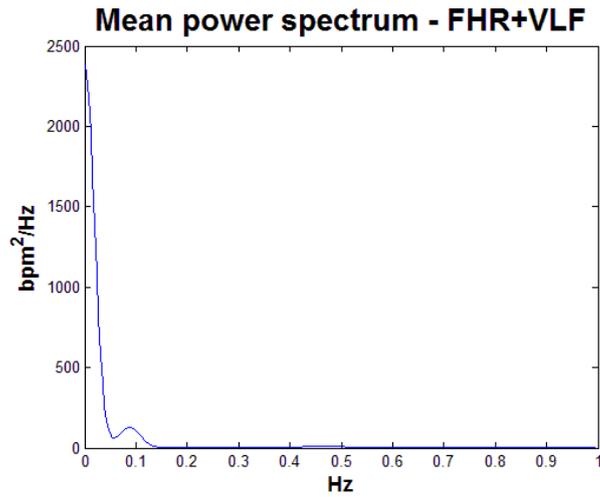


Figure 6.2 - Mean power spectrum of the FHR signal

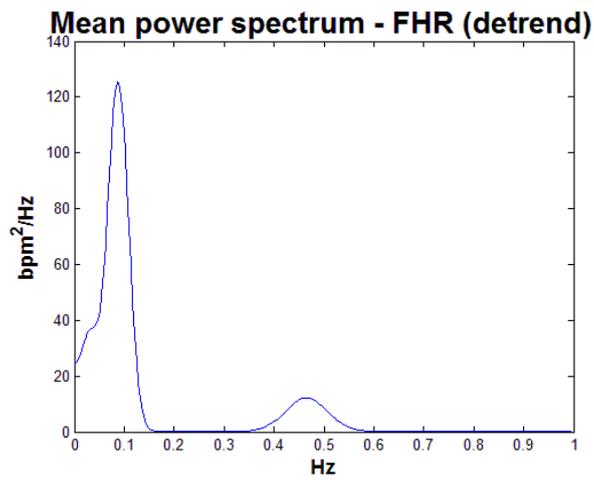


Figure 6.3 - Mean power spectrum of the detrended FHR signal

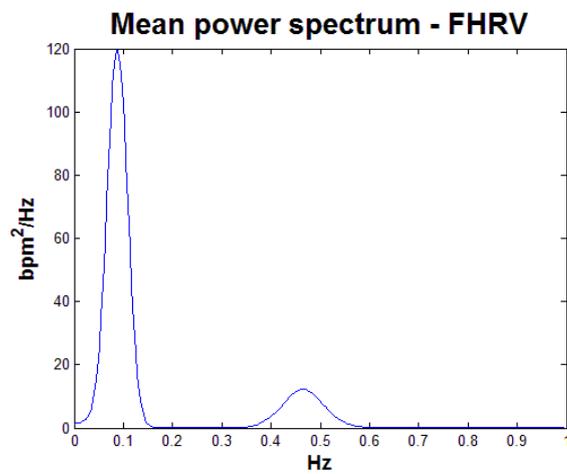


Figure 6.4 - Mean power spectrum of the FHRV (floatingline subtraction)

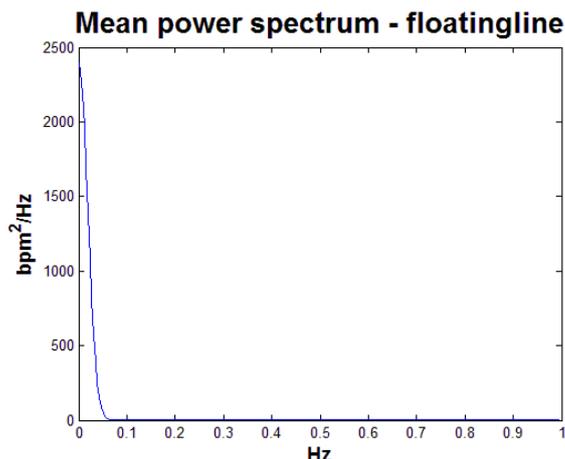


Figure 6.5 - Mean power spectrum of the floatingline

6.4.3 STFT and power computation

On the basis of the described literature study and according to the simulation analysis and our experimental results, we decided to set the three bands of FHRV power spectrum as follows:

- VLF: from 0 Hz up to 0.05 Hz;
- LF: from 0.05 Hz up to 0.2 Hz;
- HF: from 0.2 Hz up to 1 Hz.

Thus, the power spectral density (PSD) of the FHRV is estimated through the Short Time Fourier Transform (STFT) by means of a software developed in Matlab by the authors [29, 126, 131, 135, 136] using a Hamming moving window of length L (where L corresponds to 32 s), with an overlap of $L-1$ samples, after removal of any continuous and linear component [137].

Then, the power was computed in all frequency bands previously defined. Finally, the mean value and the standard deviation were computed for each of the established band. Powers percentages are calculated with the simple mathematical formula of percentage, because of the high inter-individual variability represented by the value of the total power.

Sympatho-vagal balance

Another computed frequency parameter is the Sympatho-vagal balance (SVB), which is the ratio between the power in high frequency and low frequency band (LF/HF) [172]. Sympatho-vagal balance is the balance between the two joint actions and opposing excitation and inhibition exercised on the Cardiac System respectively by the sympathetic and the vagus, in response to ongoing external and internal stimuli. Through them are put in place those processes for homeostatic regulation of cardiac parameters, implemented according to the complex physiological mechanisms of feedback control, through which the value of these parameters is maintained around a predetermined level (the average HR is, in an adult normal, about 70 bpm, whereas, in a healthy foetus at term, of about 140 bpm). Sympatho-vagal balance alterations result in fluctuations in heart rhythm that give rise to a variability of rhythm itself (the so-called signal HRV, Heart Rate Variability), whose study, in both Time Domain and Frequency Domain, allows observation of the behavior of the sympatho-vagal and then monitoring status, integrity and proper functioning of the SNA and the cardiac system.

6.5 Nonlinear techniques

The best non-linear methodologies, namely those that proved to be the most used and most effective in HRV analysis, were found to be Poincaré maps, Approximate Entropy (ApEn), Sample Entropy (SampEn), and Symbolic Dynamic Analysis (SDA). For both Poincaré and Entropy, a classic procedure was implemented through the adaptation of the following software "Heart Rate Variability Analysis Software" "HRVAS" by (John T. Ramshur, University of Memphis). With regards to the Entropy, the following parameters $m=2$ and $r=0.2$ have been set.

6.5.1 Entropy measurements

The ApEn show a close dependence from the length of the time series and therefore it lacks of consistency. In order to overcome these limitations, it was introduced

another nonlinear entropic technique: Sample Entropy (SampEn). This is able to decrease the dependence on the length of the series of data, and then the bias of the parameter. The parameter SampEn appears to be largely independent of the length of the time series and shows to have relative size even in cases where ApEn seems inadequate. SampEn a low value is indicative of a signal more predictable and less randomness [178, 183 - 185].

6.5.2 Poincarè plots parameters

From a quantitative point of view the point cloud obtained by Poincarè plot is characterized by two parameters: SD1 (minor axis) and SD2 (major axis of the ellipse) that are believed to be indicative respectively of heart variability in the short and long term. Provides both general information that details of the behavior of the heart beat by beat. The points located above the line identity, in fact, the RR intervals that appear to be longer than the previous, while points below the line indicate intervals less than the previous. Accordingly the dispersion of the points in the direction perpendicular to the line identity (width of the cloud) is related to the variability in the medium term. The deviation of the points along the line identity (length) is related to the variability of RR intervals.

6.6 Symbolic Dynamics

6.6.1 Signal Preprocessing

Because of the importance of foetal heart rate variability and accelerations in foetal wellbeing assessment and considering the well-known difficulties in the interpretation of cardiotocographic recordings, we proposed a new method of foetal heart variability analysis. In particular, we used a nonlinear technique (Symbolic Dynamics Analysis) in order to evaluate the global variability of the signal and the presence of accelerations. The dataset of real cardiotocographic recordings described in the chapter 5 was examined using the above-mentioned method. From

that database we excluded six recordings because the presence or absence of accelerations was uncertain.

CTG signals were processed by a software previously developed by the authors [29, 140, 141], described in the previous chapter and recently updated in order to implement the SDA.

6.6.2 The symbolic encoding

The criterion of symbolization is based on the choice of certain thresholds against which encode the series ΔRR , obtained by the difference between consecutive RR samples (series of inter-beat interval durations). It is worth remembering that the RR series is calculated from the FHR one by using the formula:

$$RR = \frac{60}{FHR}$$

It should be noted that the division in only two symbols is definitely too narrow, especially not taking into account the fact that, because of the resolution of the recording instrument, there is a range of uncertainty in estimating the value RR. It would obviously be wrong to consider how changes in heart rate values that fall into this category of noise on the signal. It is therefore essential to add at least a third symbol.

By comparing different kinds of transformations and according to our previous work in adult subjects [84, 174], we found that the use of five symbols is adequate [173] in order to get a more detailed analysis, since it allows to distinguish even slight and marked changes in heart rate.

The evaluation of the primary threshold stems from an estimate of uncertainty measurement calculated from the frequency resolution of the instrument, 0.25 bpm. In particular, it was chosen considering that its value must be such that below it, there have to be only samples of the signal FHR that are not due to the physiological variability of the cardiac rhythm. Therefore, it was set in order to confine between its positive and negative value samples due to noise (related to the uncertainty of the measurement). Moreover, since the uncertainty - in RR values - due to the

frequency resolution of the recording instrument depends on FHR value (in fact: $RR = 60/FHR$), we decided to choose the primary threshold value according to the FHR mean value of the FHR signal under analysis [142, 143].

About secondary threshold, it was heuristically set to 3 ms considering that the literature attests that a good part of the differences in interbeat intervals in the foetus is less than 2, 3 or 5 ms [142, 143].

The symbolic encoding then identifies as the trend of increasing variability all samples with variability above the secondary threshold ($\Delta RR > + 3\text{ms}$ or $\Delta RR < -3\text{ms}$) and the trend of decreasing variability all samples comprised between the threshold primary and secondary ($- 3\text{ms} < \Delta RR < - \text{primary threshold}$ or $\text{primary threshold} + < \Delta RR < + 3\text{ms}$). All samples that instead fall in the range defined by the single primary threshold ($-\text{primary threshold} < \Delta RR < + \text{primary threshold}$) correspond to a substantial absence of variability.

The following table summarizes matching samples and symbols associated with them. Note that with the acronym PT we have indicated the value of the "primary threshold" calculated according FHR average and expressed in milliseconds:

Table 6.3 - Coding 5 symbols

Value of ΔRR	Symbol	Meaning
$\Delta RR > + 3 \text{ ms}$	VP	High positive change
$PT < \Delta RR < + 3 \text{ ms}$	P	Positive change
$- PT < \Delta RR < + PT$	O	Absence of change
$- 3 \text{ ms} < \Delta RR < - PT$	N	Negative change
$\Delta RR < - 3 \text{ ms}$	VN	High negative change

6.6.3 Words of symbols definition (word length) and analysis

In the generation of the words of the symbols, Symbolic Dynamics on adults makes use of words long 3, 5 or at most seven symbols. These values are related to the response times of sympatho-vagal cardiac activity [144].

The choice of the window length in the foetus is difficult because, unfortunately, probably due to the inaccessibility of the foetus, hidden in the womb, the literature is not as detailed as in the case of the adult. In any case, what emerges from physiological studies on the foetus is a strong analogy with the adult.

To vagal stimulation the foetus shows a cardiac response with rapid and short latency, while, in that sympathetic, a slow response and with greater latency [140, 141, 146]. Specifically, the response to vagal stimulation is almost immediate, whereas to the sympathetic stimulus occurs after about 2 or 3 seconds [20, 141].

Therefore, in this work, the word length (L) value was chosen equal to 7 (considering a mean foetal heart rate of 140 bpm, this value corresponds to 3 s) in order to surely include in a one word the burst peak of a sympathetic response.

Thus, a sliding window of length L was shifted along the codified series, with an overlap of L-1 points, transforming it in a sequence of patterns of L samples (called words) [154].

6.6.4 Words classification

As regards the classification of words, generated by a sliding window of 7 symbols we have chosen to use the 'criterion of dominance' summarized below [154]:

Table 6.4 - Criterion of dominance for sorting the words generated with floating window of length equal to 7

Description	Meaning	Class
At least 4 symbols "VP" or "VN"	High sympatho-vagal activation	H
At least 3 symbols "VP" and 1 symbol "P"		
At least 3 symbols "VN" and 1 symbol "N"		
At least 4 symbols "P" or "N"	Moderate sympatho-vagal Activation	M
At least 3 symbols "P" and 1 symbol "VP"		
At least 3 symbols "N" and 1 symbol "VN"		
At least 4 symbols "O"	Absence of variability	O
All other cases	Random	R

6.6.5 Variability Index

Finally, a novel variability index (V.I.) was estimated from percentages of occurrence of the different words classes (p_H , p_M , p_O and p_R), with the aim to put in evidence the amount of physiological variability of the signal at the expense of that null or random, at which we assign zero weights. It will be given by the following formula [154]:

$$\text{Variability Index} = \frac{p_H}{100} * 1 + \frac{p_M}{100} * 0.5 + \frac{p_O}{100} * 0 + \frac{p_R}{100} * 0$$

In the equation with the terms p_H , p_M , p_O and p_R we refer to the values of the percentage of occurrence of the word classes H, M, O and R. We associated different weight factors depending on the class in question, notably "1" for the high activation sympatho-vagal, "0.5" for the moderate activation and zero for the absence of variability and random words, that is not covered by the classification criterion adopted.

As last step, in order to quantify FHRV, three ranges of values were experimentally set for V.I. [154]:

- Low variability: V.I. < 0.20;
- Medium variability: $0.20 \leq \text{V.I.} \leq 0.28$;
- High variability: V.I. > 0.28.

The variability index V.I is an index that provides a summary on the overall layout of the examined. It was calculated from Symbolic Dynamics technique by assigning a weight factor that enhances the information of each class of words of histogram. It has proved capable of distinguishing the signals: low variability; average variability; high variability.

Figures 6.6 and 6.7 show examples of CTG recordings, with FHR in the upper panel and uterine contractions in the lower panel; figures 6.8 and 6.9 show the related distributions of word classes. In the CTG recording # 228 (figure. 6.6) a reassuring variability and a good reactivity of the foetus can be observed, corresponding to a V.I. value of 0.53 (high variability) and to a spontaneous delivery.

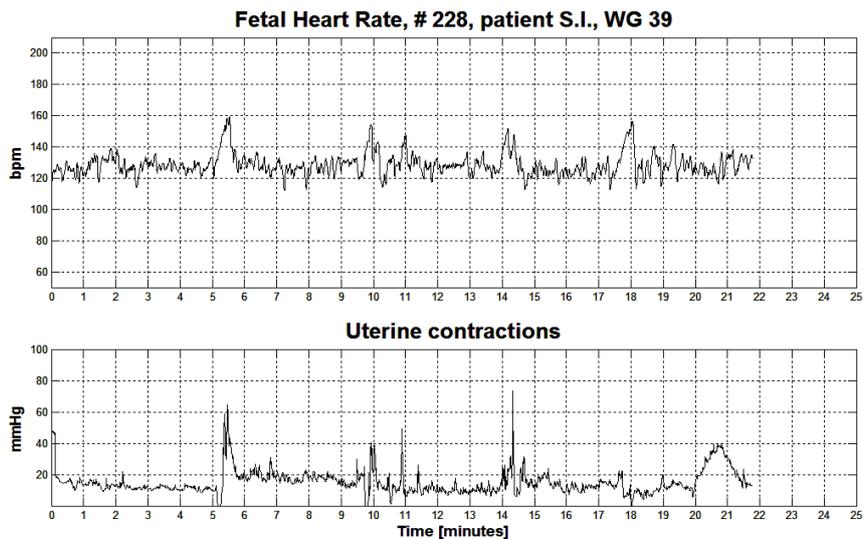


Figure 6.6 - CTG # 228 (internal numbering of our database). V.I. = 0.53

Vice versa, in CTG recording # 127 (Fig. 6.8), it is possible to note a low variability as results by a V.I. value of 0.14, despite to the presence of little accelerations. Physicians, in fact, decided in this case for a caesarean delivery.

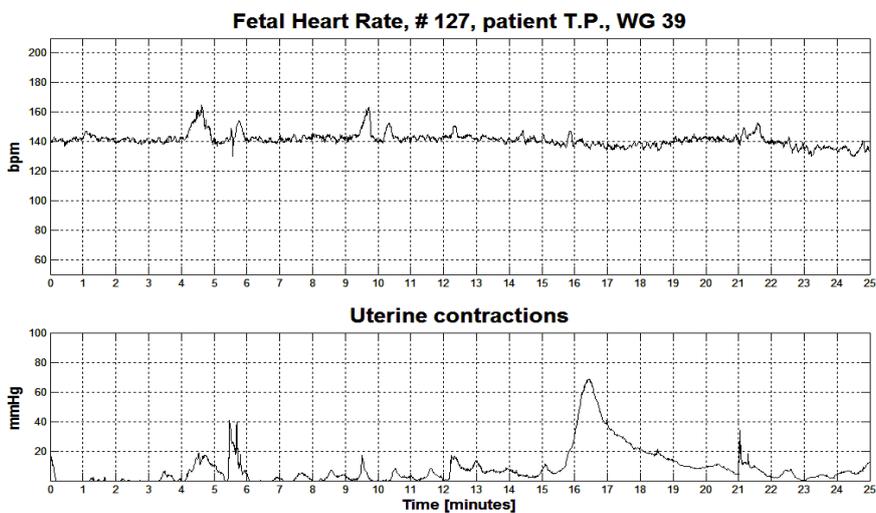


Figure 6.7 - CTG # 127(internal numbering of our database). V.I. = 0.14, CS

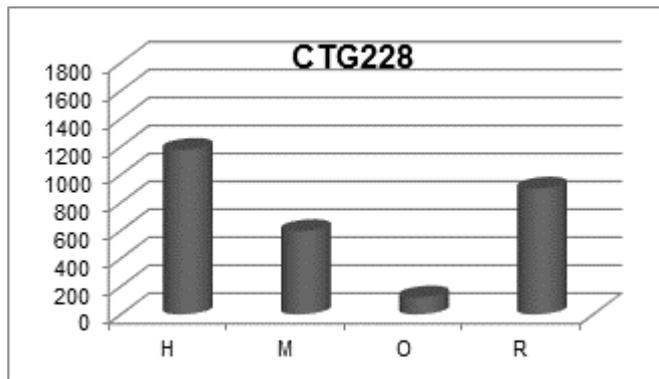


Figure 6.8 - Histogram of word classes for the CTG # 228 shown in figure 6.6

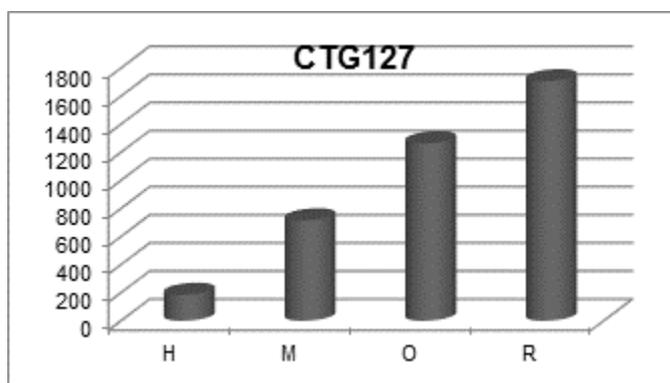


Figure 6.9 - Histogram of word classes relative to the CTG # 127 shown in figure 6.7

It is possible to observe that the occurrence of H words is more than six times less than in distribution of CTG # 228.

6.7 Statistical analysis

In order to evaluate relationships between linear and non-linear FHRV indexes and some foetal characteristics, statistical tests (t-test) and regression graphs were carried out. In particular, CTG traces were grouped according to the following parameters:

- Apgar score (high or low), which is an index of the newborn wellbeing;
- Kind of delivery (cesarean or spontaneous), as an index of a healthy foetus at term;

- Week of gestation (from 26th to 42th week), as a measure of the foetal development;
- Foetal status (active or at rest), which is an index of foetal reactivity.

On the basis of what has been discussed in the previous paragraphs, the chosen FHRV indexes for our study were the following:

Linear index in the time domain:

- Short Term Variability (STV);

Linear indexes in the frequency domain:

- Absolute and percentage power in VLF, LF, HF bands;
- Sympatho-vagal balance (SVB), equal to LF/HF power ratio.

Nonlinear indexes:

- V.I. – Symbolic Dynamics Analysis;
- SampEn – Entropy measurement;
- SD1 and SD2 – Poincarè maps.

Results of statistical tests and regression graphs will be presented in the next chapter.

CHAPTER 7

Results

7.1 Introduction

In this section of the thesis the main results obtained from the application of the methodologies described in the previous chapter are presented. In particular, as mentioned in the previous chapter, we evaluated possible relationships between FHRV indexes (linear and non-linear) and the following parameters and characteristics:

- Apgar score (high or low);
- Kind of delivery (cesarean or spontaneous);
- Week of gestation (from 26th to 42th week);
- Foetal status (active or at rest).

In particular, paragraphs from 7.3 to 7.6, after a concise description of each of the previously mentioned foetal characteristics, show the most important results achieved explaining and reporting them also by means of tables and graphs. Moreover, in order to make a comparison between the two most relevant techniques of our analysis (Symbolic Dynamics and Frequency Domain Analysis), a regression analysis between the Variability Index and the total spectral power of the FHR signal as well as the power in both LF and HF bands has been carried out and its results are illustrated and discussed.

In conclusion, a synthetic overview of the results concerning all the statistical and regression analyses carried out is presented through a summarizing table, in order to highlight the most significant values obtained and discuss from a comprehensive point of view.

7.2 Ranges of values of the chosen parameters

The following tables contains the values of the mean, standard deviation, and maximum and minimum values of the time domain index, the frequency domain parameters and the non-linear indexes (V.I., SampEn, SD1 and SD2). Since we analyzed CTG signals only related to healthy foetuses, the presented values can be considered as ranges of normality.

Table 7.1 - Range of variability of the time domain index

	STV
Mean	2, 65
SD	0, 81
Min	1, 13
Max	5, 77

Table 7.2 - Range of variability of different parameters in frequency domain

	VLF	LF	HF	P tot	SVB	VLF%	LF%	HF%
Mean	95, 45	7, 82	1, 10	104, 37	8, 63	83, 40	14, 44	2, 16
SD	198, 65	4, 88	1, 01	198, 78	3, 72	11, 67	9, 88	2, 52
Min	1, 78	0, 81	0, 11	4, 55	1, 87	30, 90	0, 18	0, 02
Max	2217, 92	31, 95	7, 31	2222, 64	24, 04	99, 79	64, 97	21, 14

Table 7.3 - Value of the mean, standard deviation, maximum and minimum value for non-linear V.I., SampEn and SD1 and SD2

	V.I.	SampEn	SD1	SD2
Mean	0, 35	0, 63	3, 51	28, 84
SD	0, 13	0, 24	1, 60	9, 56
Min	0, 06	0, 20	1, 30	9, 00
Max	0, 81	1, 67	11, 60	67, 30

Considering the great disagreement – well illustrated in the previous chapter (see paragraph 6.4.1) - about the frequency parameters definition along with the lack of literature works providing precise information about the ranges of values of other linear and non-linear parameters, the obtained values can be considered an important research achievement.

7.3 Apgar

The Apgar index is named after American anesthetist, Virginia Apgar who devised it in 1952. It is the result of some checks made immediately after birth at 1 and 5 minutes of life and aimed at assessing the viability and efficiency of the vital functions of primary. It is based on five parameters to which a score from 0 to 2 can be assigned. They are heart rate, breathing, muscle tone, reflexions and skin color. On the basis of the total score, three possible scenarios open up:

- Apgar <4: it is necessary to call the doctor;
- $4 \leq \text{Apgar} \leq 6$: indicates newborn at risk;
- $7 \leq \text{Apgar} \leq 10$: indicates normal newborn.

On the basis of this definition we have classified the CTG signals in two groups: the one with a low Apgar score ($\text{APG} \leq 6$), representing newborn at risk, and the one with normal Apgar score ($\text{APG} \geq 7$), representing healthy newborn. We considered only the Apgar score at 1 after birth.

Table 7.4 – p values for APG1. The number of CTG traces of both groups of low and normal APG1 is indicated on the first row. Time and Frequency domain parameters as well as non-linear indexes are distinguished

		APG1 – p value (Low vs Normal)
# CTG recordings		4 vs 274
Time Domain	STV	0, 356
Frequency Domain	VLF	0, 154
	LF	0, 440
	HF	0, 183
	SVB	0, 181
	VLF%	0, 277
	LF%	0, 271
	HF%	0, 394
Non-linear Indexes	V.I.	0, 304
	SampEn	0, 164
	SD1 (Poincarè)	0, 069
	SD2 (Poincarè)	0, 287

As far as the Time and the Frequency Domain analyses are concerned, after a comparison between time parameter (STV) along with spectral power indexes (absolute and percentage power in VLF, LF and HF band as well as sympatho-vagal balance) and Apgar scores, the obtained results lead us to conclude that there is significant correlation neither between time domain measures and Apgar score nor between the spectral power of the FHR signal and the Apgar score. Similar considerations are valid also for the non-linear parameters.

With regards to the Symbolic Dynamics, the association between V.I. and APG1 and APG5 has been also assessed, in a previous work [153], splitting data into three groups, regarding both Apgar score's values at birth. Normality distribution of V.I. index for all groups has been assessed by D'Agostino & Pearson omnibus normality test ($\alpha=0.05$) and difference between groups has been assessed by an unpaired one-way analysis of variance followed by Tukey's multiple comparison post-test between each groups' couples.

Apgar values ranged from 7 to 9 for APG1 and from 8 to 10 for APG5. Most of CTG recordings corresponded to an APG1 score value of 8, improving to 10 for the APG5 score value. V.I. showed a gaussian distribution in all groups ($p>0.05$) and a significant p value ($p<0.005$) of ANOVA between all the three studied groups for both APG1 and APG5. Higher V.I. values of antepartum CTG recordings are associated to early greater vitality at birth quantified by APG1 score. Particularly, Tukey's post-tests for APG1 revealed a significantly different mean V.I. values discriminating antepartum CTG recordings corresponding to APG1=7 vs. 9 and APG1=8 vs. 9. This behaviour is confirmed by the recovery at five minute after birth. Higher V.I. values of antepartum CTG recordings are associated to late greater vitality and vital primary functions' efficiency at birth quantified by APG5 index. Particularly, Tukey's post-tests for APG5 revealed a significantly different mean V.I. values discriminating antepartum CTG recordings corresponding to APG5=8 vs. 10.

Table 7.5 - Association between APG and VI (* for $p < 0.05$; ** for $p < 0.005$; ns for not significant)

	APG1			APG5		
APG value	7	8	9	8	9	10
# CTG recordings	8	44	8	8	16	36
VI index (mean±std)	0,19±0,07	0,20±0,05	0,27±0,02	0,22±0,08	0,25±0,05	0,29±0,07
D'Agostino norm. test	0,33	0,12	0,76	0,53	0,96	0,18
ANOVA	p=0,0028**			p=0,0072**		
Tukey's post-test	7 vs 8 (ns)	7 vs 9*	7 vs 8 (ns)	7 vs 9*	7 vs 8 (ns)	7 vs 9*

7.4 Kind of delivery

Type of delivery (cesarean or spontaneous): was considered because it is supposed to represent the state of healthy foetal term pregnancy and is particularly important because doctors can get information to guide him in the choice, t-tests were carried out on a large number of FHRV using different indices.

To test the association between V.I. and kind of delivery (delivery analysis), we split data in two groups: spontaneous delivery (SD) and caesarean section (CS) and, then, performed a t-test to find the best indexes allowing a distinction between the two groups.

Table 7.6 - *p* values for kind of delivery. The number of CTG traces of both groups of spontaneous deliveries and cesarean sections is indicated on the first row. Time and Frequency domain parameters as well as non-linear indexes are distinguished

		Kind of Delivery - <i>p</i> value (Cesarean vs Spontaneous)
# CTG recordings		97 vs 237
Time Domain	STV	0,467
Frequency Domain	VLF	0,034
	LF	0,462
	HF	0,496
	SVB	0,280
	VLF%	0,007
	LF%	0,010
	HF%	0,011
Non-linear Indexes	V.I.	< 0,001
	SampEn	0,003
	SD1 (Poincarè)	0,290
	SD2 (Poincarè)	0,145

As results of our analysis, only the Frequency Domain and SampEn allow a significant separation between the two groups. In particular the percentage power in all the three frequency bands of the FHRV offers a relevant distinction between a spontaneous delivery and a cesarean section.

With regards to the Symbolic Dynamics, the associations between V.I. and type of delivery had been also evaluated, in a previous work [154], with a Kolmogorov-Smirnov test, since the CS distribution resulted not normal.

Table 7.7 - Spontaneous and caesarean values for V.I. and p value

	<i>Spontaneous</i>	<i>Caesarean</i>
# CTG recordings	97	237
V.I. index (mean)	0,32	0,27
V.I. index (standard deviation)	0,09	0,07
V.I. index (standard error of the mean)	0,009	0,005
D'Agostino norm. test (p value)	0,07	0,005
Kolmogorov-Smirnov (p value)	< 0,001	

The Kolmogorov_Smirnov test revealed that V.I. values (computed for SD and CS groups) are not drawn from the same population ($p < 0.001$). In particular higher V.I. values of antepartum CTG recordings are associated to the set of spontaneous deliveries.

From the above showed table it can be state that using the VI it is possible to discriminate the type of birth; in particular, the readings of V.I. higher in correspondence of parts spontaneous. Distributions of average occurrences of the word classes obtained for the two set of CTG signals, corresponding to caesarean sections (237 recordings) and spontaneous deliveries (97 recordings), are shown in figures 7.1 and 7.2 respectively.

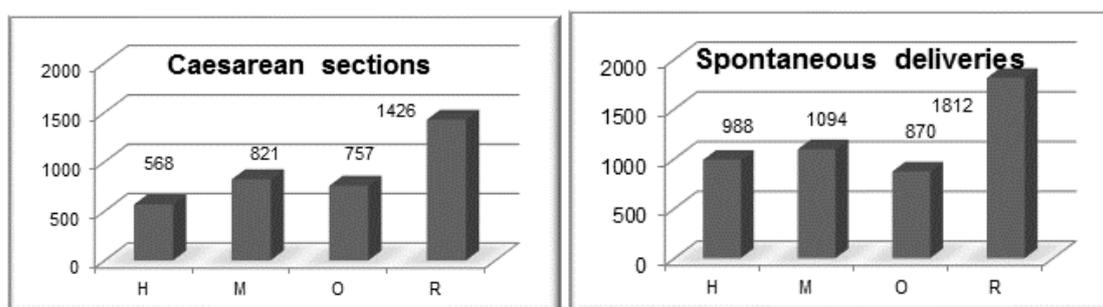


Figure 7.1 - Distribution of average occurrences of WC computed for CS (on the left) and for SD (on the right)

Following are presented some boxplots, which are been calculated to evaluate the ability of V.I. to discriminate CTG signals by a spontaneous or by a caesarian delivery.

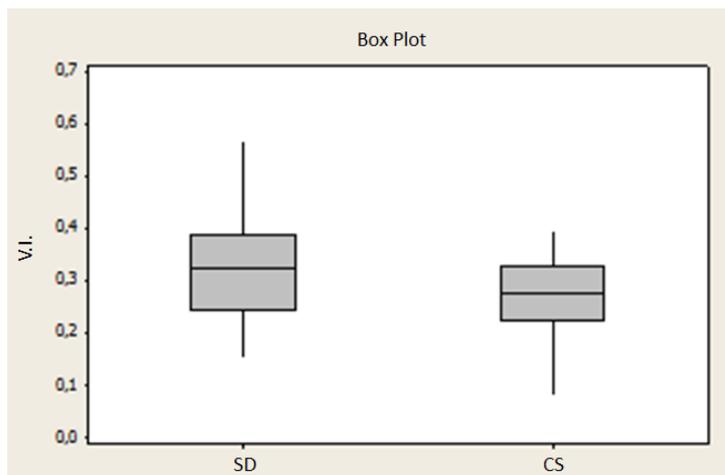


Figure 7.2 - Box-and-whisker plots of V.I. values for spontaneous deliveries (SD) and caesarean sections (CS).

7.5 Week of gestation

Week of gestation (WG) is the simplest index of development and, therefore, of foetal well-being.

7.5.1 Time domain parameters

The following figure shows the average values of Short Term Variability (STV) trend in relation to the Week of Gestation, indicator of foetal development and, therefore, of foetal well-being.

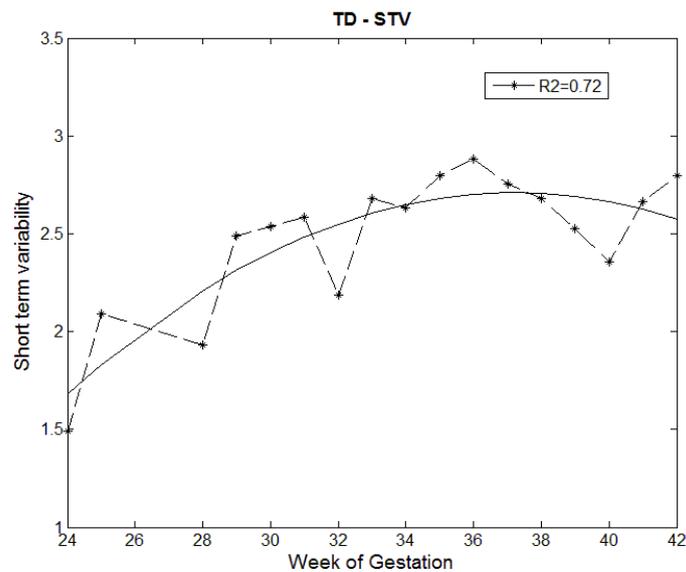


Figure 7.3 - Short Term Variability in relation to Week of Gestation

As regards the classification of weeks of gestation, the coefficient calculated (R^2) showed a good reliability in the time domain with the STV index (R^2 equal to 0.72).

7.5.2 Frequency domain parameters

The regression analysis was also carried out for estimating the relationships among the power mean values and the gestational week. Three regression analyses were done, one for each frequency band. The three regression graphs plot the power mean value (along y axis) vs the gestational week (along x axis). A second order polynomial regression equation was used to define the trend line and for each graph the coefficient of determination (R^2) was computed. The mean power values in each band were correlated, by means of a regression analysis, to the gestational week in order to study their variations with foetal development. Figures from 7.4 to 7.7 show regression graphs with trend line equations and coefficients of determination (R^2).

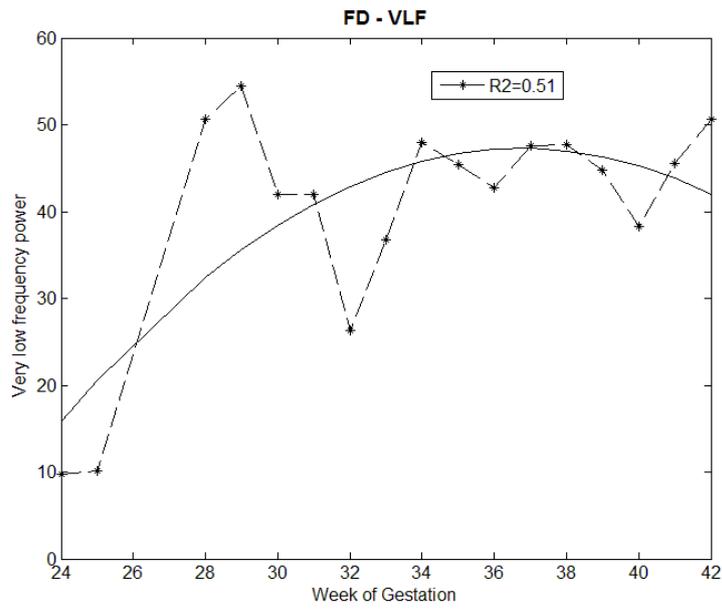


Figure 7.4 - Regression graph with trend line equation and R2 relating the power mean value in VLF (0-0.03) Hz to the gestational week

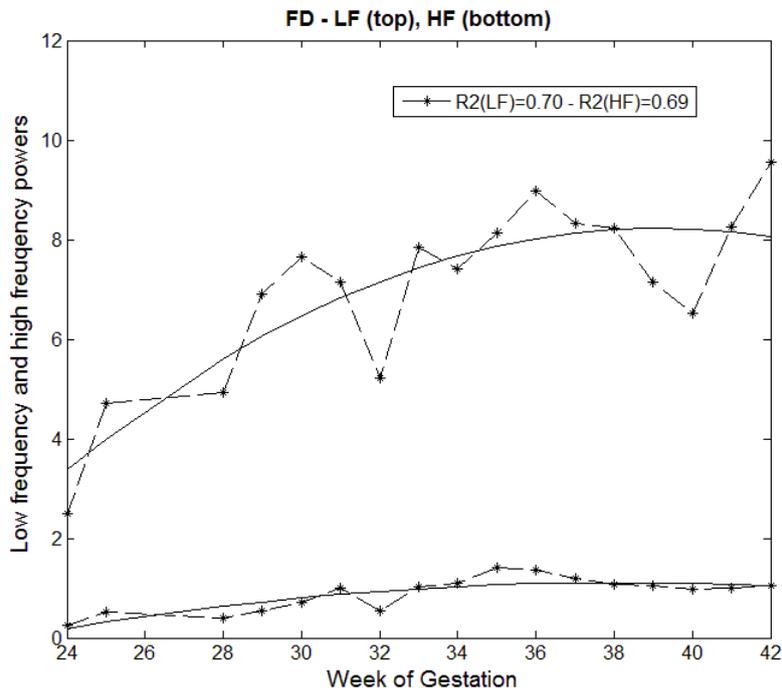


Figure 7.5 - Regression graph with trend line equation and R2 relating the power mean value in LF and HF to the gestational week

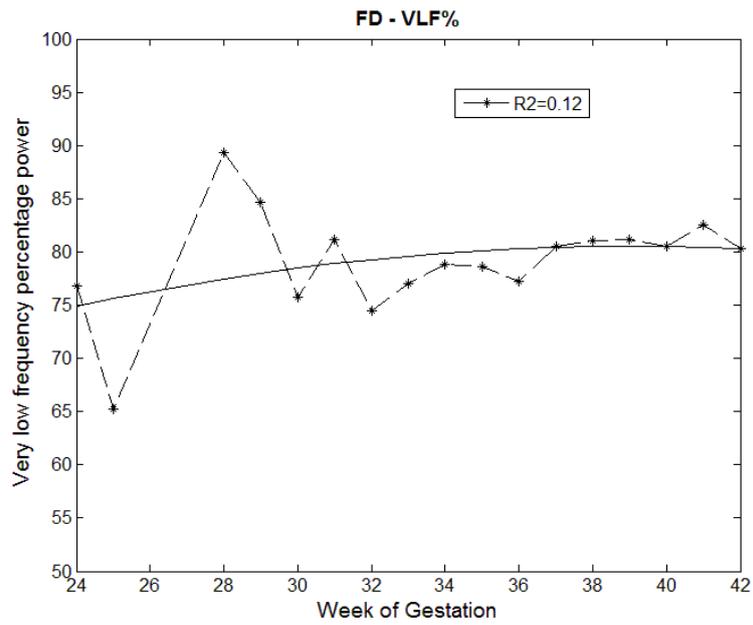


Figure 7.6 - Regression graph with trend line equation and R2 relating the percentage power value in VLF to the gestational week

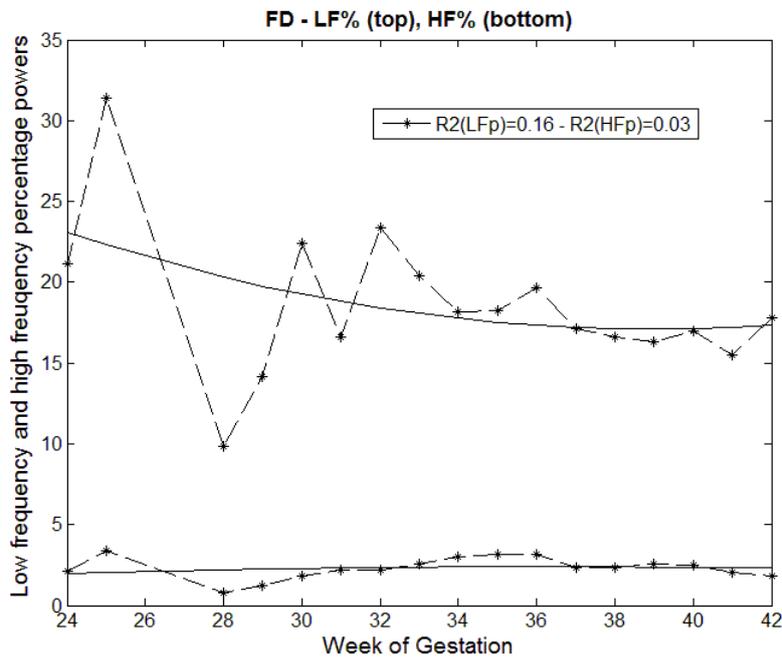


Figure 7.7 - Regression graph with trend line equation and R2 relating the percentage power value in LF and HF to the gestational week

The analysis showed that the highest mean power values correspond to the LF and the HF bands. However R2 value is slightly lower for the correlation with HF power;

this result is not surprising since it is known that, in the foetus, HF band has characteristics very variable and often is not present [125].

Concerning LF power, the R2 obtained is the highest and also greater than values found in literature [128], confirming in this way that the power can be a useful index of the changes which occur during the pregnancy.

This result also confirms that an efficient pre-processing has to be employed before any FHRV analysis is carried out. In fact, the better fit found here suggests that the PSD computed by the authors should be scarcely affected by errors [138, 139].

To sum up, the analysis of the trends confirms that, according to literature, power increases in the course of pregnancy [125, 127] but the increment decreases in the late weeks of pregnancy. In particular, the trend line obtained for the LF is comparable to literature [128].

7.5.3 Non-linear indexes

Regression graphs for non-linear indexes are showed below:

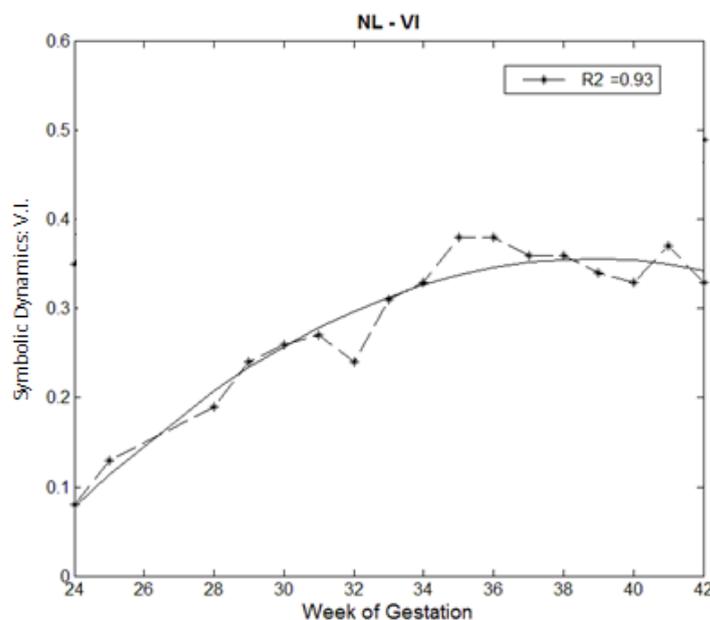


Figure 7.8 - Regression graph with trend line equation and R2 relating the V.I. to the gestational week

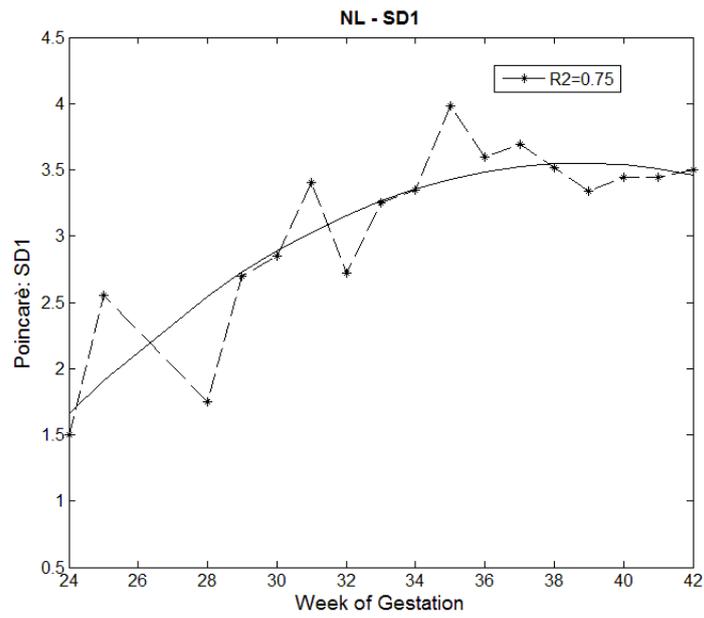


Figure 7.9 - Regression graph with trend line equation and R^2 relating the Poincaré SD1 to the gestational week

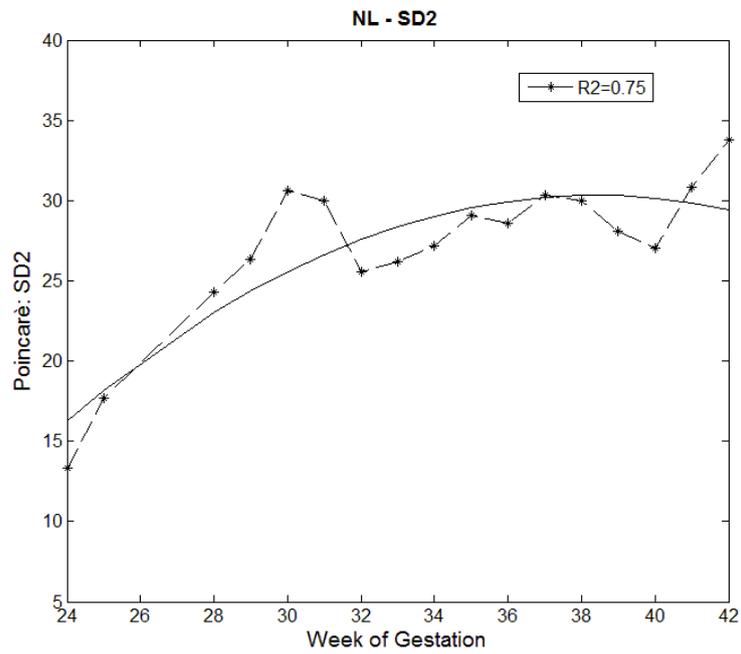


Figure 7.10 - Regression graph with trend line equation and R^2 relating the Poincaré SD2 to the gestational week

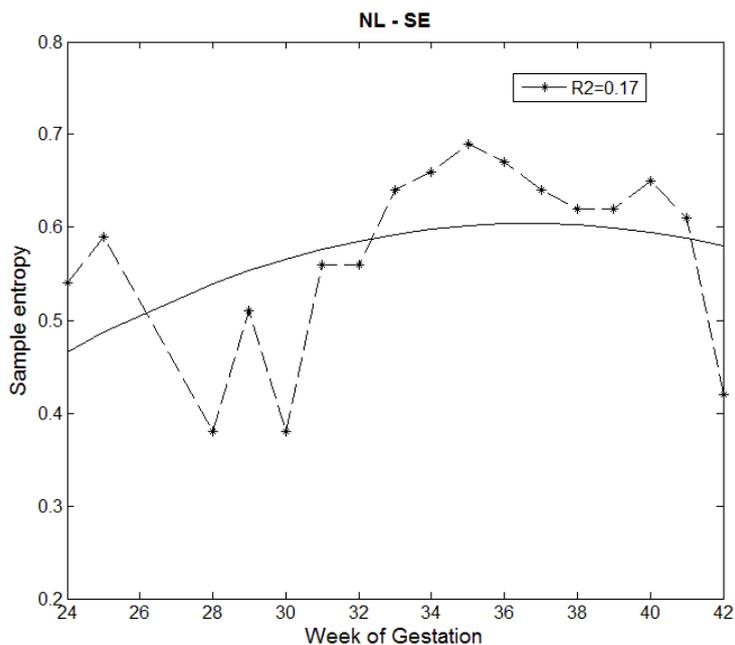


Figure 7.11 - Regression graph with trend line equation and R2 Sample Entropy to the gestational week

With the exception of the SampEn, all the regression curves show a high regression coefficient (R2). In particular, the results obtained with the SDA (Variability Index – R2 = 0.93) lead us to conclude that the VI, growing so evident with the pregnancy progresses, may be a good indicator of foetal development. Similarly to the LF trend line, as the pregnancy progresses, the average V.I in the LF band grows according to an equation of quadratic polynomial regression until the 36th to 37th week at which it has a maximum of the trend curve; then tends to stabilize.

In the following table we summarize all the coefficients (R2) obtained from the regression analysis between gestation weeks and both linear and non-linear computed FHRV indexes.

Table 7.8 – Regression coefficients for all the computed parameters

		<i>Regression coefficient (R2)</i>
Time Domain	STV	0.72
Frequency Domain	VLF	0.51
	LF	0.70
	HF	0.69
	VLF%	0.12
	LF%	0.16
	HF%	0, 03
Non-linear Indexes	V.I.	0.93
	SampEn	0.17
	SD1	0.75
	SD2	0.75

In conclusion, the regression coefficients (R2) that showed the best fitting with the data are:

- The STV index, equal to 0.72, as time domain index;
- The LF absolute spectral power, equal to 0.70, as frequency domain parameter;
- The Variability Index, equal to 0.93, as parameter from nonlinear analysis.

It is important to highlight that these results, with particular regard to the V.I. and the Poincarè indexes, represent an absolutely new insight in non-linear FHRV analysis.

7.6 Foetal status

Foetal intrauterine behaviour is not stable but it consists of continuous alternation of states characterized by significant changes in foetal motility, heart rate, hemodynamics, metabolism and response to stimulation [148]. Characteristic behavioural states do exist for the human foetus. These states have been called 1F to 4F and resemble states in the neonate. States 1F and 2F are similar to non-REM sleep or quiet sleep and REM sleep or active sleep respectively. The foetus spends most of the time in these two states.

Behavioural states are defined as combinations of physiological and behavioural variables, repeatedly recurring, not only in the same subject [148]. In particular, each state can be characterized by a particular combination of 3 variables: presence or absence of foetal eye movements and body movements, and FHR patterns. From about 36 weeks these combinations can be recognized during longer periods without interruptions, and with clear state transition. The four foetal behavioural states were defined as follows [149]:

- State 1F: quiescence, which may be regularly interrupted by brief gross body movements. Eye movements absent. Stable FHR pattern, with a narrow oscillation bandwidth. Isolated accelerations occur, strictly related to movements.
- State 2F: frequent and periodic gross body movements, mostly stretches and retroflexions, and movements of the extremities. Eye movements continually present. The FHR shows a wider oscillation bandwidth with frequent accelerations in association with movements.
- State 3F: gross body movements absent, and eye movements continually present. The FHR is stable, but has a wider oscillation bandwidth than in state 1F and a more regular oscillation frequency than in state 2F. No accelerations.
- State 4F: vigorous, continual activity with many trunk rotations. Eye movements present. The FHR pattern is unstable, showing large and long-lasting accelerations, often fused into sustained tachycardia.

It is possible to classify foetuses in relation to: active or resting state. It is important to emphasize that these states are clearly established only near term, by about 36 week of gestation [148, 150]. The conditions that allows dividing a foetus in an active or in a rest state are described below:

Rest is characterized by: low variability and absence of marked accelerations, while Activity is characterized by: good variability and reactivity, signal responsive (at least two accelerations every 20 min - automatic classification) and normal variability (≥ 5 bpm - automatic classification). The rest-activating classification is particularly important because it is highly regarded in the literature that signals of a foetus at rest "resemble" those pathological and, therefore, this analysis can be

preliminary to the healthy-medical condition which, at the time, was not carried out for lack of data.

Considering from the 30th week of gestation, we can define:

- “Resting state” characterized by low variability and absence of marked accelerations (visual grading);
- “Active State” characterized by good variability and reactivity (according to visual grading), signal responsive (at least 2, accelerations every 20 minutes automatic classification), normal variability (≥ 5 bpm, automatic classification).

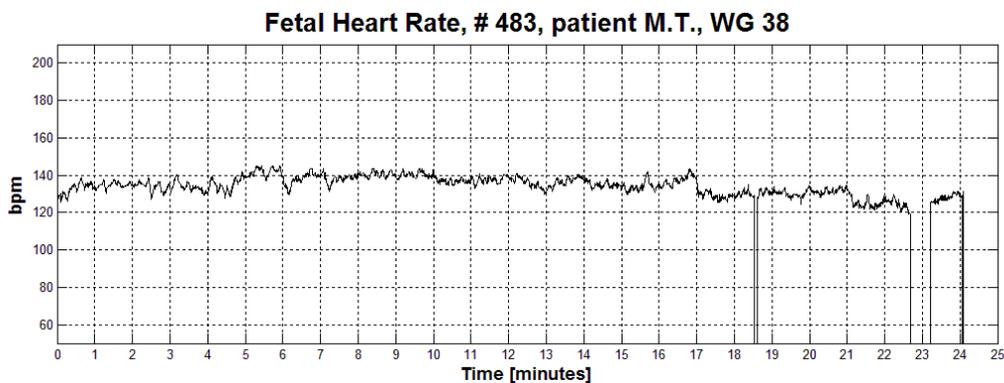


Figure 7.12 - Example of FHR recorded from a foetus at rest

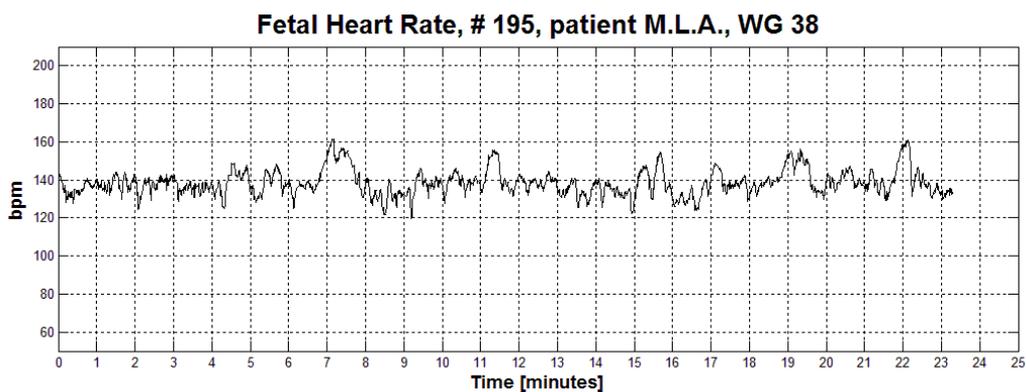


Figure 7.13 - Example of FHR recorded from a foetus in an active state

To test the association between V.I. and foetal status, we split data in two groups according to the above described definitions: fetuses at rest (Rest) and active fetuses

(Active). Then we performed a t-test to find the best indexes allowing a distinction between the two groups.

Table 7.9 - p values for APG1. The number of CTG traces of both groups of low and high APG1 is indicated on the first row. Time and Frequency domain parameters as well as non-linear indexes are distinguished

		<i>Foetal status - p value (Rest vs Active)</i>
# CTG		55 vs 384
Time Domain	STV	6, 20E-23
Frequency Domain	VLF	1, 03E-23
	LF	2, 02E-17
	HF	5, 21E-16
	SVB	1, 92E-08
	VLF%	0, 357
	LF%	1, 52E-06
	HF%	1, 69E-06
Non-linear Indexes	V.I.	0, 0008
	SampEn	5, 72E-18
	SD1 (Poincarè)	0, 0004
	SD2 (Poincarè)	4, 77E-09

As showed in the above table, the index STV, along with the V.I. and the SD2, were the most reliable to distinguish foetuses at rest than in the waking state. However, with the exception of the SVB, all the computed parameters seem to be excellent (very low p value) to distinguish an active or resting foetus.

7.7 Comparison between Variability Index and frequency parameters

The relationship between V.I. and frequency parameters is shown in the following figures [155]:

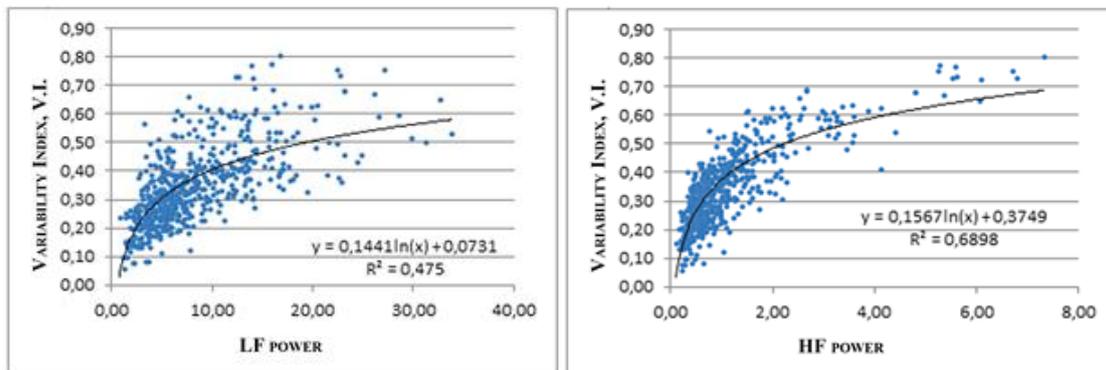


Figure 7.14 – Variability Index and as a function of LF (left) and HF power (right)

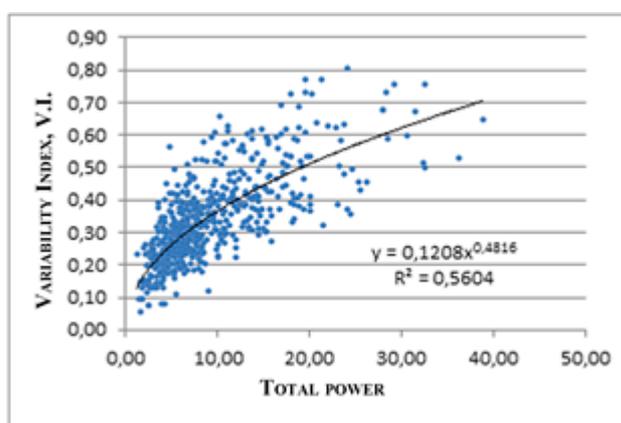


Figure 7.15 - Variability Index and its relationship with total FHR spectral power

Curves in figures 7.14 and 7.15 show that the Symbolic Dynamics index (V.I.) increases in correspondence of higher values of the power parameters. V.I. may then be considered also as an indicator of signal power.

However, the correlation between V.I. and frequency parameters is stronger for HF. This observation highlights that, for providing at least a rough explanation, the computation of the ΔRR series corresponds to perform a high-pass filtering of the signal and, hence, an underestimation of the low frequencies.

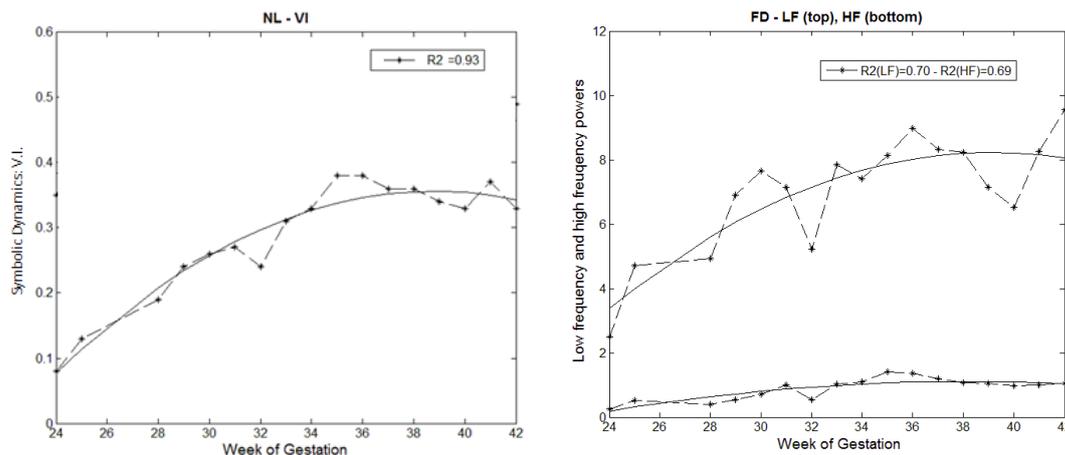


Figure 7.16 - Variability Index (left) and LF and HF power (right) as function of week of gestation

Particularly interesting are results shown in figure 7.16, which represent the regression graphs reported in figures 7.5 and 7.8 and here presented again in order to make a comparison. Focusing on the V.I. and the LF power trend curves, it can be observed that both curves have a trend comparable with literature result [128], for the analysed gestation weeks, but with higher R2 values. This coefficient computed for the V.I. is far higher than frequency parameters' one (LF power).

In summary, the obtained results, also considered together with the previous one, indicate that Symbolic Dynamics, as much as Frequency Domain Analysis, could be a helpful tool in foetal monitoring.

Moreover, considering the presented regression analyses (shown in paragraphs 7.5 and 7.7), we can conclude that indexes derived from Symbolic Dynamics resulted, on average, a more reliable indicator of foetal development during the course of pregnancy not only than the frequency parameters, but also than all the other evaluated parameters.

7.8 Summary table of the statistical results

The following table presents all the results obtained carrying out the statistical t-test to evaluate, for each computed FHRV parameters, the capability of discerning different foetal characteristics.

Table 7.10 - Statistical test (t-test) for Apgar1 (Low vs Normal), Kind of Delivery (Cesarean vs Spontaneous) and Foetal Status (Rest vs Active)

		Apgar1 <i>p value</i> <i>(Low vs Normal)</i>	Kind of Delivery <i>p value</i> <i>(Cesarean vs Spontaneous)</i>	Foetal status <i>p value</i> <i>(Rest vs Active)</i>
# CTG		4 vs 274	97 vs 237	55 vs 384
Time Domain	STV	0,356	0,467	6,20E-23
Frequency Domain	VLF	0,154	0,034	2,02E-17
	LF	0,440	0,462	5,21E-16
	HF	0,183	0,496	1,92E-08
	SVB	0,181	0,280	0,357
	VLF%	0,277	0,007	1,52E-06
	LF%	0,271	0,010	1,69E-06
Non-linear Indexes	HF%	0,394	0,011	0,0008
	V.I.	0,304	< 0,001	5,72E-18
	SampEn	0,164	0,003	0,0004
	SD1	0,069	0,290	4,77E-09
	SD2	0,287	0,145	2,95E-28

The first evidence is that none of the computed linear and non-linear parameters allow differentiating the Apgar score.

As far as the linear parameters are concerned, all of them show significant p values to represent the foetal status, with the only exception of the SVB. In the specific case of the delivery, only the spectral power percentages are significant to differentiate the two types of delivery.

As we can note in the previous table, each non-linear index that is reported into the table is significant to distinguish the foetal status. In the specific case of the delivery, only the SampEn index is significant to differentiate the two types of delivery.

CONCLUSIONS

Regardless of its limitations and the high number of false positives, nowadays the Cardiotocography (CTG) is still the most widespread foetal monitoring technique, having legal value in Italy and in some other countries [2]. On this basis, researchers and scientists keep on studying to support physicians and improve the CTG traces interpretation.

This thesis has the main aim of applying a non-linear technique, Symbolic Dynamics Analysis (SDA), to the foetal heart rate variability analysis. A multiparametric approach to analyze the FHRV, which includes the use of indexes originated from Symbolic Dynamics, in addition to more traditional ones, for example from Time and Frequency Domain Analysis and from other nonlinear methods such as Poincarè maps and Sample Entropy, is a possible interesting way to validate the SDA usefulness and capabilities and improve the evaluation of the foetal development and distress.

An in-depth literature research proved to be necessary for identifying the most used and reliable techniques and parameters for HRV and, in particular, FHRV analysis.

Moreover, further essential tools have been developed, updated or simply used to carry out the proposed study. Among these it is worth highlight the following tools:

- A software for CTG analysis, conveniently updated from a previous version in order to implement the different chosen and employed methods (Time Domain Analysis, Frequency Domain Analysis, Symbolic Dynamics Analysis, Poicarè plots and Sample Entropy).
- A software for CTG simulation, developed to support and validate the CTG analysis.

From the literature review it has been shown that, despite their recognized values, none of the investigated methods can be considered a “gold standard” in the FHRV analysis. This is also true for the time and frequency domain methodologies. Thus, it can be concluded that there is still not a unique standardized method to analyze

CONCLUSIONS

FHRV as well as there is still not a shared and confirmed definition of the FHRV itself. On the basis of what has been highlighted, this work proposed a definition and characterization of the FHRV and some methods to analyze it by means of the most reliable techniques emerged from the literature review.

In particular, regarding the time domain analysis the computation of the Short Term Variability Index has been considered the most suitable and reliable choice due to its recognized usefulness as valid support to the diagnosis of foetal health. As far as the frequency domain analysis, the spectral power has been evaluated through the Short Time Fourier Transform after an in-depth bibliographic study to define frequency band values of the FHRV.

With regards to the non-linear techniques, our study seems to confirm that, despite the good results achieved, there is not a single non-linear method or index that stands out among the others or that give better analysis results.

On the basis of these considerations, we focused our study on the application of Symbolic Dynamics Analysis, already applied with positive results to the analysis of HRV of the adult, to the foetus. Since its simple logic of implementation and its preliminary promising results, the SDA allowed us to define a new index of heart rate variability that could be useful for clinicians in foetal monitoring and wellbeing assessing.

In this work 580 antepartum recordings of healthy foetuses from the 24th to the 42th gestation week were examined. CTG traces were recorded by healthy patients during the clinical practice, using commercially available cardiotocographs. The database was completed with other clinical information of patients and newborns. CTG signals were processed using the developed and updated software previously mentioned. Finally, statistical tests and regression analysis were performed for estimating the relationships among indexes extracted from the adopted methodologies of FHRV analysis and other clinical data, such as Apgar score (low or normal), kind of delivery (cesarean or spontaneous), week of gestation (from the 24th and the 42th) and foetal status (active or at rest).

The obtained results confirm that:

CONCLUSIONS

- None of the chosen indexes and employed techniques is more suitable or reliable than the others. Differently, each one should be used along with the others, complementing them in order to improve the FHRV evaluation.
- In agreement with the literature, each implemented analysis should take into account two relevant parameters for the foetal monitoring, i.e. the foetal status (active or at rest) and the week of gestation.

As far as the Symbolic Dynamics is concerned, results confirm, on one hand, its usefulness and promising capabilities in the FHRV analysis. In fact, it allows recognizing foetal status and - in some cases - the kind of delivery and it is strongly correlated with the gestation week and, therefore, with the foetal development, showing a correlation coefficient far higher than the one calculated for the other parameters. On the other hand, further studies are necessary to establish and definitively confirm the reliability of this parameter. In particular, the study should be extended by including the analysis of pathological cases in order to compare the reliability of linear and non-linear parameters in distinguishing healthy from non-healthy foetuses.

For sake of simplicity, the following table summarizes the goals that have been achieved or not with this work:

Table 7.11 – Objectives of the thesis achieved or not

Goal	Description	
Developing new tools	Two software have been developed: <ul style="list-style-type: none"> • A software for CTG analysis, which is effective and useful to process and elaborate CTG traces; • A software for CTG traces simulation, which is a helpful testing tool. 	✓
Gathering new data	No possibilities to extend the dataset.	✗
Time Domain Analysis – method and results	The previously developed methodology confirmed its efficacy in foetal health assessment.	✓
Frequency Domain Analysis – method	A new definition of the FHRV has been established and a method of estimation has been developed.	✓
Frequency Domain Analysis – results	No possibilities to standardize spectral power measurements (too high variability of the spectral power percentages).	✗
Non-linear analysis – method	The bibliographic research revealed none of the chosen indexes and employed techniques is more suitable or reliable than the others.	✗
Non-linear analysis – results	Ranges of variability in physiologic conditions were defined for each of the computed indexes. The computed indexes proved to be good indicators of foetal health.	✓
Symbolic Dynamics Analysis – method	The Symbolic Dynamics has been applied to the foetal HRV following the main works on adult subjects.	✓
Symbolic Dynamics Analysis – results	Satisfying results have been achieved with the new computed index (Variability Index) from the Symbolic Dynamics Analysis	✓
Identifying the best predictor of foetal health	No possibilities to identify a parameter that can significantly improve CTG specificity. To this aim, pathologic CTG traces should be considered and further analyses should be carried out.	✗

APPENDIX A

Non-linear methods for HRV and FHRV analysis: further details

A.1 Principal Dynamic Models

The Principal Dynamic Models (PDM) method is another method nonlinear which was introduced first by Marmarelis. The estimate of the PDM is obtained using the Volterra series-Wiener, in discret time. The report output-input of a nonlinear system dynamic invariant time is described by the Volterra series:

$$y(n) = k_0 + \sum_{m=0}^{M-1} k_1(m)x(n-m) + \sum_{m_1=0}^{M-1} \sum_{m_2=0}^{M-1} k_2(m_1, m_2)x(n-m_1)x(n-m_2) + \dots$$

where $x(n)$ is the 'input, $y(n)$ is the' system output and M is the memory of the system. The Volterra series (k_0, k_1, \dots) Describes the dynamics of the system as a hierarchy of nonlinear systems [A-1].

The method PDM is based on the principle that among all the possible choices of the bases of expansion there are some that require a minimum number of bases to achieve a given approximation of the mean square value of the system output. In literature Y. Zhong [A-1] uses this method to separate the activities of the sympathetic and parasympathetic nerve.

During this study, data were collected from nine healthy subjects between 19 and 38 years of age and the data consisted of simultaneous recordings of electrocardiogram of surface ECG and instantaneous changes in blood pressure and venous blood. The data were recorded for 13 minutes in the supine position is that in lying position. With administration of different drugs, and antopine propranololo, were found significant decreases the amplitude of the wavelengths.

It was also observed the elimination of these dynamics when both drugs are administered to the subject. This non-linear method has the advantage of allowing a clear separation of the two autonomous nerve activity.

A.2 The Lyapunov Exponent

Lyapunov exponents are useful to classify the asymptotic behavior of the orbits of a dynamic system, and thus can be used to determine the stability of quasi-periodic and chaotic regimes, as well as that of the equilibrium points and periodic solutions of a given field vector. The sensitive dependence on initial conditions is one of the features highlighted in the definitions of deterministic chaos more accepted. It manifests itself in a flow in which the trajectories on the attractor have at least one direction of the exponential divergence: in such a situation the ability to forecast the change of the dynamic system is rapidly lost. In other words, small differences in the initial conditions (the limit undetectable) will amplify enormously to produce orbits completely different (unrelated to the limit). Any dynamic system, whose attractor has at least one positive Lyapunov exponent, is defined to be chaotic, and the numeric value of the exponent provides a precise indication on the scale of the time after which the system dynamics become unpredictable [A-2]. Lyapunov exponents can be defined both for continuous, that for discrete systems, and they define as many as the size of the physical space (or phase space), which describes the system in question. Recall that Lyapunov exponents measure the rate of divergence of initially close trajectories in the phase space, and the positivity of these parameters is an indication of the presence of deterministic chaos signal [A-3, A-4, A-5].

A.3 Hypothesis tests based on surrogate data

A surrogate is a dataset artificially generated by modifying some features of the original series. Usually, the surrogate data is constructed so as to preserve the linear properties of the original signal. If the difference between the results obtained from the original signal and those obtained from the series surrogate is statistically significant, one cannot exclude the hypothesis that the original series is generated by a stochastic process [A-6]. Of course, the effectiveness of the surrogate data depends on the choice of surrogates themselves, which should be made on the basis of the algorithm used to perform nonlinear analysis. In order to preserve the dynamic properties of each series and their interaction, one can resort to surrogate data that have the same spectral and cross-spectral original data. This operation can be carried out by the procedure of randomization of the phases:

- the two series are Fourier transformed;
- generates a random number uniformly distributed between 0 and 2π , which is added to both phases of the original Fourier transforms of the two series in order to preserve the difference (cross-spectrum);
- the two series are then anti-transformed.

The comparison of the results obtained with the original data and the surrogate data, the hypothesis of a stochastic dynamics may be rejected. The technique of the surrogate data is commonly used in combination with other non-linear methods: method fractal, fractal and multi method by entropy or complexity represented by the fractal dimensions. In this method there is an output from a linear system with input in a white Gaussian noise [A-6, A-7].

APPENDIX B

Non-linear methods for HRV and FHRV analysis: literature details

B.1 Summary table – HRV analysis: literature review

Ref	Scholar	Techniques	Notes	# Citations
[B-1]	M. Morse, G.A. Hedlund	Mathematical study		950
[B-4]	Makikallio	Degree of complexity with correlation dimension		20
		Lyapunov's exponents		
		Kolmogorov's entropy		
		Fractal properties		
		Law gradient power		
		DFA		
[B-5]	A. Voss et al.	Study on nonlinear methods		117
[B-6]	P.Guillen	Lyapunov's exponents	Positive Lyapunov exponent indicates a significant dependence of the initial conditions and is consideratun relevant index for the presence of chaos.	1
		Symbolic Dynamic	The sequence of symbols is estimated using the algorithm proposed by Mrowka	

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-11]	J.A.Palazzolo	ApEn and Symbolic Dynamic	The study was carried out on dogs.	48
[B-12]	S. Lau et al.	Discussion on methods nonlinear entropic	Applications for patients with myocardial infarction.	
[B-13]	F. Valencia	Entropy rate	In the present study the factor NYHA was useful to identify 'high risk of cardiac death.	10
[B-14]	N.Wassel et al.	ApEn	Useful in the prediction of atrial fibrillation. Presents advantages as the insensitivity to noise and the use in series of short time	0
[B-16]	Goldberger	Fractal dimension	General discussion of chaos theory and fractal dimension	39
[B-18]	Lake et al.	Sample and Approximate Entropy	The entropy sampling unlike ApEn shows good characteristics as the independence of the data length and the implementation without problems.	3
[B-21]	Voss et al.	Maps of Poincaré	Methods of nonlinear dynamic. The time series have been created through the resolution of 'differential equation Roessler.	275
		Sym Dyn		
[B-22]	D'Addio et al.	Maps of Poincaré	The analysis does not require Poincare normal distributions or stationary.	0
		Symbolic Dynamic		
[B-23]	D'Addio et al.	Symbolic Analysis	In this study were analyzed 24 hours Holter recordings	11
		Pattern Construction		
[B-24]	Yeragani et al.	fractal dimensions	Most of these studies used data arising from linear measurements as the standard deviation SD for assessing the variability in the time series.	40
		Approximate entropy ApEn		
[B-25]	Wessel et al.	Symbolic Dynamic	The combined use of different indexes can improve the identification of potential arrhythmias	104
		Shannon's entropy		

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

		Growth rates in finite time	imminent. Limit of this type of study concerns the reduced number of time series, and then the reduced statistical analysis possible.	
[B-26]	D'addio et al.	Maps of Poincaré Sym Dyn	The results in this study were analyzed by ANOVA test	0
[B-27]	A.Porta et al.	Analysis of irreversibility	In this study were analyzed 24 hours Holter recordings	0
[B-28]	A.Porta et al.	Analysis of irreversibility	The usefulness of this method even when applied under experimental conditions uncontrolled	21
[B-29]	Wajid Aziz Loun	Treatment of non-linear methods and measures of complexity		
[B-30]	Cammarota	Symbolic Dynamics		5
[B-31]	S.Guzzetti et al.	Symbolic Dynamic	It is estimated an increase of sympathetic or parasympathetic modulation through the study of the data collected.	93
[B-32]	J. Kurths	Symbolic Dynamic	Data were collected from patients who have found low risks with traditional investigative methods.	249
[B-33]	R.Maestri	Symbolic Dynamic	The indices of SymDyn are not associated with the activation of the sympathetic system.	1
[B-34]	M.Vallverdù et al.	Hidden Markov models	Were analyzed two different types and two different length of HMM structures, in the same way were considered different lengths of the RR series.	4
		Symbolic Dynamic	It has been considered an alphabet of four symbols {1, 2, 3, 4} to codify the standard RR	
[B-36]	J.J.Zebrowski et al.	Lyapunov's exponents	KS entropy is used only for purely deterministic systems.	
		Entropy of Kolmogorov – Sinai		
[B-37]	Akselrod	Power Spectrum	Study carried out in 1981.	0

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

	Mandelbrot	Monofractal surrogate data	The term fractal was coined by Mandelbrot to describe a fragmented geometric shape that can be split into parts.	
[B-38]	Spilka	Analysis of 'fetal electrocardiogram		0
[B-39]	Porta et al.	Coarse Graining and Pattern Construction Entropy of Shannon Surrogate data	The rate of entropy of the complexity of short-term HRV is designed to decrease the risk stratification and to predict cardiac death of patients with ischemic dilated cardiomyopathy.	123
[B-40]	Theiler et al.	Surrogate data	Numerous scholars have compared the results obtained from this study with their methods using non-linear indexes.	1827
[B-41]	Parlitz et al.	Symbolic Dynamic	The results were analyzed for short stories intervallic (5 minutes) and longer intervals.	19
[B-42]	Cysarz et al.	Approximate entropy ApEn	In this study were differentiated results obtained during the day and at night.	22
[B-43]	Meyerfeldt et al.	Growth rates in finite time	The data analyzed were collected from patients with a high cardiac risk, it is shown that circadian variations did not significantly influence the 'analysis.	31
[B-44]	M.G. Frasch	Review		5
[B-45]	D'Addio et al.	Mappe di Poincarè	The study population was extracted from Non Linear Time Series Analysis	3
[B-46]	Steffen Schulz	Review	Cardiovascular and cardiorespiratory analysis.	5
[B-47]	Y.Zhong	Fourier Power spectral density	Method for recognizing the presence of interactions between the nervous system and the parasympathetic nervous sympathetic system	0
[B-48]	S.Guzzetti	Exponents of Lyapunov	Application of methods for the non-linear noise reduction.	0

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-49]	M.G.Signorini	fractal dimension Exponents of Lyapunov	The parameters found in this study can be obtained at the same time by 'analysis of the classification of various diseases.	6
[B-50]	K.L.Kalon	Approximate entropy DFA	For each recording was made a division into segments in portions of 15 minutes	
[B-51]	G.Magenes	Approximate entropy and DFA	Data derived from a vibro-acoustic stimulations that are able to produce a response in fetuses.	
[B-52]	D.Cysarz	Approximate entropy	The symbolic binary is give a clearer interpretation of the regularity of the HRV.	49
[B-53]	M.Costa	multiscale entropy	MSE is able to distinguish the difference in complexity due to age and to heart attack.	19
[B-54]	K.Phyllis	DFA1 Maps of Poincarè Slope of the power law	In this study we demonstrate how the combination of several non-linear methods may be optimal for assessing the risk of stratification.	29
[B-55]	M.P.Tarvainen	Power spectral density Fourier transform	Components arising from respiratory sinus arrhythmias are separated from the other components of HRV through the variation of some parameters.	251
[B-56]	A.Porta	Fourier transform	Changes in the complexity of short-term HRV are induct from the modification of experimental conditions.	56
[B-57]	N.Wassel	Symbolic Dynamic	The best way to analyze the data is to calculate complex physiological parameters in the time domain and frequency as well as the parameters that describe the dynamics in the time series.	8
[B-58]	N.J.Dabanloo	Zeemann's model	This model also has the ability to accurately simulate important diseases associated with autonomous HR regularity.	1

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-59]	Y.Zhong	PDM Principal Dynamic model	This approach has allowed a clear separation of the two activities autonomous nerve, sympathetic and parasympathetic.	50
[B-60]	C.Shanthi	PDFA Principal dynamic fluctuation analysis	The results show that the linear analysis does not distinguish the signals fluctuation NSRDB and SDDB and their control activities.	0
[B-61]	H.Ding	Entropy Pattern and VNDP	The significant increase in the discriminating power, compared to that of conventional RQA analysis, shows that the analysis VNDP can quantify the dynamics of nonlinear and non-stationary.	14
[B-90]	V.Magagnin	Symbolic Dynamics and Conditional entropy	The data were obtained from Holter recordings obtained by making recordings during daily activities	26
[B-91]	J.S.Perkiomaki	Measurements in the time domain	General discussion on the evaluation of HRV	7
[B-92]	F.C.Pivatelli	ApEn SD SDNN	The procedures used were approved by the Ethics Committee in Research	12
[B-93]	Y. Shiau	DFA	General discussion on the evaluation of HRV with DFA methodology	2
[B-94]	M.G. Signorini	DFA ApEn SampEn MSE	Data were obtained through ECG recordings	3
[B-96]	C.K. Karmakar	Measurement of complex correlation CCM	The CCM method was used to distinguish between Poincare plot with the same shape	11

B.2 Summary table – FHRV analysis: literature review

Ref	Scholar	Techniques	Notes	# Citations
[B-2]	P.van Leeuwen	ApEn	ApML If this exponent is smaller than 1, the trajectories converge, or diverge.	21
		ApD1		
		approximation ceiling Lyapunov's Exponents ApML		
		Speed of the divergence of the trajectory ρ		
[B-7]	P.Van Leeuwen et al.	ApML	The strength of this study was to combine the methods of recording and CTG FEC to examine changes in heart period variability during pregnancy	77
		ApEn		
		ApD1		
[B-8]	D.G.Chafflin et al.	Reconstruction phase-space	Dimensional analysis was performed using the method of Grassberger and Procaccia	39
[B-9]	D. Borserio et al.	Correlation dimension	In that study was not carried out any assumption of linearity.	1
[B-10]	Di Rienzo et al.	Fractal Dimension	FHRV The data were obtained through the process of Dawes-Redman.	22
[B-15]	V.Baier et al.	HMMs	The observation of the sequence was generated by the transformation of the RR interval and dale time series resulting from systolic blood pressure using the SymDyn	6
[B-17]	P.A.Hopkins et al.	Fractal Dimension	The fractal dimension appears to be the most suitable technique for fetal investigations than any other nonlinear technology such as Lyapunov exponents or size correlational for evaluating the fact that smaller datasets it has a better resolution in time.	0
[B-19]	M.Ferrario et al.	Multiscalar Entropy MSE	In this work the techniques have not proved suitable to distinguish fetuses sick and healthy fetuses.	74
		ApEn		
		Sample Entropy SempEn		

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-38]	Spilka	Analysis of fetal electrocardiogram	Dealing generic valid also for studying adult	
[B-44]	M.G. Frasch	Review	Nonlinear modulation properties vagal and sympathetic.	5
[B-62]	G. Magenes	Review	Analysis of fetal HRV	15
[B-63]	U.Schneider	SD	The data were obtained through the MCG	16
[B-64]	D.Cysarz	ApEn	If you can show the nonlinear component and reproducible be stable in normal pregnancies, the calculation of the strength of the component can be useful for identifying pathological conditions.	10
[B-65]	M.Ferrario	Complexity of Lampel Ziv ApEn SampEn	The complexity of Lampel Ziv is a stable parameter, and is capable of discriminating IUGR severe to moderate and those from healthy fetuses.	17
[B-66]	G.Magenes	ApEn Detrended Fluctuation Analysis	In this work we focused on the division of fetuses with IUGR through a multiparametric analysis based on recordings CTG.	7
[B-67]	M.G.Signorini	ApEn PSD	Implementation of a new system of clinical classification for early diagnosis of the most common fetal pathologies.	117
[B-68]	H.Goncalves	ApEn SampEn	The indices are not significantly different in the linear initial segments.	42
[B-69]	P.Van Leeuwen	ApEn Dati Surrogati	The increased complexity of FHRV during pregnancy can be attributed to the time structure nonlinear and irregular.	0
[B-70]	H.Goncalves	ApEn SampEn	Linear and nonlinear indices are evaluated in each segment.	31
[B-71]	J.Bernardes	ApEn SampEn	The results obtained were processed going to compare male and female.	25
[B-72]	P.Hopkins	SampEn HMM Shannon Entropy	In this study different techniques are used to check their discriminatory power of the various patterns.	0

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-73]	V.Baier	HMMs	The observation is a probabilistic function of a state that is not observable but which can be observed through a 'another set of stochastic processes.	6
[B-74]	M.Ferrario	MSE Complessità di Lampel Ziv	The results show that the complexity of Lampel Ziv and MSE can be useful for identifying the 'current IUGRs and to separate them from healthy fetuses.	29
[B-75]	D.Hoyer	Shannon Entropy	Objective of this work is to write fetal maturation based on indexes of autonomic modulation of HRV.	17
[B-76]	N.S.Padhye	DFA MSE	Both measurements have shown That the fetus is Subjected to a change in the autonomic nervous system controls That the HRV from the 26-th to 30-th week.	1
[B-77]	H.Shono	PSD	FFT has been applied to individual sequences	12
[B-78]	M.Akay	Matching Pursuits	The present study shows the advantages of the method Matching Pursuits 's signal analysis FHR in' time-frequency analysis	11
[B-79]	H.Shono	PSD	It used the Fourier transform to analyze such data	18
[B-80]	G.Morren	DFA	It was analyzed the DFA method to evaluate the behavior of the series of neonatal RR intervals.	3
[B-81]	J.C.Echeverria	DFA	Using DFA there has been a long-term HRV in fetal around 24 weeks of gestation normal.	8
[B-82]	U.C.Lee	UTBE	Through this method it is possible to compare the entropy data without any ambiguity due to the non-stationary.	0
[B-83]	D.M.Mooney	Poincaré's Maps	The observations were carried out for an interval of time ranging from 3 to 5 minutes.	8
[B-84]	J.Kalda	CD	Dealing generic valid also for studying adult - Analysis of fetal HRV	

APPENDIX B: Non-linear methods for HRV and FHRV analysis: literature details

[B-85]	J.C. Echeverria	EMD	Essentially EMD is able to perform a general separation of the original signal components in non-overlapping temporal scale	
[B-86]	G. Magenes	Contributions linear and nonlinear	Dealing generic valid also for studying adult - Analysis of fetal HRV	15
[B-87]	J. Lim	Apen SampEn	One of the disadvantages of Apen is to depend on the number of the input sequence	0
[B-88]	Young-Sun Park	Complessità di Lampel -Ziv ApEn SampEn	There were no differences in fHRV registered	2
[B-89]	E. Moraes	ApEn SampEn	The variability of the parameters are useful for differentiating between states of stillness and activity states.	2
[B-95]	P.Van Leeuwen	Apen SDNN	It found a greater influence in cardiovascular regulation by measuring Apen	5

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