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The development of motor activity:
observing spontaneous behavior in fetuses, preterm and term infants

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Angela Valente

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Introduction

Recently, the observation of spontaneous motor behavior from the beginning of prenatal life has been always more interesting for students and scientists. This is caused by the newborn's skills and abilities that he proves to have just after birth, and it is assumed they emerge during prenatal life. In the Seventies the advent of the new ultrasound technologies made possible the observation of human fetal behavior and also the increasing of knowledge of embryo and fetus that has opened a new window to observe human development. Nowadays, ultrasound examination helps to promote the concept of continuity in human motor behavior from prenatal to postnatal life.

The main aim of this study is to observe spontaneous motor behavior from the beginning of movement *in utero*. Human motor behavior is related to the normal function of central nervous system and these observations can detect early, or even before birth, the integrity of central nervous system (Prechtl, 1997). It is also important to note that presently there is not a neurobehavioral scale universally accepted and recognized as valid for the observation of spontaneous fetal behavior yet (Di Pietro, 2005).

Spontaneous behavior is an activity endogenously generated by the central nervous system which reflects the state of neural development. As behavior is sensitive to changes in physiological and motivational functions, the study of spontaneous motor activity allows to understand the evolutionary trajectories of specific functions, and therefore, to identify atypical paths as well as newborn well-being. Therefore, it is possible to note that atypical motor patterns, characterized by monotonous, stereotyped and less fluent movements, may predict adverse neurological outcomes.

Over the past thirty years, even the way to observe and evaluate infant behavior has changed. Until the Sixties, newborn was considered a passive receptor unable to respond to environmental stimuli, and his assessment was predominantly made by observations of neurological reflexes. Presently, newborns prove to play an active role thanks also to their behavioral repertoire already showed after birth. The modern medicine challenge is especially in the field of developmental techniques of life support and these are allowing survival of fetuses aged even less than 28 weeks of gestational age. Since preterm newborn was not able to spend the last trimester of pregnancy *in utero*, he is called "premature" for his structural conditions of immaturity. Therefore, it could be interesting to study behavior in fetuses and their typical developmental trajectories to better understand how this development can occur in cases of premature births.

During our first experiment in order to describe behavior both before and after birth, we are going to code spontaneous motor patterns in a group of fetuses and to compare to a group of preterm infants, at the same gestational age. To specify each pattern we developed a new coding scale predominantly made on anatomical basis. We considered as a starting point the micro-analytic facial coding system for babies and children Baby FACS, written by Harriet Oster (2007). This new motor patterns scale was designed for the detection of specific complex movements coming from the behavioral categories originally described by de Vries (1982), Prechtl (1985), Kurjak et al. (2003), Einspieler et al. (2008) and Wolff (1987), and observed in fetuses, preterm and term neonates. Therefore, we described in detail 22 complex motor patterns as much objective as possible, in order to ensure reliability regardless of the subjective interpretations of individual encoders. Our interest comes from the recent opportunity to observe spontaneous behavior in preterm infants at a very low gestational age, and it also comes from the opportunity to discover fetal behavior. The observation of spontaneous motor activity will help us to understand the ontogeny of human behavior, but it will also help us on the way to the evaluation of the activity of central nervous system and human well-being.

In order to describe spontaneous behavior both before and after birth, we are going to dedicate the first experiment to code spontaneous motor activity in a group of fetuses and preterm infants at the same gestational age.

The theory of dynamical systems in the study of motor development emphasizes that changes in spontaneous motor pattern can be determined by changes in any factor subject to interaction, such as biomechanics growth, functional, motivational or cognitive changes (Kamm, Thelen& Jensen, 1990). According to this perspective, we can hypothesize that the fulfillment of a basic need, as a motivational factor, can influence the manifestation of behaviors. Therefore there could be a relationship between the onset of the condition of appetite and spontaneous behavior. In the second experiment we are going to test the same spontaneous motor activity coding scale already used before. Our intention in this second experiment is to test the sensitivity of this scale, coding two groups of preterm newborns video-recorded before and after meal. We wonder how the preterm behavior may be modulated by the necessity to satisfy the appetite basic need and what are the differences emerging from a comparison in spontaneous behavior observed before and after meal.

The second experiment is concerned with the observation of spontaneous motor behavior in premature infants in order to investigate how the primary condition of appetite can modulate the behavioral performance. We are going to observe preterm newborns behavior through the observation of video recordings of two groups of premature infantsrecorded before and after the meal.

The medical-technology advances led to an improvement of care and an increase of the indices of neonatal survival (Fava Vizziello, 1992). Today, they can ensure survival for infants at less than 400 grams and a gestational age of 24 weeks. Preterm birth is a quite common phenomenon. According to the latest OMS "Born too soon: the global action report on preterm birth," published on May 2012, every year 15 million children born premature, with a ratio of more than 1 in 10 preterm births, showing an alarming increase in cases in the last 20 years in almost all countries surveyed by OMS experts. Unequal cases were detected according to the different geographical areas analyzed, and it is found that more than 60% of premature births occurs in Africa and South Asia, and this variability also affects the countries of the North. In the United States of America there are 12% of infants born prematurely at a mean ratio of 9% of high-income countries, and 7% in Italy (World Health Organizations, Born too soon, the global Action Report On Preterm Birth, 2012). Despite the survival of preterm infants, the quality of that survival is certainly improved thanks to the advances in care of preterm infants obtained in the last twenty years with the application of new knowledge of neonatal pathophysiology, technology monitoring of vital functions, the new possibilities for respiratory care, parenteral feeding and other modern diagnostic and therapeutic techniques.Among the survivors, the effects of premature birth can occur throughout the entire life and they can influence the neuro-functional development. The arrangement of the premature is different from a full-term newborn, where the focus is on the interaction with the family, relatives and friends. In case of preterm birth instead the focus is on health, on cardiac, respiratory, and brain functions. The premature baby is immediately accepted as a "different"child.

In the light of the last considerations in both previous studies, during the third and last experiment we are going to test the new motor coding scale for the observation of spontaneous behavior in a group of full-term newborn infants and to compare to a group of preterm newborn infants at the same gestational age. The last intention is to underline the sensitivity of this scale in revealing differences between preterm and full-term newborns. After the assessment of the actual sensitivity of the motor patterns scale specifically designed for the detection of spontaneous behavior, we are now wondering how the preterm newborn’s spontaneous behavior may differ from the full-term newborn behavior observed at the same gestational age.

The collected material was used in the respect to the privacy (in accordance to the Legislative Decree no. N. 196 of June 30, 2003 "Code concerning the protection of personal data").

Part I Origins and behavior

1. The origins of motor behavior

ἧτοῦ δυνάμει ὄντος ἐντελεχεία ή τοιοῦ τον κινησις ἐστίν

“The actuality of being in power, as a being in power, is the movement”

Aristotele, *Fisica*, III (G), 1, 201a10-11.

In the systematic thesis of movement and change (Phys. III 1-3) and the different types of them (De Gen. et corr. I 1-5), Aristotle takes up and develops themes that emerged in the survey on the principles. Nature is the principle of movement and change and do not know what movement is would, therefore, ignore what nature is, but, on the other hand, there is no movement outside of the things that are (pragmata) in motion, because there is nothing outside of these, then: to know what movement is you have to investigate what and how many ways they are and are said to be things that are.

Each of these things can then be in power or act (entelecheia).

The movement is the act of what is in potency itself. The generation and corruption of a substance, which is the coming to be of something that was not, and his cease to be while before it was, constitutes what may be called for Aristotle generation and corruption in an absolute sense, but this does not imply a generated by or corrupted in what absolutely is not.

Finally dismissing the old Eleatic aporia (according to Parmenides, in fact, being is and is not possible that it is not; non-being is not and it is necessary it is not), Aristotle says (De Gen. et corr. 3 I, 317 a 32-b 33) that even what is not, anyway is: it is in power, not in place. That 's why we can say that man is born from man, things are created by something that is in place (which is a substance), but that is in power the same that arises from it, nor it could be in power if it had not in place.

1.1. Historical background

The term “quickening” is the moment of pregnancy in which a woman first feels fetal movements and they have been attributed to the beginning of individual life. This is the criterion historically regarded to determine the starting point which it is conceded to the fetus the right to life.

Hippocrates (460-370 a. C.) had already suspected that fetal movements may set a few weeks earlier than expectant mother feels them, around 70-90 days after conception which correspond to a gestational age of 12-15 weeks.

Rebecca, Isaac's wife, in the Bible is probably the first written account of human fetal movement (Luke 1:39-44)[1] .

Actually, it is enough interesting to notice that charting the mothers' perception of fetal motion is the oldest and simplest method to monitor fetal well-being during the second half of pregnancy.

The modern age contribution to embryology comes from Leonardo Da Vinci, with his famous drawings of a fetus in a womb (1510-1512), where we can see the illustration of embryos and fetuses, according to the ability discovered for the first time the real measure and assess dimension and development related to the observation in all the different gestational ages. Leonardo's anatomical drawings, discovered in 1900, should have been collected in a treaty never completed and left to his assistant Francesco Melzi, after the author's death. In 1690 Charles II bought them and remained in the Royal Collection. At that time, dissection was not forbidden by the Church and the artist inspired by Avicenna and Galeno di Pergamo studied and the discoveries made in hospital and medical schools. during early dissections of human bodies. The beautiful studies of human skull and fetus give us the greatness of his ability and interest in neurology and represent the first and the most detailed testimony of human's anatomy[2].

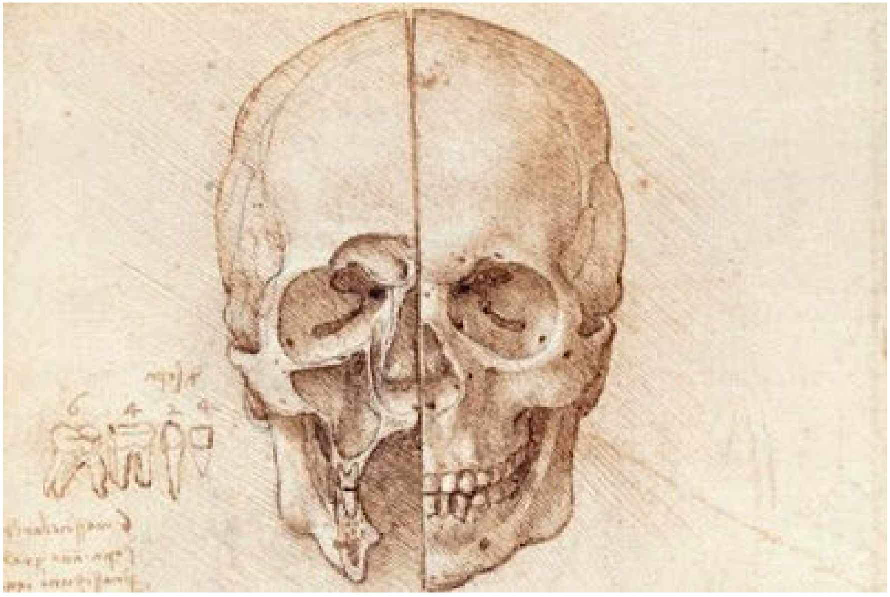

Figure 1. Leonardo Da Vinci – Studies of the skull

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[1] "Now at this time Mary…entered house of Zacharias and greeted Elizabeth. And it came about that when Elizabeth heard Mary's greeting, the baby leaped in her womb;…and she cried out with a loud voice…when the sound of your greeting reached my ear, the baby leaped in my womb for joy" (Luke 1:39-44).

[2] From Royal Collection, Buckingham Palace.

Figure 2. Leonardo Da Vinci – Embryological Drawings of the fetus

In 1885 the English-German physiologist William T. Preyer (1841-1897) “heard” the fetal movement placing a stethoscope on a mother’s abdomen and noticed that movements were definitely present around 12 week of gestational age or even earlier. At that time scientists were convinced that these movements had to be evoked, but in spite speculation of that period, Preyer believed those early movements were spontaneously generated.

First non-invasive study of fetal behavior was conducted by Fels Longitudinal Study (the Fels research Institute was founded in 1929) originally designed to analyse the effects of the “Great Depression Time” child had development, posing the question: “what makes people different?”, derived from the study of individuals from prenatal life to adulthood. Then Sontag and Wallace (1934) were able to differentiate between slow squirming or writhing movements, which increased until 28 week of gestational age and then decreased. They assumed there is a great differnce among fetuses as well as s difference in fetus himself as he changes day by day (Sontag 1941), and realized first classification of fetal movements as kicking and punching, which decreased with the fetus growing and with the age, and rare, small rhythmic movements and possibly hiccups. This study also confirmed that maternal emotional stress is linked to fetal behavior as those infants remained irritable and hyperactive for weeks, cried a great deal and slept for short periods (Sontag 1941).

Through the perspective of the reflexology doctrine that considered the motility a merely response to exogeneous stimuli, we can classify other systematic studies on human fetal motility carried out after spontaneous miscarriages or after Cesarean sections. Examination of exteriorized embryos and fetuses were conducted by Hooker (1938, 1952) and Minkowsky (1928), and at the same time Coghill (1929), they applied tactile stimulations on amphibians embryos and fetuses, Barcroft et al. (1940) on sheep and Windle and Becker (1940) on cats. According to these studies there were two different thesis: Coghill believed that reflex mass movements preceded individual reflex movements, while Windle (1940) suggested that complex coordinated movements develop by integration of local reflex circuits, according to the reflex-integration approach to reflexology of 1930s and 1940s.

It is due to Hooker Davenport the beginning of study on human fetal activity (in 1932 at the University of Pittsburgh) that he was influenced by previous studies conducted on animals and summarized by George E. Coghill. Although for those studies motility was considered to be evoked, it was common to observe movements without any evidence of prior stimulation and to classify them as "spontaneous reflex which the stimulus of was not known yet" (Hooker, 1952). It is also important to notice that these observations were conducted on dying fetuses, so presumably the nervous system was in a depressed state and activity almost ceased (de Vries et al. 1982; Prechtl 1989).

The use of B-mode linear scanner allowed the Viennese obstetrician Emil Reinold in 1971 to study fetal movements obtaining first sonar observation of cross-sections through uterus, although not yet simultaneously. He described two types of movements: a lively movement beginning with a short impulse and ending with a motionless phase and a slow and lazy one, generally followed by a pause of 1-5 minutes (Reinold, 1973). He stated fetal movements were not forceful enough to alter position of fetal body before 10th weeks of gestational age, and for the first time he was able to concluded that observed movements of the fetus were spontaneous rather than caused by external influences (Reinold, 1973). By these earliest reports, Birnholz was influenced and he made a categorization scheme of fetal motion with a particular attention to the extension of the head or limbs relative to the trunk, the rotation of the torso and to independent limbs movements, combined/repetitive and respiratory movements (Birnholz et al. 1978).

After the advent of new ultrasound scanners it has been possible to see simultaneously fetal movements in uterus. These new technologies allowed to determine correlation between fetal movements and the correspondent age. Having a great number of recorded images per second that can provide real-time recordings, some obstetricians such as Ianniruberto and Tajani in 1981, de Vries et al. in 1982, first of all have carried out examinations to evaluate the onset of motility and behavior in collaboration with neuropaediatrician Adriano Milano Comparetti and the developmental neurologist Heinz Prechtl. Real time observation opened a view to a new kind of studies in human behavior as well as to the possibility of neurological assessment in human fetus (de Vries et al 1982, 1985, 1988, Nijhuis et al. 1982).

High resolution in real scanning images became a new tool to observe movement patterns, underlying differences between quantitative and qualitative analysis, as a basis to understand condition and assessment of nervous system. It was due to the Groningen's study founded by Prechtl, the beginning of investigation with few very important shared questions that we can summarize as follow: how should fetal movement patterns be classified? At what age do they appear at the first time? Do they change in the course of intrauterine development? Are there any age-related preferences with regard to the fetal position? Are there any specific motor patterns which are responsible for changes in the fetal position? (Prechtl and Schleidt 1950, Prechtl 1953, 1958, 1977, Prechtl et al 1979). It was surprising to assume that all the observed patterns in uterus were repeated after birth in neonates (Prechtl 1984, 1985, 1989, 2001).

1.2. Ontogeny of human behavior

At the beginning, the study of human nervous system has been investigated in early human fetuses at the operating table. The reactions of many human fetuses older than 9 weeks of gestational age have been studied both by Minkowski (1928) and Hooker (1939). First reflexes are established by 8 ½ or 9 weeks, when the human nervous muscle system have attained degrees of maturity compatible with functioning before 8 weeks g.a. (Windle and Fitzgerald, 1937). In this study, James E. Fitzgerald and William F. Windle have had occasion to observe 15 fetuses of 7 weeks to a little over 8 weeks of gestation age under various operating conditions. They supposed that it was probable that the infrequent observation of early human fetal movements were related to the use of general anesthesia and narcotics as well as to the rapid onset of fetal anoxia during the usual surgical procedures. The three fetuses which did react were observed at operations performances under spinal cord anesthesia. No preoperative medication, other than 1|120 grains of atropine sulphate was given to the patients and no pituitrin was used and continuous motion picture records of the experiments performed at the operating table were made. Tapping upon the amniotic sac induced no movements of the fetus and no responses were obtained after touching and stroking the body and limbs with a needle passed through the amniotic sac. When the regions of the mouth and the nose were strongly stimulated with a needle, a contralateral flexion of the trunk appeared. When they tried to touch strongly the region of the entirely face, the whole of the trunk musculature contracted and the arms and legs moved with the body at their attachment. Homolateral responses predominated but contralateral movements were observed, too. At no time there were no movements elicited touching the limbs or the trunk; otherwise movements elicited touching the whole region of the face last for more than 3 minutes.

This lateral movement of the head and trunk, following stimulation of particular parts of the face supplied by the trigeminal nerve, principally maxillary division, has been reported by Hooker (1939) after removing from uterus.

These experimental observations were interpreted by the concept of a stage in development during the eight weeks of gestational age compatible with a limited degree of function These movements are essentially of reflex nature and although if we cannot say what receptors has been stimulated, it is possible to see that some receptors or synapses are more easily rendered non-functional than others: a stimulus applied to the face may induce a trunk flexion reflex or, if strong enough, a mass movement of trunk, arms and legs. So they were able to say that after afferent connections involved in local responses cease functioning, some of the muscle units which were first activated can still be activated by adequate stimulation of the other afferent elements, as we can see for the face (James E. Fitzgerald and William F. Windle, 1942).

Human behavior has been studied since the early origin in prenatal life by Fitzgerald & Windle (1942) and Hooker (1952, 1954, 1958) thought the observation of the human embryo abilities of movement since the 7.5 weeks of menstrual age. They found that by the age of 14the weeks of menstrual age the fetus is able of a wide variety of reflex movements. Cinematographic pictures have been used by Hooker and have been widely used by psychologists and various authors even if for the most part of fetal reflexes were elicitated by stimulation of the cutaneous surfaces by touching or stroking with a needle or a plume.

The first reflexes observed by Hooker (1944, 1952, 1958) were obtained by stimulation of areas supplied by trigerminal nerve and were comparable to the total pattern movements described by Coghill (1929) in amphibians. He found that when the extremities developed they first moved in conjunction with the trunk and only later acted independently. The first response to sensory trigerminal stimulation in development of human fetal reflexes is the early total pattern type of activity. Hooker defined these reflexes as stereotyped and he found that repetition of the stimulus elicited the same response. He also found that local reflexes following trigerminal stimulation begin to appear by 9.5 weeks and as they increase in number the total pattern type of activity begins to disappear and is seen only as anoxia begins to suppress reflex activity (Hooker, 1952, 1958). Then he understood that as the movements become less stereotyped the same stimulus may produce a local reflexes, before anoxia appears. These combinations appear in sequences functional to postnatal life. After 13-14 weeks the movements become graceful and flowing (Hooker, 1952) and lose the more jerky appearance often seen earlier. In vertebrates we can see the connection between motor neurons and their target skeletal muscle fibers. Skeletal muscles allow animals voluntary movements that are controlled by the peripheral nervous actions. This system requires neuromuscolar conjuctions made by striated muscles and confined to a narrow endplate band in the central region of the muscle. At the moment, the process of neuromuscular synapse formation has been extensively studied and well described on a molecular level, but we don't know what part of the nerve and muscle play in regulating this spatial arrangement. Of course we understand the importance of confining neuromuscular junctions to the central muscle region but the functional meaning of this activity is still unclear.

At the moment, one of the most important question is how this characteristic and stereotyped pattern of innervation is established and what extend motor neurons and muscles contribute to shaping this arrangement.

1.3. The concept of neuro – behavior

The concept of neuro-behavior reflects the idea that all human experiences have a double matrix: a biological matrix and a psychosocial one. These two systems dynamically influences each other and they are dependent on a neural response that determines the optimal synergic operation. Therefore the concept of neuro-behavior translates, a dynamic relationship between behavior and physiology.

A neuro-behavioral evaluation criterion should be sensitive to differences in behavior exhibited both in a typical and atypical development.

A tool for assessing the broad neuro-behavioral spectrum, observable from the earliest stages of the motor system development represents a possible allocation of evolutionary ontogenesis of motor activity.

The advent in the 70s of ultrasound technology made possible the observation of human fetal behavior, increased knowledge of embryo and fetus, and helped to promote the concept of continuity of fetal behavior from intrauterine life to the neonatal life (Almli, Ball & Wheeler, 2001; Stanojevic, Kurjak, Salihagic'- Kadic, Vasilj, Miskovic, Shaddad, Ahmed, & Tomasovic, 2011) .

1.4. The reasons for complexity

Many researchers observed a particular variation of the movement in the early stages of neuromuscular development. These studies were mainly related to the observations of infants during the first days after birth (Thelen, 1995). An atypical development of motor system, as well as in cases of cerebral palsy (c .p.), is associated with a lower variability of movements in the first months of the infant's life (Prechtl, 1997). The importance of the qualitative variability criterion is widely recognized in the observation of the motor system developmental trajectory, even during the earliest stages of development.

In 1978 Touwen, for example, argued that variability in movement was an important feature for newborn well-being, and he attributed the origins of this criterion to the properties of the nervous system. Therefore a brain damage was reflected in the form of a reduction in variability, due to the lack of "hardware", or available programs .

Even Prechtl (1990) noted that a reduced movements variability in subjects at risk was sloped in the repetition of monotonous and stereotyped patterns, that were not aimed at achieving a specific objective. These studies interpret the origin of the variability as a phenomenon determined by pre-programmed patterns already present at birth and, in the light of this theoretical perspective, they justify the less of variability in subjects at risk as a result of reduced availability of motor patterns caused by brain damage.

The theory of dynamical systems are strongly influenced by ecological perspective, which emphasizes the dynamic relationship between action and perception. Such a perspective sees in new forms of movement and behavior, emerging from the interaction between different factors, more than a particular predetermination of motor patterns chosen within the central nervous system. Thelen and Smith (1994) argued, in fact, that particular individual conditions are determined by the interaction of several factors of human behavior and specify that this approach is the "cornerstone of a dynamic theory of development".

From the point of view of dynamic systems theory variability would indicate a greater stability in behavior in general and not, as for the maturation approach, the index of an atypical development. In recent decades researches on the development of children's motor behavior abandoned the focus on developmental milestones and they focused on the concept of spontaneous behavior observed in early infancy. Therefore spontaneous movement has been recognized as an important precursor to the development of motor control. (Piek, 2002; Thelen, 1995).

Complexity in development means that dynamic systems interaction increases over time, that systems self-organize from subcomponents and context, and finally that development is not predetermined but emergent (Thelen & Smith, 1994). The notion of regulation also includes the concept of plasticity, according to the innate variability of regulating functions among the scenery of different contextual influences (Damasio, 1994). This approach emphasizes the interaction role of bottom-up component, such as physiological, emotional, attentional and self-regulatory functions, as well as the integrative component of brain stem, limbic and cortical system (Tucker et al., 2000).

This model suggests that development is generally gradual and it is also constrained by the system's state initial conditions of a previous point. The evolutionary perspective adapted by these interactive models is the theoretical comparability of ontogenesis and phylogenesis.

Although the concept of development is still argued in an open debate, it is defined as the change in a specific function over time, underlying the regulation mechanism applied to the multifaceted and multilevel constructions across time (Goldsmith, 1964).

1.5. Continuity in development

During the past many textbooks of developmental psychology ignored the functional significance of prenatal life in relation to development of postnatal behavior, and to the ontogeny of development. Researchers believed that prenatal life was an interlude of rapid physical growth, during which the fetus can experience a succession of spinal reflexes related to different gestational ages, as if the ten lunar months of pregnancy represent a limbo, or a state of suspended life, from which the fetus would have been released only after birth.

Around 1980, we can see a revival of observations and studies of prenatal behavior, especially thanks to the psychobiological and behavioral neurosciences (Smotherman & Robinson, 1988).

This renewed interest in prenatal life was preceded by the development of the new ultrasound examination technologies. The first important change was the birth of the subject's point of view, thanks to the direct observation of fetal behavior in his intrauterine environment (Campbell, 2002). Direct observations of fetal life gave rise to an important line of research, with a particular attention to the study of individual motor patterns and human development trajectories from prenatal to postnatal life. Several following studies on development shared the fundamental assumption that neonates have a sophisticated repertoire of behavioral skills ready just a few minutes after birth, and that the mechanisms for the organization of behavior or appear ex novo after birth, or they take part in the trajectories of development during prenatal life (Kurjak et al., 2003).

According to the theory of the motor activity neural control, (Cioni et al., 1997; Birnholz et al.,, 1978), we assume that fetus has a simple control system that allows him to begin the path of action coordination, and the regulation of behavior. This simple system of coordination and regulation is responsible for the complexity in the specification and differentiation of behavior, which is of great relevance for the development of complexity in infant's behavior as well as in adult's (Smotherman & Robinson, 1988).

Research on prenatal behavior produced some interesting methodological advantages. For example, we know that infant is strongly dependent on mother's behavior, who represents a source of nutritional balance, as well as for adjustment of food, water and salt; moreover, this relation is the dyadic latest form of behavioral and physiological regulation of the very early postnatal period. Unlike the baby, the fetus in fact bases his nutrition on the connection of the umbilical cord and the placenta, that provide the transmission of nutritional and immunological properties, temperature control and protection. Therefore mother is indispensable to the satisfaction of fetal needs, physiological conditions, and her behavior is necessary and sufficient to provide the main elements of regulation during the entire period of prenatal life. The main advantage of the new technologies in the observation of fetal behavior is the chance to observe fetal life system independently from maternal narrative (Kurjak et al., 2002).

There is a long tradition in behavioral studies that interprets the phenomena of coordination and stereotyped behavior in species-specific motor activity as a model of a pre-existing plan or predetermined patterns in the central nervous system (Coghill, 1929). These interpretations explain the concept of fixed action patterns, or the concept of innate release mechanisms in the field of ethological classic studies. These interpretations were after declined in the modern neurobiological concepts of "power or generators of movement" (Grillner, 1985) or "command neurons." This paradigm may be useful to promote an adequate description of control in adults' behavior but, it is not adequate to describe the development of control during the early stages of development, yet. For example Smotherman & Robinson, 1988 showed that some movement patterns are assembled both in real-time, and during the time of the future development. From the contingency point of view, we can observe a collection of continuous events precursor of movement that specify the organization of movement.

From the evolutionary point of view we can observe some behaviors occur at a specific age, and that other movement patterns appear later.

The amniotic fluid environment acts as a support or "scaffolding" to a series of physical movements later recognized as motor behavior. The most important conclusion of these assumptions is that systems that specify motor development are not only dependent on the actions (activity-dependent), but are also the result of experiences (experience-dependent). Consequently, we can assume that neural spontaneous activity and sensory experiences are indispensable for behavioral development. All these conditions contribute to the development of a set of motor experiences across time and they are functional to the life after birth.

2. Describing human fetal motor behavior

2.1. What is ultrasound technology?

Ultrasound technology is an electronic tool that allows clinicians to see organs of our body thanks to the high frequency sound waves (ultrasound). This system is composed by three parts: the electronic tool, the monitor and probes system. The electronic system sends the pulses of sound waves through the body, then the sound waves are partially reflected by the abdominal wall, the pelvic organs or by the fetus, and they creates echoes of return; finally, the electronic system reprocesses these echoes transforming them into images on monitor. Thanks to this system it is possible to observe the uterus, the ovaries and in pregnant women the fetus and placenta.

Ultrasound in pregnancy has different purposes depend on fetal gestational ages. The Ministerial Decree Guzzanti-Bindi of September the 10th in 1998 stated that in Italy three ultrasounds exams have to be performed (one for each trimester) during pregnancy. In some cases (for example in case of slow fetal growth), it could be necessary to perform a greater number of examinations.

During the first trimester (up to 13 weeks of pregnancy) ultrasound allows to define the location of the pregnancy, the number of embryos or fetuses, to visualize cardiac activity and to assess whether the gestational age corresponds to the date of the last menstrual period (embryo is the product of conception up to the 10th week and fetus from the 11th week onwards), and finally to measure the thickness of the nuchal translucency. In the second trimester of pregnancy (from the 14th to the 26th week) we can observe fetal anatomy and assess whether fetal size (head, abdomen, thigh) corresponds to the reference gestation values. During this period, the exam will display the site of placental insertion and the amount of amniotic fluid. This examination is commonly called morphological ultrasound or ultrasound screening of fetal malformations. During the third and last trimester (from the 27th week until delivery) ultrasound is used to assess fetal growth, the amount of amniotic fluid and the insertion of the placent.

Therefore, ultrasound is tool to detect images that took a very important role in medicine in general and its role in the field of obstetrician is even central, as it is certainly a method not detrimental to the fetus (unlike X-rays, CT scans, etc..), and it allows to study the fetus in motion (unlike MRI). Thanks to ultrasound is now possible to study the "patient-fetus" (replacing the semiotic traditional observations, such as palpation, auscultation, etc.).

After 40 years of ultrasound in 2D, we wonder what the 2D ultrasound can reveal. We were able to investigate sufficiently certain development issues, such as birth, development of motor system and its continuity after birth. Ultrasound definitely improved our knowledge of neuromuscular development, but what are the limits of this technique?

This technology is very useful to describe fetal behavioral repertoire and topography of movement, but it can provide a narrow field of view which sometimes is not sufficient to characterize temporal and sequential patterns of fetal behavior (Smotherman & Robinson, 1988).

During the last twenty years researchers has focused on the characterization of behavior, as well as on the identification of the process, mechanisms and experiential factors of development (Kisilevsky & Low, 1998). Several studies on the so-called "fetal learning", including memory, habituation and intentionality, were mainly conducted by the vibro-acoustics-stimulation (VAS) (James, 2010; Hepper, 1996; Hepper, 1991; Visser et al.,1989).

Recently, the use of ultrasound in 3D and 4D has connected the assessment of facial expressions and body movements with the assessment of fetal neurobehavioral development and fetal well-being (Hata et al.,2011).

2.2. Ultrasound 3D and 4D new technologies

The term 4D imaging was created by a craftsman to represent the addition of the time to the first static images in 3D.

Nowadays , the 4D scanning frame-rate is about 18/24 frames per second and it depends on the size of the region of interest and on the number of floors involved. However, there are considerable advantages in 3D and 4D.

Firstly, the impossibility to study fetal anatomy frame-by-frame has been removed, and secondly, the high rate of failure in 2D anatomical surface images is significantly reduced. However, it should be clearly stated that 3D/4D imaging is not an alternative to the 2D scan. Two-dimensional images of the interior of the fetal anatomy with rapid fetal biometry, will remain the cornerstone of prenatal display for the near future. However, according to Stuart Campbell (2002), the biggest benefit in terms of 4D scans are in two areas relatively unexplored: the parents behavior and fetal behavior. It is now recognized that the attachment relationship between mother and child is of critical importance to the future development of emotional and social development and parental attachment relationship begins during pregnancy, in response to fetal movements images.

The ultrasound images in early pregnancy can contribute to a greater sensitivity of attachment to the unborn child, as well as an improvement of maternal health behavior.

There are no empirical studies that show ultrasound examination improves the maternal-fetal bonding, but at least in the short term, and there are some evidences of added value with ultrasound in 3D (Sedgmen et al, 2006).

2.3. Mapping the face: craniofacial development

To understand and to explain variations and anomalous arrangements of facial muscles and the general patterns of development in the head and neck regions is useful to describe the morphogenesis and histogenesis of muscles innervated by the fifth and the seventh cranial facial nerve in man. This muscles are important in phonation, mastication, deglutition, audition and vision and they play an important role in postnatal life of man. After Hooker (1952, 1958) and Humphrey's (1964) studies, we can see a new attention on prenatal life and a more specific knowledge of human fetal reflexes and their correlations with head and neck areas. These studies were conducted on aborted fetuses, segregated according to their state of development and sectioned in various planes.

The sections of facial pre-muscles masses were plotted and examined microscopically. Gasser R.'s article named "The development of facial muscles in man" ,published in 1967, presented five sequences stages of facial muscles development. Each stage is anticipated by some important developmental changes. When an individual muscle develops, it separates into a superficial and a deep group and only after this separation we can observe the development of the superficial muscles. The muscles innervated by the facial nerve are grouped according to their location (superficial or deep), and their common pre-muscle condensation (lamina, mesenchymal collection or complex). This study shows that the development of the peripheral branches of the facial nerve follows simultaneously the development of the facial muscle masses (Gasser, 1967). Since there is a close relationship between muscles and nerves, as the muscle masses are formed, the nerves supply to differentiate them and this process influences the histo-genesis and the morpho-genesis.

According to the ontogenetic perspective (Oster;1997; Oster et al.; 1992), that takes its starting point from the observation of provoked motor behavior, as a way to determine the origin of movement, we can look at the continuities and changes in human spontaneous movements repertoire as a way to understand both origins and meanings of human behavior. This ontogenetic perspective (Oster, 2005) suggests to interpret development of fetal abilities and skills as an important step in behavior, necessary to life after birth. Moreover according to challenge of the new epigenetic models, it seems to be necessary to ask how genes, uterine environment and maternal emotions can influence or act in development of emotional reactions, even in this very early development stage of prenatal life.

2.4. Fetal abilities and skills

The following description is based on longitudinal studies performed by (Prechtl et al., 1979, Prechtl et al., 1989; Prechtl et al., 1997) and his colleagues to understand fetal motility from the neurology point of view. First movement observed around 7.5/8 weeks of gestational age was the sideward bending of the head. At 9 weeks the so-called "complex and generalized movement patterns" and startles appeared (de Vries et al., 1982). Complex and generalized movement pattern is a slower, general and complex sequence that involves different part of the body and becomes extremely important for early diagnosis of brain dysfunction as well as prediction of later neurological outcome. The startle reflex is a quick and phasic movement that involves limbs, neck and trunk (Einspieler, et al., 2004). One week later, at 10 weeks, isolated movements of one arm and leg emerge, and they are more difficult than global activity of complex movements for a young nervous system. As we noted from Hooker (1952) in stimulations of fetal response, the ontogenesis of sensory system develops from cranial to caudal, isolated arm and leg movements occur at the same time, even if there is an higher frequency of arm movements than leg movements. At 10 weeks hiccup emerges and it is described as a short and repetitive contraction of diaphragm that can last for several minutes and it can be so forceful that the whole fetus can be passively moved in the amniotic cavity. At 11 weeks we can see head movements that can be differentiated in: head ante-flexions, head retro-flexions and rotations.

We can also see the first hand-face contact but we can't discuss the beginning of the intentionality yet, and they are still considered accidental. At 12 weeks of gestational age the episodically presence of breathing movements appear. This pattern made an exception despite other fetal movements as it depends on the maternal glucose level and it more easily seen after mother's meal. At the same age (12 weeks) we can see stretches and yawns, described as complex patterns maintaining their aspect throughout the entire life without changing in form. During these weeks fetus starts to drink amniotic fluid with rhythmical movements called sucking and swallowing patterns.

Head and trunk rotations, alternating leg movements and general movements are responsible of fetal position changes in utero. This is a very frequent event especially during the first half of pregnancy and may run up to 25 changes per hour. It is also very interesting to notice the functional meaning attributed to this pattern useful for the event of birth, underlying the ontogenetic adaptation of these motor patterns. Alternating leg movements can find correspondent patterns in postnatal life in newborn stepping phenomenon. At 20 weeks we can see slow eye movements and at 22 weeks a rapid eye movement (Birnholz et al. 1978, Nijhuis et al.,1982). Smiling movements are also present both in preterm fetuses (Kurjak et al., 2005).

The term "neuro-behavior" reflects the idea that all human experiences have both psychosocial and biological reasons and human behavior is a consequence of genetic and specific experiences made by infants during and prior birth. There is a dynamical interaction between biological and behavioral systems that influence each other. Starting observation of human fetal behavior Salisbury, Lester and

Salisbury and colleagues (2005) published a study on fetal motor patterns classification divider in an face view and a chest/upper body view.

Face view:

1. Fetal Eye Movement: Clear movement of the pupil or eyelid when a view of the eyes is obtained.

2. Suck/Rhythmic mouthing: Rhythmical bursts of regular jaw opening and closing at a rate of approximately one per second (or more). Regular and irregular sucking is coded.

3. Drinking and Mouthing Movements: Mouth opening and closing that is isolated or limited to less than 3 at one time, often with tongue protrusion. You may see swallowing of amniotic fluid.

4. Yawn: This movement is similar to the yawn observed after birth: prolonged wide opening of the jaws followed by quick closure often with flexion of the head and sometimes elevation of the arms.

5. Hand to Face: The hand slowly touches the face or mouth, the fingers frequently flex and extend.

6. Isolated Head Movement (IH), head rotation, extension : Movement of the head that is not accompanied by limb or trunk movement, either small jerky movements in the vertical plane, rotation from side to side, or extension.

Fetal behavior coding definitions: Chest/Upper Body View

1. Breathing Movements: Displacement of the diaphragm lasting less than 1s that is either small or large.

2. Isolated Limb Movement (IL): Movement of any limb or combination of limb movements that does not include trunk or head movement. Scored as smooth or jerky in quality.

3. General Body Movement (GB): An indiscriminate pattern of movement involving at least one limb, the trunk and the head. Scored as smooth or jerky in quality.

4. Startle: A quick, generalized movement, always initiated in the limbs and sometimes spreading to neck and trunk.

5. Back Arch: Extension of the trunk and maintenance in this position for greater than 1 second.

6. Stretch: Always carried out at a slow speed and consists of a forceful extension of the back, retroflexion of the head, and external rotation and elevation of the arms.

7. Hiccup: Consists of a jerky, repetitive contraction of the diaphragm.

8. Hyperflexion: Flexion of the trunk and maintenance of this position for greater than 1 second.

Fetal motor patterns have been documented by ultrasound from 8–9 weeks of gestational age. Nadja Reissland and Brian Francis'contribution to the quality of the upper limb movements distinguished movements in healthy fetuses that are "performed strikingly similarly and fluently throughout prenatal life" with the exception of startles, hiccups, isolated twitches and head retro-flexion. The different quality of these fluent or smooth fetal movements are not only indicative of healthy development but they are positively correlated with postnatal behavior, too. There is continuity in motor behavior development from prenatal to postnatal life, as discussed by deVries and Hopkins for general movements that increase from 8 to 28 weeks, and then decline from 28 to 36 weeks. Other researchers suggest that there is continuity in movement patterns before and after birth starting from 8th week of postmenstrual age, around three months of postnatal age. The quality of fetal movements has been argued as a sensitive manifestation of central nervous integrity (Einspiele et al., 2012) and it predicts not only brain dysfunction but also points to specific lesion sites in the central nervous system of compromised fetuses. Reissland's contribution starts assuming the idea that the quality of fetal movements provides a means of assessing fetal well-being. Since it is not clear yet whether the quality of fetal limb movements in terms of being jerky is related to fetal stress level or is a normal age-related phenomenon in the human fetus, the most important question that aimed this study is: are jerky limb movements predicted by the age, stress or both? Therefore, the different quality of upper limbs movements is declined as: jerky-movements, smooth-movements e no-movements.

Kurjak, and colleagues (2003) differentiated:

1. Hand to head: when hand movement ends at contact of fingers with the parieto-occipotolo-temporal region of the head;

2. Hand to mouth: when movement ends at contact of thumb or finger with mouth, lips or the immediate oral region;

3. Hand near mouth: when movement ends with fingers in fluid between nose and shoulders/nipples or between both shoulders. Hands must be below eyes and within the area defined by the ears, less than a hand away from the mouth;

4. Hand to face: when movement ends with hand in contact with the face (cheecks, chin, forehead);

5. Hand near face: when movement ends with finger in fluid in front of the face but not in mouth region;

6. Hand to eye: when movement ends with hand or palm or fingers in the eye region;

7. Hand to ear: when movement ends at hand contact with the ear.

Facial activity and expression

1. Isolated blinking: consisted of eyelid movement;

2. Mouthing movements: consisted of a series of rhythmic movements involving the mandible and tongue, characterized by constant frequency and duration until disappearance;

3. Mouth and eyelid movement: simultaneous eyelid blinking and jaw movement;

4. Yawning: slow and prolonged wide opening of the jaws followed by quick closure with simultaneous retroflexion of the head and sometimes elevation of the arms of exoration;

5. Tongue expulsion: facial activity characterized by mouth opening with protruding of fetal tongue;

6. Pouting: full extension of the lips in a pout consisting of straightening the fibers of musculus orbicularis oris;

7. Smiling: the expression consists of the bilateral elevation of the mouth angle;

8. Scowling: the expression consists of bilateral contraction of eyebrows and mimic musculature between them.

2.5. Behavioral states from fetus to neonates

The behavioral state is a coordination of physiological parameters described for the first time in fetuses by Nijhuis and collegues in 1982. Behavioral states are a constellations of physiological variables, which are repeated over time, and are similar to those observed in premature infants by Prechtl and associates in 1979. As early as 36 weeks of gestational age, it is possible to observe a repetition of cycles of each variable observed in individual states; while starting from 38 weeks up to 40, we can observe a consistent synchronization of variables cycles in each behavioral state.

Fetal and neonatal behavioral states have been classified analogously, using four categories and excluding indeterminate sleep and crying state. Although intra-uterine and extra-uterine environment is profoundly different, their distribution is very similar at the same gestational age.

Fetal behavioral states are classified using heart rate pattern, eye movements and gross body movements and after 36 weeks of gestation they are well coordinated and almost indentical at the newborns correspondent age.

A particular attention is referred to fetal breathing movements, also defined as paradoxical. They start around 28 weeks of gestational age and from 36 weeks they seem to be reduced during quiet sleep, reflecting also development of quiet sleep state during late pregnancy. They are characterized by inward chest movements and outwards abdomen movements during inspiration, and occur also in neonates during REM sleep. This phenomenon is probably caused by a lack of intercostal muscle tone and a compensatory increase in diaphragmatic excursion.

Classification of fetal behavioral states

Behavioral state 1f:
Quiescence, which may be interrupted by sudden movements of the entire body, mostly startle.
Absence of rapid eye movements.
Heartbeat stable. Occasionally there might be small accelerations of heart-related movements. This pattern of the heartbeat is called FHRP A.

Behavioral state 2f:
Frequent and regular movements of the whole body especially retroflection, bending and movements of the extremities.
Eye movements are always present.
FHRP B characterized by a wider oscillation with respect to FHRP A and a frequent acceleration during movements.

Behavioral state 3f:
Eye movements continuously present.
FHRP C heartbeat stable, but with an oscillation in the band wider than in FHRPA and devoid of accelerations.

Behavioral state 4f:
Continuing strong activities which also includes the trunk rotations.
Eye movements are always present (where it is possible to observe them).
And FHRP beat is unstable, with large and sustained accelerations, which often result in a sustained tachycardia.

Neonatal behavioral states

The states of sleep-wake affect every physiological function and neuro-behavioral. In newborn we can identify six behavioral organized pattern: two states of sleep (quiet and active), a state of drowsiness and three states of waking (quiet, active, crying) (Brazelton and Nugent, 1984):

- Quite Sleep: the child is completely relaxed, the breathing is regular, the eyelids are closed, and there areno eye movements;

- Active Sleep: breathing is irregular, movements of the body are made up of limited limbs movements and occasional body, arms and legs movements. In this state, child often contracts facial muscles, sometimes smiles or frowns. They are often recognizable sucking movements, and there are rapid eyes movements;

- Drowsiness: occurs in sleep-wake transition, the outlook is not bright, and the eyes are half-closed;

- Quite waking: the gaze is alert, the child is able to follow with the look and orienting to the sound, establishing interaction with the adult's voice, face and attention;

- Active waking: the child moves briskly arms, legs, trunk and head, the eyes are kept open and active;

- Crying: the baby has vigorous physical activity with eyes open or closed and cries clearly.

In preterm infant sleep, wakefulness and periods are difficult to classify. Breathing tends to regularize progressively by gestational age. Davis & Thoman, 1967 defined three types of sleep in preterm infants: quiet, active and transitional. The increased frequency of active sleep in preterm infants has been associated with biochemical mechanism aimed to ensure the growth of central nervous system. Wakefulness state in premature are less defined than sleep, and may be better defined by behavioral observations: for example, lower gestational age prematures demonstrate almost exclusively drowsiness, while by age we can see the capacity to fix and follow (Dall'Antonio and Paludetto, 1987).

Several studies showed that (Dell'Antonio and Paludetto, 1987) when preterm infant is cared in a setting with a temperature equal to his body, he has more prolonged periods of quiet sleep; when premature infants of 29 weeks are subjected to tactile stimulation they show more prolonged periods of wakefulness.

3. Prematurity

Nowadays thanks to new the technologies, the number of infants that survive in spite of a very low birth weight keeps increasing. It is well known that preterm infants are at a great risk of developmental deficits and motor impairments, as well as of severe visual and hearing impairments. Although the most common deficit area of development is observed in cognitive domain, the 40% of 6-years-old children born with an extremely low birth weight have moderately to severely decreased IQs, while the 30% of children have mild cognitive impairments. there is also an important risk for the so-called "long-term" deficits, such as attentional, motor and visuo-spatial abilities. For these reasons it is very important an early identification of neurodevelopmental deficits. For example the quality assessment of General Movements (GMs) is a non-intrusiveness method for the assessment of fragile and physiologically unstable preterm infants.

During the last 15 years most researchers have proved the functional integrity of the young nervous system founding that abnormal GMs from preterm age onwards up to 3-4 months post term were associated not only with an high risk for cerebral palsy, genetic disorders, neurological deficits and behavioral problems but also lower intelligence.

Premature birth is a particular phenomenon that seems to prefigure the future development of children.

The World Health Organization (OMS) defines preterm a baby born before 37 weeks of gestation, that means 259 days from the first day of last menstruation. Pregnancy normally lasts 38-42 weeks, but if it stops before the 37th week, the baby is called premature. Nowadays statistically the vitality limit to ensure survival in preterm newborns is 23 weeks of gestation (OMS, 2012) .

A pregnancy that starts normally leads to a child's birth whose average weight in our population is about 3000 - 3400 grams. The baby with low birth weight (Low Birth Weight, LBW) is a child with a body weight less than 2500 grams. This can happen for two main reasons:

1. the birth took place before the 38th week of pregnancy and the baby is premature;

2. during pregnancy, there was a situation of suffering and poor growth of the baby. The placenta and the bodies were not able to feed adequately the fetus.

In the first case we can assume that baby is premature, in the second case we can assume the child is small for gestational age. These two situations can be combined together, and so we would have a baby preterm small for gestational age (Pignotti, 2006).

These children have a higher risk of morbidity and mortality compared with term infants, although today with the modern techniques of intensive care the very real risk of death or chronic illness is limited primarily to premature infants of gestational age less than 33 weeks and children extremely small for gestational age (Pignotti, 2006).

Within the large group of children born with low weight we can distinguish four groups:

- LBW - Low Birth Weight: low birth weight babies with a birth weight less than 2500 grams;
- VLBW -Very Low Birth Weight: very low weight children with a birth weight less than 1500 grams;
- VVLBW-Very Very Low Birth Weight: weight children very, very low, less than 1000 grams;
- ELBW Extremely Low Birth-Weight: children of extremely low weight with a birth weight of less than 750 grams(MS Pignotti, 2006).

Considering the gestational age we can distinguish three groups of children:

- EXTREMELY preterm before 28 weeks;
- VERY preterm ≥28 ≤ 32 weeks;
- MODERATE preterm ≥32 weeks.

Many of these children that are extremely premature (from 23-33 weeks of gestation) may be at increased risk of:

- perinatal mortality;
- diseases in the first days of life;
- feeding problems;
- changes in intellectual, psychological, neuromotor development;
- chronic diseases.

There are complex medical-care problems related to prematurity that require the intervention of specialized and very different prepared figures such as doctors, nurses, physiotherapists and specialists ready to ensure an immediate intervention for child and for parents (MS Pignotti, 2006). These children require prolonged hospitalization in neonatal intensive care unit, where there are all the modern systems for life support (Fava Vizziello, 1992), such as mechanical ventilation, nutrition and parental care.

One of the main medical problems is the temperature control, which is at birth the first problem to be addressed (MS Pignotti, 2006). In the last months of pregnancy baby lives in a small but comfortable, warm and soft place. The womb of the mother is a safe place away from the noise and light, allowing the child to prepare for his transition to extra-uterine life. During the months next term, the child is curled up on himself and he is able to touch and explore the boundaries of his environment by his hands and the body. After premature birth, the child is in an open space, he can feel the cold temperature, noises and sudden manipulations often painful. At birth the temperature control system is not developed yet and for this reason preterm infants are placed in an incubator (incubator) or "caps Plexiglas", in which it is possible to adjust temperature, humidity and oxygenation.

The small low-weight as a consequence of the low adipose tissue, of reduced mass muscles (which allows the contraction of muscles and the "creation" of chills to generate heat) and of the scarce resources of sugar don't allow him to adjust his body temperature independently. In the absence of heating the child may experience acidosis, increase in acidity of blood and tissue, the decrease of sugar and oxygen in the blood, and loss of water and of weight. It is necessary to produce and control the heat by two probes: one located in the cradle which detects the temperature of the environment, and one placed on the skin indicating the body temperature. In cases of body lowered temperature, the thermostat of the cradle is raised to bring temperature to its normal state. Conversely, if the temperature is high, the thermostat works less to bring the temperature in the average values (MS Pignotti, 2006).

According to E. Verhagen (2005), the aim of the intensive care is not only to ensure the newborn survival, but also an acceptable quality of life. The advanced technologies and the development of clinical practice are increasingly deleting the limits of medical intervention: the survival of severely premature infants is a paradigmatic situation. For many years, the dominant approach has been to "let nature takes its course." During Sixties, coinciding with the spread of departments dedicated to neonatal intensive care, neonatal and perinatal mortality took a drastic reduction, as one of the real success in the history of modern medicine. However, the same techniques used to support the vital functions necessary to life can cause the onset of diseases in the future health conditions. In this situation it is not easy to decide (Pignotti, 2008) and even though the technologies of modern medicine significantly reduced the mortality rate, there is still a risk of neurological damages.

Nowadays in Italy and in the rest of developed world, there are structures at different levels that can accommodate preterm newborns and they can offer them as much support as possible including:

- to ensure the survival when necessary;
- to evaluate and provide neonatal care of healthy children;
- to stabilize and treat children born between the 35th and the 37th week of gestation (late preterms) that remain physiologically stable;
- stabilization of sick newborns and those born before 35 weeks of gestational ages until transfer to a facility that can provide an adequate level of service;
- to assist children weighing more than 1500 grams at birth but born after 32 weeks of gestation; care of convalescent children;

- survival and stabilization of preterm and / or ill prior to transfer to a neonatal intensive care unit;
- monitor brain function, cardiovascular, respiratory, with the help of diagnostic tools such as MRI and CT scans;
- to assist children born after 28 weeks and weighing more than 1000 grams; mechanical ventilation;
- perform basic surgery;
- to support children born after 28 weeks but with a birth weight less than 1000 grams; respiratory support;
- access to numerous pediatric specialists;
- diagnostic imaging including ultrasound, CT, MRI;
- support congenital heart defects by extracorporeal circulation and surgical repair of (Pignotti, 2008).

3.1. Preterm newborn characteristic

A premature baby should not be confused with a small infant for gestational age (SGA) that is not necessarily preterm, and can also be born at the end of the pregnancy only with a low weight. Gestational age is the most precise indicator to assess prematurity. However, it is important to emphasize that both prematurity and low weight are factors of risk for development of post-natal and perinatal diseases (Vizziello Fava et al, 1992).

Prematurity is not a uniform condition but it can varies depending on gestational age and on weight of intrauterine growth development. The high risk of respiratory diseases for preterm newborns is related to the incomplete development of organs: under 24 weeks of gestational age the pulmonary alveoli necessary for gas exchange are not developed yet; under 35 weeks preterms have frequent episodes of apnea related to the immaturity of the cerebral respiratory center. If the gastrointestinal tract is not fully developed it can cause problems in power supply, or correct serious diseases.

The whole system of preterm newborns is still fragile and may also be unable to control the basic functions, such as heartbeat and breathing. The failure in the homeostatic control is also reflected in the irregularity of behavioral states, that are difficult to classify. With the increase of gestational age the duration of quiet sleep and active wakefulness increases, and the active sleep decreases; the ability to focus attention increases and there is a gradual transition from active wakefulness to drowsiness state. Before 30 weeks it is hard to distinguish between active sleep and quiet sleep (Wolff, 1987). From 31 weeks attention and sensory abilities change too: newborns exhibit a more prolonged fixation of complex stimuli and start to pay attention to visual stimuli. Responses to auditory stimuli are already organized at 28 weeks and they reach the 35 levels of response similarly to term infant. With the increase of gestational age also the alertness becomes more frequent and ready to effective social interaction.

Considering preterms facial muscles, one of the largest differences is the absence of “Bichat’s bullet” under the cheeks and around the mouth, that contributes to the physical maintenance of body temperature and can cause tenderness in caregiver, that is essential to the newborn survival.

Figure 3

Even the preterm mimicry can be quite poor and difficult to interpret. The premature infants show more negative emotions in interaction with the mother (Segal et al. 1995) and cry is rather weak and easily up to 32 weeks. In face-to-face contact preterm newborn is less involved in communication with the caregiver, showing less eye contact with the mother and a greater number of looks of aversion (Van Beek et al, 1994). Even in this case, with the increase of gestational age there is also an increase of the positive affects of joy and interest, and a relative decrease of the negative ones.

3.2. Effects of preterm birth on the development of behavior

The appearance of a premature baby is very different from the aspect of a term newborn. Premature baby is smaller in size, he has a thinner reddish-purple skin for transparency of the underlying blood vessels. There is a very little subcutaneous tissue and the head seems to be larger compared to the body and thin limbs. The eyes often appear protruding, the abdomen is large and there may be an umbilical hernia. The chest seems to be very solid, with retractions between the ribs and along the breastbone. A premature baby is released too early from his mother’s womb and he is not ready to extra-uterine life, yet. Since premature infant is unable to self-regulate his body temperature, the incubator seeks to reproduce the womb environment and to support those functions that he is not able to do, yet. The length of stay can be very variable depending on the gestational age and on the occurrence of complications and specific problems too.

Studies on premature babies' development reveal that these retain a lower height and weight until the age of five or six years and during the first five years of life premature babies also tend to score lower results on tests of general cognitive and motor development. There is no doubt that the premature baby is slightly different from the newborn at term and if he is extremely premature, he can manifest a slightly delayed motor and cognitive development during the first year of life. Only in cases of extreme prematurity, the child may have brain lesions and serious psychological defects. Finally, we cannot exclude the possibility that behavioral differences in premature babies depend at least in part by maternal behavior.

In cases of very low gestational age we can look at the motor system as divided into a phase of akinesia and apostural. The premature infant can lie prone or in supine position and he does not show attempts of head or limbs movements. These steps can be followed by phases of asymmetric movements and by startles or genetically programmed movements that can influence behavioral states.

As for the suction in newborn at term we can distinguish a kind of nutritive mouthing movements and a kind of nonnutritive sucking movements, functional to the homeostatic regulation, that can play the role of self-regulation and crying inhibition (Oster, 2005). Thanks to Harriet Oster we can distinguish a particular facial configuration called pre-cry face (AU 17 + AU 24 or AU 10, AU or AU 11 + 17 + AU24). The non-nutritive sucking movement is already observed around 15 weeks of gestational age, and it becomes stable at around 31-32 weeks; the nutritive sucking movement matures around 32-33 weeks of gestational age.

3.3. Behavioral states differences between term and preterm infants

In 1971, Anders introduced scoring principles for behavioral states in full-term newborn defining six different behavioral states: quiet sleep (non-rapid eye movements, NREM sleep), active sleep (rapid eye movements, REM sleep), indeterminate sleep, quiet awake, active awake and crying.

The regularity of breathing movements is an important criterion to define behavioral states; for example, in quiet sleep we can find regular breathing movements and during active sleep irregular breathing movements. The breathing maturation is also connected with the development of behavioral states. During early developmental stages, while in determinate sleep decreases, the proportion of quiet sleep increases.

Although for every gestational ages the percentage of a behavioral state can vary depending on both infant and environmental factors, through the first year of life we can register an increasing proportion of quiet sleep and a decreasing proportion of active sleep.

Recently, thanks to the new advances in technology and in preterm newborns intensive care units, our understanding of behavioral states in preterm infants improved, too. Nowadays, several studies on breathing regularity of preterm suggest that this is not a tightly connected criterion for defining states, in spite of the earliest concordant criteria for behavioral states, such as EEG and REM criteria. As early as the 27th week of gestational age, sleep states can be differentiated using EEG and REM criteria, and we are able to differentiate a continuous activity with REMs (active sleep), from a discontinuous EEG activity without REM (quiet sleep). It is also important to underline that behavioral states are more undifferentiated in preterms, although a cycling of sleep states has been observed from 24/28 weeks of gestation. A large portion of indeterminate sleep characterizes sleep in preterms, but we can also distinguish an indeterminate sleep defined as a state that does not meet criteria of either active or quiet sleep(…). Sometimes indeterminate sleep is included in preterms active sleep and in NREM sleep of infants after term. In very immature infants, it is hard to distinguish wakefulness from arousal and the amount of crying is very small. There is an increase of crying from the 31rst week to term, with a peak in the second month of full-term infants.

In preterm newborns breathing movements are more regular during quiet sleep, but irregular breathing can also occur. During active sleep breathing movements are always irregular. The maturation of breathing occurs in synchrony with other behavioral state criteria.

The premature infants had more QS (quite sleep), longer QS (quite sleep) bouts, and less AS (active sleep). The normal developmental course includes increased QS and decreased AS over age, and for this reason premature newborn seem to have more mature characteristics than full-terms. One explanation could be that postnatal age is more important than conception age for the early development of sleep.

Rather than interpret premature or full-term infants sleep as a gradient of more mature or less mature, Behrman & Howard (1976) and Parmelee (1975) suggested that the notion of development is uneven for preterms. Compared with full-terms newborns, preterms differ in such a way that indicate more advanced development (increased QS, decreased AS), whereas other differences indicate delayed development (more movements in QS). Since premature infants is considered a population at higher risk for later developmental problems (Gill et al., 1986; Bornstein & Sigman, 1986), it seems reasonable to conclude that the uneven development of preterms early sleep state patterns are indicative of less-than-optimal CNS organization and function. This is a fundamental approach to specific differences is an area of important investigation for the future.

During the preterm period the major developmental changes are a decrease in the amount of active sleep and an increase in quiet sleep and waking states, especially crying (Ardura et al., 2008; High & Gorski, 1985; Holditch-Davis, 1990; Holditch-Davis & Edwards, 1998; Sahni et al., 1995, Stefanski, Myers, & Fifer, 1995). Throughout preterm period we assist the organization and increase of sleep states. Temporal organization is an already known characteristic of sleep in older infants (Coons & Guilleminault, 1984; Thoman & McDowell, 1989; Whitney & Thoman, 1993), but it is still unclear whether temporal organization develops primarily before or after term and how it is related to brain development.

In fetuses the quiet sleep cycle increases from 30 to 37 weeks of gestational ages and it remains constant. In preterm infants there is an evidence that the amounts of waking states, active sleep, and quiet sleep change over preterm period (Ardura et al., 1995; High & Gorski, 1985; Holditch- Davis, 1990; Holditch-Davis & Edwards, 1998; Sahni et al., 1995), but it is still unclear how these changes occur. According to these researches, as preterm infants mature their state bouts become longer, less frequent, and less variable in duration (e.g., Fajardo et al., 1999), but there is little empirical support for this view.

Probably caring interventions in a special nursery with diurnal cycles are involved in these changes, too (Fajardo et al., 1999; Korner et al., 1982) and we don't know whether the effects of interventions on temporal organization has to be beneficial for the infant.

From the neuroscience point of view the concept of regulation involves a complex and hierarchical relation between three core brain systems: brainstem, limbic and cortical. Even if regulation is not a term with a comprehensive definition, obviously it implies a systems perspective. Therefore researchers on early development emphasize the cooperation between regulatory components of different functions emerging from infant and external influences. Regulation requires an integrate and hierarchically system including the parameter of time to synchronize the interplay between self-regulated and co-regulated processes. The circadian time-keeping system is monitored by biological clock located in a small region of the hypothalamus called the suprachiasmatic nucleus (Antle, Foley, Foley, & Silver, 2007). The cardiac vagal tone measured the effects of respiration on heart rate variability mediated by the parasympathetic system to regulate functions. From mid-pregnancy period to term, biological clock and vagal tone trajectories are assessed weekly and they cooperate to the maturation of the sleep wake cycles (Oppenheim et al., 1997; Rutter, Kim-Cohen, & Maughan, 2006). Over preterm period, temporal organization of sleeping and waking cycles develops and preterms exhibit changes in sleep-wake lengths, in frequencies and in their percentages of transitional states. While there is an increase of bout lengths, we can observe a decrease of their frequencies.

Recent researches suggest probably both lengths and frequencies of sleeping and waking states are not set during preterm period, yet and developmental patterns can vary depending on infant characteristics and environmental circumstances.

Therefore, although the developmental patterns of each state is biologically determined, preterm infants sleep-wake cycles may be influenced by different factors. These environmental differences are an example of epigenetic mechanism that involves developmental trajectories (Gottlieb, 1996).

Nowadays we can also assume that regulation of lengths and frequencies of developmental patterns during sleep and wake states may depend also on medical and nursing care received by preterm infants (Fajardo et al., 1990; Korner et al., 1982; Korner et al., 1990; Thoman et al., 1990).

Preterm newborn development is an interesting point of view to observe a particular human being who according to his post-conception age is still a fetus, but he is surviving and growing in an atypical neonatal environment. Since developmental trajectories are contingent and related to environment, it could be inappropriate to compare these infants progresses to the developmental milestones arising from neonatal and fetal development. It should be more appropriate to identify a new method to understand, to care of and to evaluate this particular developmental process. Preterm infants represent also a source to understand the fragility of the early development and they can augment our considerations on normal development in full-term newborns.

Reciprocal influences studying full-term and preterm infants should be an important exchange between theories and clinical practices to promote health and well-being also in preterm newborns at risk.

3.4. The assessment of preterm and term infants

The spontaneous motor activity is extremely important for the early detection of brain dysfunction. When there is a positive assessment in terms of both quantity and quality of movement, it is associated with a good neurological activity (Einspieler, Prechtl, Bos, Ferrari, & Cioni, 2004). However, it is surprising that this so delicate and important factor has been overlooked for so long. Nowadays, the neurophysiology suggests that central nervous system is not just a passive mechanism. It is directly linked to the variety of movement patterns that it produces, so we can say that a good spontaneous motor activity reveals a correct development and proper functioning of the child central nervous system and his neurological development (Einspieler & Prechtl, 2005). The requirement of methods to assess behavior from the very early stages of development has been widely recognized as indispensable for understanding the functioning of the central nervous system.

The developmental psychologist Di Pietro studied very closely the methods to evaluate behavior \and neurological development, even before birth. Along with her, also other scientists and colleagues developed a rating scale of fetal neuro-behavior useful to assess gestational age and neurological maturity, also paying attention to the premature babies (Salisbury, Fallone et al., 2005). Many researchers devoted to these delicate studies compared the development of motor behavior and CNS of preterm and term newborns (Kurjak et al., 2004).

The purpose of these studies was to bring results and data useful to understand the continuity between prenatal and postnatal life, the fragility of the fetus and his motor and neurological development from the first phase of pregnancy, across the advent of birth and throughout life (Nijhus et al., 2003).

The most important data in this field have been reported by Nijhus (2003), and by Prechtl (1997), and they are based on detailed studies on the sequences of normal fetuses activity. In these studies researchers observed that fetal body and eyes movements are characterized by a series of stable and recurring sequences, which become clearer as gestation progresses and that together with the heart activity, both body and eyes may constitute behavioral states (Nijhus et al.; Prechtl et al., 2003).

Naulaers and colleagues (2002) showed that, among premature infants with a good coordination between the intake and breathing, only the 12% infants had poor control cardio - respiratory, while this figure reached 79% among those not able to coordinate these two activities. Therefore, he concluded that the observation of coordination between sucking and breathing may be a method to assess the neurological behavior of premature babies. It is very important to fully understand movements and behavior that characterize human development from the very early stages. Therefore, looking at the preterm newborn birth, the question is when does the assessment of motor behavior start (Prechtl, 1997). We can start to observe human motor behavior also during prenatal life and we can continue to study and to compare motor action patterns even after birth, observing full-term newborns, but how should we look at the premature behavior and what kind of assessment is needed to understand these particular newborns? For example, the so-called generalized movements are part of the spontaneous human movements repertoire and they are recognizable from the very early stages of fetal life (deVries, Visser & Prechtl, 1982). Generalized movements are complex patterns, they occur frequently, and they can last long enough to be observed and studied. They involve the whole body in various sequences of the skull, arms, legs, neck and trunk movements and they develop with the increase of speed and complexity. In cases of compromised central nervous system, they are abnormal and they tend to decrease becoming monotonous and rigid. These movements are also an excellent indicator of impairment and / or proper functioning of the central nervous system (Salisbury et al. 2005).

Human nervous system constantly generates a variety of endogenous motor patterns that continue even after birth (Hopkins & Precthl, 1984, 2001) .

Over the last thirty years, even the way to observe and evaluate infant behavior has been changed. Nowadays, the baby from birth proves to play an active role, thanks to the behaviors repertoire that he is able to manifest. Therefore, it is necessary to develop new tools to quantify and evaluate neonatal skills, and to design a new scale to assess the entire neurobehavioral structure and its sensory, motor, cognitive and relational components. Generally neurobehavioral scales complete the diagnostic tests, and they help to identify the normal infant adaptive strategies as well as the normal infants reactions influenced by prenatal factors (such as drugs administered to the mother during pregnancy, or using drugs and alcohol), by perinatal factors, or still postnatal.

Among the different neurobehavioral scales we underline the Neonatal Behavioral Assessment Scale (NBAS) of Brazelton (1985), which is the most widely used both in the application and in the research, especially thanks to its completeness. NBAS is an instrument born in the early Sixties to assess behavioral, thanks to a group of students guided by the American pediatrician TB Brazelton. This scale for the evaluation of newborn is based on the observation of spontaneous behavior and on responses that child makes in relation to a series of tests conducted by the investigator. This scale can lead to two different types of evaluations: the first is of neurological level, with a series of tests (reflections). The NBAS is not a complete neurological evaluation, but it is able to make a rapid clinical screening as well as to understand the physiological integrity of the child. The second evaluation allows the assessment of the first extra-uterine adaptation with a particular attention to the individual characteristics and responsiveness towards the caregiver. This type of evaluation provides a broad measure of behavior expressed during the responses to various types of stimulations (Brazelton & Nugent, 1985). The NBAS mainly deals with social behavior, as a measure of the juvenile characteristics best suited to encourage interaction and appropriate care (Packer, Rosenblatt, 1979), and it underlines all the innate components of child behavior that can give rise to a relationship with his caregiver (Brazelton, 1982). At birth in fact, the baby has a rich nonverbal repertoire, which has a profound adaptive value (Brazelton, 1982). Thanks to these studies both in fetal and neonatal assessment, we can improve our knowledge in the field of spontaneous motor behavior development.

The assessment of vital signs at birth is useful to understand the child's response to the stress of birth and to the survival efforts as well as to evaluate his chances of life (Pignotti, 2008). The premature baby is neonate at risk due to his evolutionary and / or neonatal disease. He needs a multidisciplinary follow - up to provide continuity of care and to follow his physical and psychomotor development, promoting the best quality of life in the short and long term. The available data in Italy and in European countries as well as also in the North of America show that during the first year of life these children present a great risk of hospitalizations for respiratory failure or because their body and their senses did not complete a normal development in utero. Preterm newborn lives in a real shock uncomfortable condition especially if the birth occurs very early.

Preterm birth is a global challenge in the field of obstetrics. Approximately 13 million preterm births occur each year worldwide. Medical advances have led to an increase of survival cases of births really dramatic with a very low birth weight infants (<1250 g) (Mk. Anulty et al., 2009). In South Africa, currently available data reflect that only 10% and 11% of surviving infants with birth weight less than 1500 grams can grow up properly. Changes in health care has been slow, especially in poorer areas, and medical care are not easily accessible especially with regard to the prematurity. Nowadays, in the rest of the developed world day care and attention to the pre-natal life are gradually growing more and more. The costs associated with prematurity, including the neonatal intensive care (NICU), however, are still significant. Today, we can say that the premature baby is surrounded by a welfare system that acts as a special support for the early brain development. The monitoring and continuous treatment show that in cases of latest prematurity there is also a reduction of mortalities, an increase of the weight, the promotion of an independent power supply, with a consequent shorter hospitalization (Mc . Anulty et al., 2009).

3.5. Neurological and neurobehavioral scale

Over the last thirty years, as a result of radical transformations of the theoretical perspectives the way to observe and to assess neonatal behavior have changed greatly (Valenza & Dondi, 1994). To consider the child as an individual with perceptive and interactive skills since birth, has allowed the development of new tools for the quantification and assessment of these skills. Nowadays, there are many neuro-motor behavioral scales useful to evaluate the entire neurobehavioral structure and the integrity of neurological components such as motor, cognitive and relational (Valenza & Dondi, 1994).

Neuro-motor and neurobehavioral examinations are used for several purposes: to assess the relationship between neurological behavioral and motor function, to detect early injury to the central nervous system, to predict future outcomes and the impact of the interventions on future development (Harijan et al., 2012; Hantually et al ., 2009; Salisbury et al., 2005; Noble & Boyd, 2011). A longitudinal assessments of neonate may be useful to build a picture of infants developmental trajectories and it can also be useful to understand the effects of the interventions on newborns development. Recently, our improved knowledge of the brain structures (coming from the combination of the use of magnetic resonance imaging and the use of rating scales of neuro-motor development) has shown the best prediction of future motor dysfunction (Noble & Boyd, 2011).

Nowadays, there are many neurological and behavioral rating scales and each operator can choose the scale that best suits with his needs and his area of investigation (Valenza & Dondi, 1994).. As we said before, one of the most popular and well-known scale is the NBAS (Neonatal Behavioral Assessment Scale Brazelton), which is based on the observation of spontaneous behavior and the evaluation of the responses that child makes as a result of tests conducted by the investigator (Brazelton, 1985). Then we can cite the GMs "Prechtl's Assessment of General Movements" of made by Prechtl and based on the observation of spontaneous behavior, with some little intrusive techniques and a few manipulations of child, followed by the observation and video recording of behavior (Prechtl, 1999). Researchers interested in the evaluation of behavior in preterm infants at high risk, will use the "APIB" (Assessment of Preterm Infant's Behavior) or NAPI (Neurobehavioural Assessment of Preterm Infant). Other researchers interested in the evaluation of muscle tone, reflexes, social skills and self-regulative behaviors, will adhere to the protocol of the scale NNNS (Neonatal Intensive Care Unit Network Neurobehavioural scale) developed by Barry M. Lester and Edward Tronick in 2004 (Salisbury, Fallone, Lester, 2005; Noble &Boyd, 2011; Mc Antually et all., 2009).

As we can see there are many scales available for both clinicians and researchers and there are many areas of interest related to behavioral states, interaction and social infant skills, assessment of posture, muscle tone, and reflexes. As we already said, the evaluation scales are also used as a tool for early diagnosis of the various functional and structural abnormalities in the central nervous system.

For a long time the integrity of the central nervous system and the interpretation of the neural functions of the nervous system of children, was based on the observations of reflections (Prechtl, 1999). Reflexes are involuntary and automatic response to a sensory stimulus. The infantile reflexes are present at birth and usually they disappear after the 5th-8th month and the maturation of the nervous system coincides with the disappearance of many reflections. The dramatic reappearance of some newborn reflexes in adulthood, for example as a consequence of severe head injuries, is a sign of diseases of the central nervous system (Valenza & Dondi, 1994). Therefore to assess the presence, absence or excessive expression of certain reflexes can be helpful to understand the normality of the nervous system development in children. In fact, the central nervous system works as a center supplement receiving information from the senses organs and sending messages to the effector organs. To generate a reflex action the sensory receptor that perceives a stimulus is appropriately stimulated and then the information is transmitted to the CNS via an afferent neuron (transmitter of information to the brain); the central nervous system sends signals to efferent neurons (from the brain to the periphery) which transmit them to the organs effector that generate a precise answer. A brain damage can block this chain.

In the nineteenth century Preyer was fully aware of the importance of the spontaneous movement of the fetus, and newborns as indicator of "appropriate neural structures”. These activities are in fact endogenously generated in the CNS, but reflexologists and behaviorists, underestimated this observation (Prechtl, 2001).

During the Eighties Prechtl and De Vries described fifteen different patterns of fetal movement (De Vries & Prechtl, 1984; De Vries, 2006) The advent of ultrasound scans in real time provided a safe way to view the fetal activity in utero (Kurjak, 2010). Until few years ago in fact, the only way to understand and to assess the physical activity of the fetus in utero was given by maternal perceptions fetal movement. The development of 2D ultrasound allowed the direct visualization of the fetal anatomy and fetal activity. Thanks to the subsequent advent of 3D and 4D ultrasound we are now able to observe the fetus in its real features (like 3d shows) but they also allow to see the specific fetal movement patterns , his extremities, his facial expressions, the movement eyelids, his arm movements and directions (Kurjak, 2003).

Since motor behavior reflects the development of the central nervous system, it has been suggested that an assessment of spontaneous behavior in different stages of life can help us to distinguish between a brain development, normal and abnormal, and therefore to produce early diagnosis of various structural and functional abnormalities, as well as to understand motor development trajectories from fetus to newborn.

Part II Experimental Section

4. Experiment I: Comparing fetuses and preterm infants at the same gestational age

4.1. Introduction

In recent years, the study of prenatal life is becoming more and more interesting for students in the field of developmental psychology. The new interest in this particular line of research comes from the great skills that newborns have just a few hours after birth, and it seems that these skills emerge during prenatal life (Einspieler et al., 2012). Nowadays, thanks to the new ultrasound technologies we are able to detect a greater number of information and data more accurate in fetal life.

The new ultrasound techniques in 3D and 4D are not only able to provide three-dimensional images of fetus, but also allow us to assist in real-time to fetal behavior, opening a new window of observation in the study of movement, behavior and change (Campbell, 2002). Nowadays we can collect information about different fetal movements: such as the limbs movements, the facial expressions (smiles and distress) that have a very important role in the attachment and relationship with the future parents, and that scientists consider of great interest to the assessment of the functioning of central nervous system, and therefore, of fetal health (Di Pietro, 2005).

On the other hand, one of the most interesting achievements of modern medicine has been the sharp reduction of the phenomenon of neonatal and perinatal mortality. Since the Sixties, in fact, the spread of departments dedicated to neonatal intensive care, the availability of ever more advanced technologies and the development of clinical neonatal care practice, have ensured the maintenance of vital functions, including premature infants characterized by extreme fragility (Pignotti, 2008).

Preterm infant is called premature, as a result of his early birth. In recent years there have been many studies on the development of the new Neonatal Intensive Care Units, receiving an increasing number of preterm births, unfortunately even in tricky conditions. Thanks to the technological improvements and to the emergence of new neonatal structures, we are witnessing the increased survival of preterm newborns , even those born with an extremely low gestational age (Fava Vizziello, 1992).

Our interest, therefore, comes from the recent opportunity to observe spontaneous behavior in premature infants with a gestational age also very low, and in part by the opportunity to observe spontaneous behavior even during prenatal life. Observing spontaneous motor activity can help us to understand the ontogeny of typical and atypical human behavior, as well as the evaluation of the central nervous system both in fetuses and preterm neonates (Prechtl, 2001).

Thanks to new ultrasound technologies, it is now possible to study a wide range of facial expressions including smile, distress, eyelid movements or even the typical cry facial expressions, opening a way for a new approach in the observation of behavior, both scientific and diagnostics. The aim of this study is to detect spontaneous fetal behavior characteristics, through the ultrasound scans observation of fetuses between 28 and 29 weeks of gestational age, and to compare this spontaneous behavior with a group of newborns preterm, at the same post-conceptional age. To observe newborns behavior we adopted a new coding scale made by 21 complex behavioral motor patterns, especially designed for the detection of behavioral categories originally described in fetuses, preterm and full-term newborns by de Vries (1982), Prechtl (1985), Kurjak et al. (2003), Einspieler et al. (2008) and Wolff (1987). The description of the complex behavioral motor patterns was conducted using the Facial Action Coding System (FACS) by Ekman and Friesen (Ekman and Friesen,1978; Ekman, Friesen & Hager, 2002), with the insights and information added by Harriet Oster (Baby FACS in press). These methods have shown a documented reliability in the detection of individual units of facial actions, in the first months of life as well as in preterm born infants at a very low gestational age. The opportunity to code facial spontaneous behavior so early in life, comes from the early ontogeny of the facial mimic muscles that develop from 16th week of gestational age, and that is directly connected to the activity of the V° and VI ° cranial nerve (Fitzgerald & Windle, 1942; Hooker, 1958; Humphrey, 1964, 1971).

The most recent studies on the activities of central nervous system are focused on the observation of general movements, as well as on the rules that regulate the so-called peripheral behaviors, such as facial expressions, in order to provide an additional criterion for the evaluation of fetal and preterm neonates health (Kurjak et al., 2011).

4.2. Method

4.2.1. Participants

Twelwe participants were involved in the study: 6 fetuses and 6 preterm newborns. The group of fetuses were examined at a gestational age between 28 and 29 weeks (M = 28.33). The evaluation parameters of development were considered normal and fetuses examined showed no particular physiological characteristics defined abnormal. All the pregnant women that participated to this study were Caucasian, in the absence of high-risk pregnancies, not hiring drugs or alcohol, not depressed and that did not follow a special diet. Ultrasound's examinations recordings were performed at the Center for Prenatal Pregnancy at Risk, of Padua Hospital, under the supervision of Dr. Maria Teresa Gervasi.

Consent to participate at this research was obtained by mothers and the study was approved by the Ethics Committee for Experimentation of Padua Hospital.

The group of preterm neonates were video-recorded when the gestational age (GA) was between 28 and 29 weeks (M = 203.66 days, SD = 3.88). The mean age at birth was between 26 and 28 weeks (M = 27.33, SD = 0.82). The mean weight at birth was 993,33 gr. (DS = 137.21) appropriate for gestational age (AGA; Appropriate for Gestational Age; Lubchenco, 1976). None of them had serious disease at birth that involved the heart, the brain and the organs of sense. In particular, the exclusion criteria were: chromosomal abnormalities, heart disease or other congenital abnormalities, fetal infections, metabolic disorders, obvious teratogenic factors, APGAR score < 4 at 5°min., and bleeding of III° and IV° degree. Informed consent was obtained from parents of all children involved in the study. Preterm newborns were recorded at the NICU of the Annunziata Hospital of Cosenza. Infants were observed while they were in the ICU and when they were in Neonatology and pre - discharge. Written informed consent was signed by one or both parents and it was obtained for all participants.

4.2.2. Procedure

Ultrasound examinations were performed by a gynecologist expert with the use of Voluson 730 (Expert GE Healthcare) ultrasound in 3D and 4D, with a trans-abdominal transducer of 5MHz. In particular, the Voluson 730 can acquire volumetric images in real-time up to 40 volumes per second. It allows the reconstruction in real time of an anatomical volume that is able to display precise and quantifiable information useful in diagnostic phase. In particular, in order to investigate the fetal behavior, it was essential to use 3D and 4D images obtained thanks to the automatic scan of the body volume every two seconds. Sequences of images were recorded on DVD. In the first two hours before the exam food was not provided and recordings were conducted mainly in the early afternoon, when the mother was in semi-supine position. Recordings have a total duration of 20 minutes, and for each individual subject it was calculated a coding time, functional to the procedure of coding behavior. The length of the coding period is closely related to the realization of images in 3D and 4D by the ultrasound electronic device, and it is also related to the variability of fetal movement, that do not always permit a clear observation.

Children born preterm were filmed in one session, under conditions of non-stimulation, midway between two meals, and for a period between 10 and 30 minutes (*M* = 15.7, *SD* = 5.7). Video recordings were performed by two experimenters. Particular attention was paid to the head position, since the camera objective was focused on the face for the entire length of observation. Codings and data analysis were conducted at the EARLY INFANCY LAB, of Ferrara University.

4.2.3. Coding spontaneous motor behavior in fetuses and preterm infants

The analysis of video recordings was conducted frame by frame independently by two expert coders in behavioral micro-analysis. They separately viewed the video material and, with the aid of a timer, identified in sequences videotaped the presence of the following behavioral motor patterns:*1. Rooting; 2. Occipito – frontalis reflex; 3. Chin tremble; 4. Shivers; 5. Yawning; 6. Startle; 7. Sneezing; 8. Hiccups; 9. Swallow; 10. Drooling; 11. Hand to the face movements; 12. Finger-sucking; 13. Retro-flexion of the head; 14. Rotation of the head; 15. Ante-flexion of the head; 16. Blink; 17. Mouthing movements; 18. Non-rhythmic mouthing movements; 19. Tongue expulsion; 20. Smile; 21. Distress.* Objectives aspects that ensure recognition on observational basis are reported in detail in the Appendix.

4.2.4. Reliability

We calculated the agreement between the two coders using the Cohen's Kappa coefficient, within a time window of 5 seconds. The agreement between the two observers in identifying the action was good. All disagreements were discussed and, in most cases, they have been solved. Statistical analyzes were conducted on the basis of a common protocol agreed between two coders.

4.2.5. Analysis of data

Data analysis were conducted on the frequency of occurrence per minute calculated for each of the 21 motor patterns. Since the different video recordings length, this measure was obtained by dividing, participant per participant, the total number of observations of each behavioral motor pattern into the total duration of the video recordings. According to the internal variability characterizing preterm newborns behavior and taking into account the sample size, comparisons between these two groups (fetuses vs preterm newborns) were tested using the nonparametric Mann-Whitney U-Test for independent measures. In order to identify the statistical significance it was assumed a conventional value of alpha equal to .05.

4.3. Results

Table 1 shows for fetuses and preterm infants, the mean (*M)* frequencies per minute and standard deviations (*SD*) registered for each behavioral motor pattern. Regarding the sample of fetuses, the most frequently observed behavior was the *HAND TO FACE MOVEMENT* (*M* = 0.31, *SD* = 0.31); regarding the sample of preterm newborns the most common exhibited behavior was the expression of *DISTRESS* (*M* = 1.16, *SD* = 1.17). None of the fetuses and preterm infants showed, during the period of observation, the following categories: *ROOTING REFLEX (1.), OCCIPITO-FRONTALIS REFLEX (2.), HICCUPS (8.), SHIVERS (4.), SNEEZING (9).*

TABLE 1
Behavioral motor patterns coded in Fetuses and Preterm newborns observed at the same gestational age

	Number	*Chin tremble*		*Yawning*		*Startle*		*Swallow*		*Drooling*	
	No.	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FETUSES	6	0.00	0.00	0.00	0.016	0.00	0.00	0.00	0.00	0.00	0.00
PRETERM INFANTS	6	0.038	0.060	0.017	0.042	0.193	0.256	0.141	0.129	0.90	0.163

	Hand to face movement		*Finger - Sucking*		*Head retro-flexion*		*Head rotation*		*Head ante-flexion*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FETUSES	0.317	0.310	0.00	0.00	0.056	0.088	0.086	0.075	0.00	0.00
PRETERM INFANTS	0.037	0.060	0.015	0.036	0.168	0.281	0.117	0.153	0.017	0.042

	Blink		*Mouthing movements*		*Tongue protrudes*		*Smile*		*Distress*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FETUSES	0.00	0.00	0.066	0.070	0.111	0.160	0.014	0.033	0.256	0.234
PRETERM INFANTS	0.370	0.410	0.463	0.415	0.054	0.086	0.085	0.164	1.167	1.173

Note. Behavioral motor patterns coded in fetuses and preterm infants. In vertical column Means (M) and Standard Deviations (SD) refer to the frequency per minute of each motor pattern observed. Statistical comparisons are found in the text.

The statistical comparisons performed with the Mann-Whitney U Test, showed significant differences between the two groups according to 5 behavioral motor patterns. In particular, preterm infants exhibited the facial expression of *DISTRESS* (*M* = 1.167, *SD* = 1,173) more frequently than fetuses *df* = 1, *U* = 32, *p* = .025 (see Figure 1). Moreover, preterm infants showed a greater number of *STARTLE* (*M* = 0.193, *SD* = 0.256) *df* = 1, *U* = 30 , *p* = .022 and movements related to *SWALLOWING* (*M* = 0.141 , *SD* = 0.129) *df* = 1, *U* = 30, *p* = .022. In addition, preterm infants showed also a greater number of episodes of *BLINK* (*M* = 0.370, *SD* = 0.410) *df* = 1, *U* = 30; *p* = .022 (see Figures 2, 3 and 4). Fetuses showed more frequently the *HAND TO THE FACE MOVEMENTS* (*M* = 0.317, *SD* = 0.310), compared to preterm infants *df* = 1, *U* = 4, *p* = .022 (see Figure 5 and Figure 7).

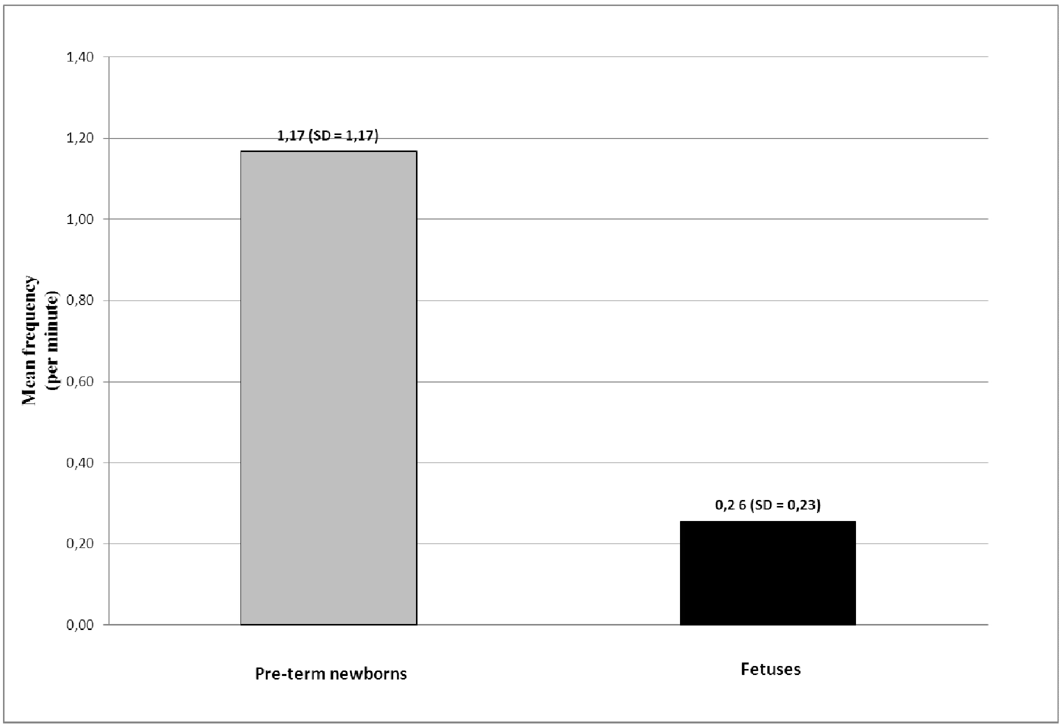


Figure 1. Graphical representation of Distress mean frequency (per minute) between fetuses and pre-term newborns; SD = Standard Deviation.

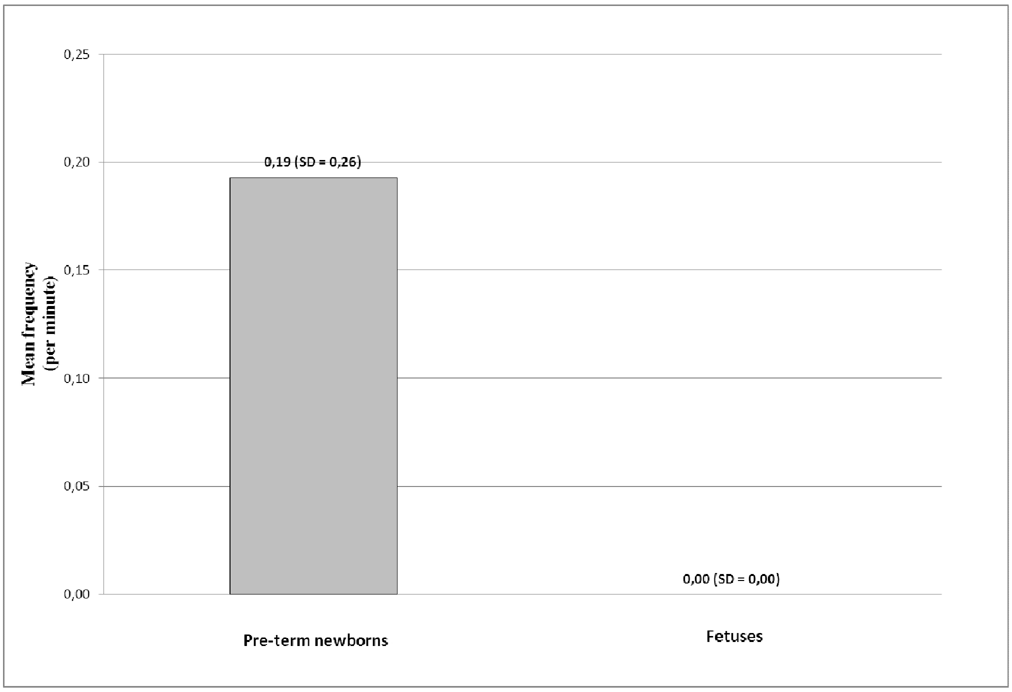


Figure 2. Graphical representation of Startle mean frequency (per minute) between fetuses and pre-term newborns; SD = Standard Deviation.

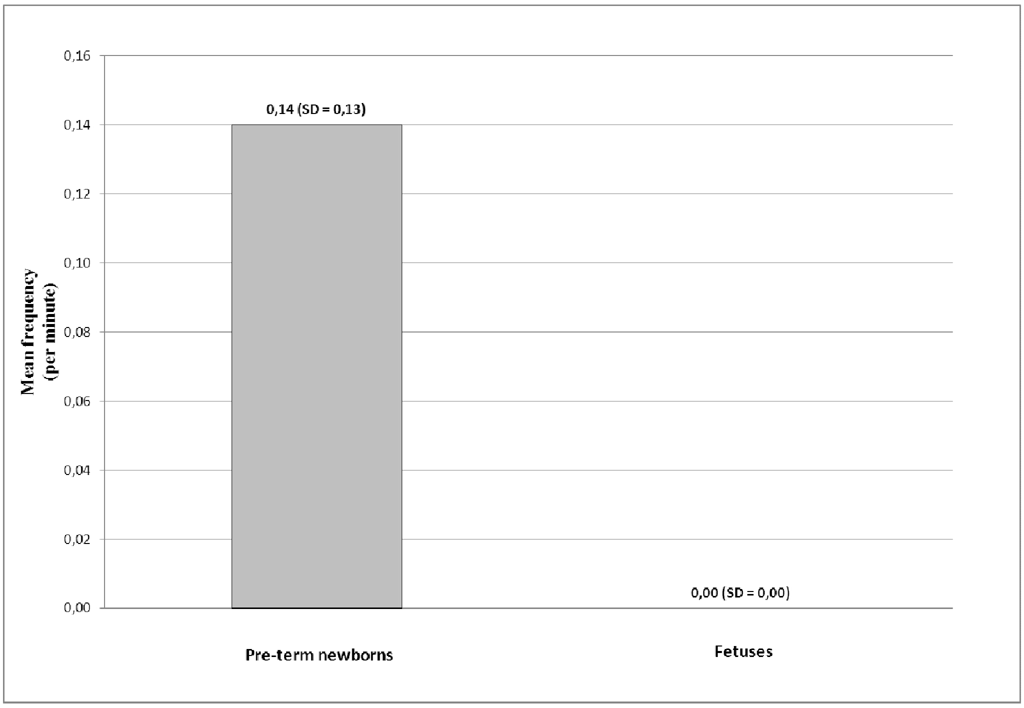


Figure 3. Graphical representation of Swallow mean frequency (per minute) between fetuses and pre-term newborns; SD = Standard Deviation.

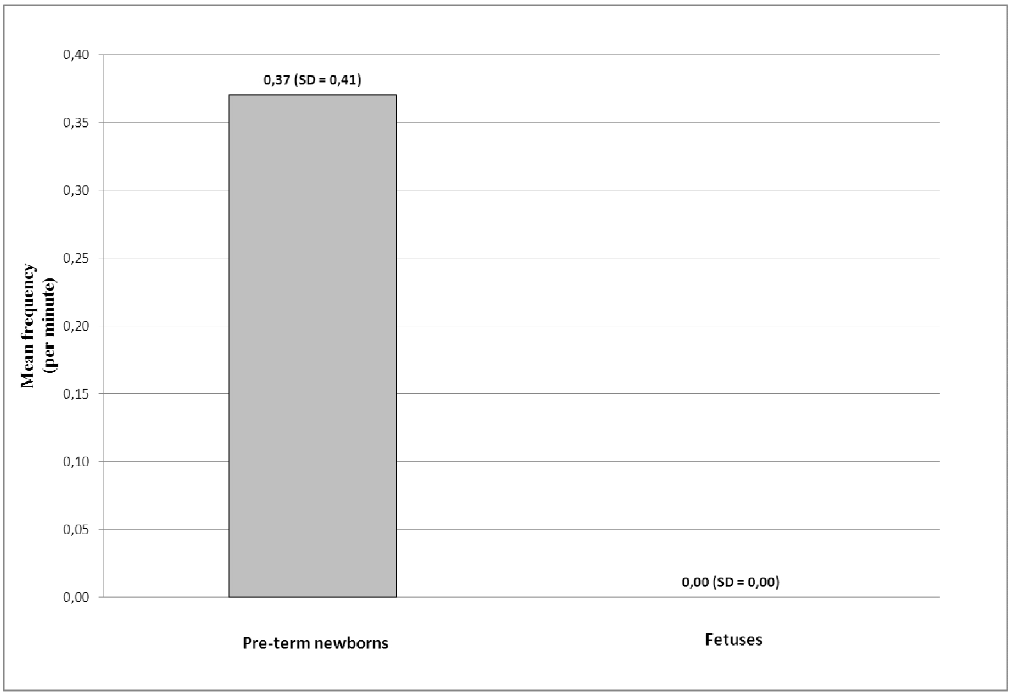


Figure 4. Graphical representation of Blink mean frequency (per minute) between fetuses and pre-term newborns; SD = Standard Deviation.

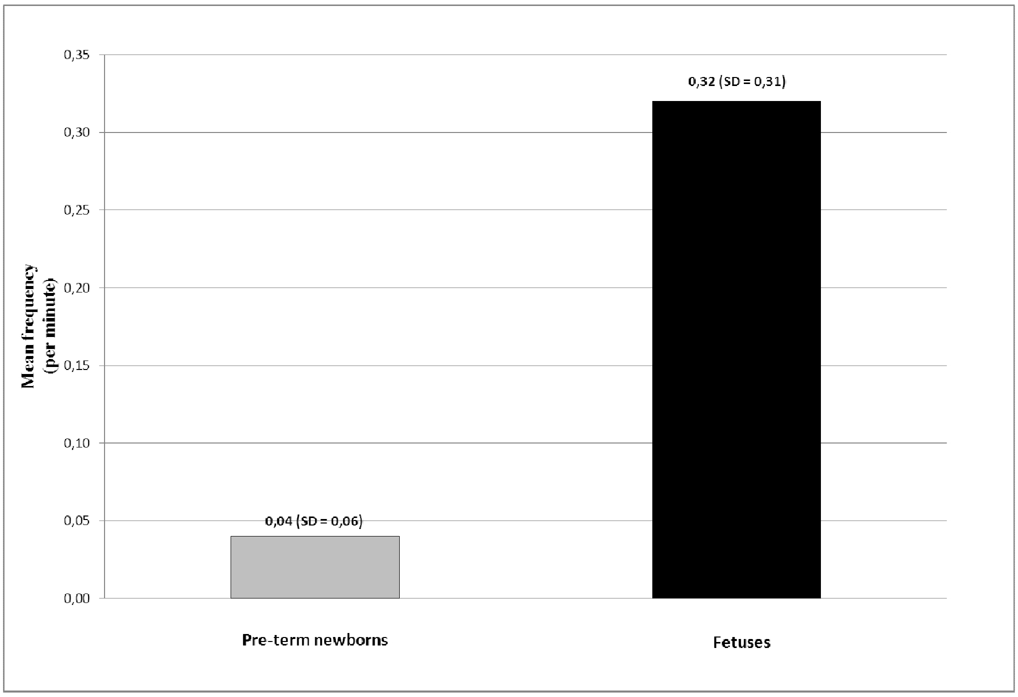


Figure 5. Graphical representation of Hand to face movement mean frequency (per minute) between fetuses and pre-term newborns; SD = Standard Deviation.

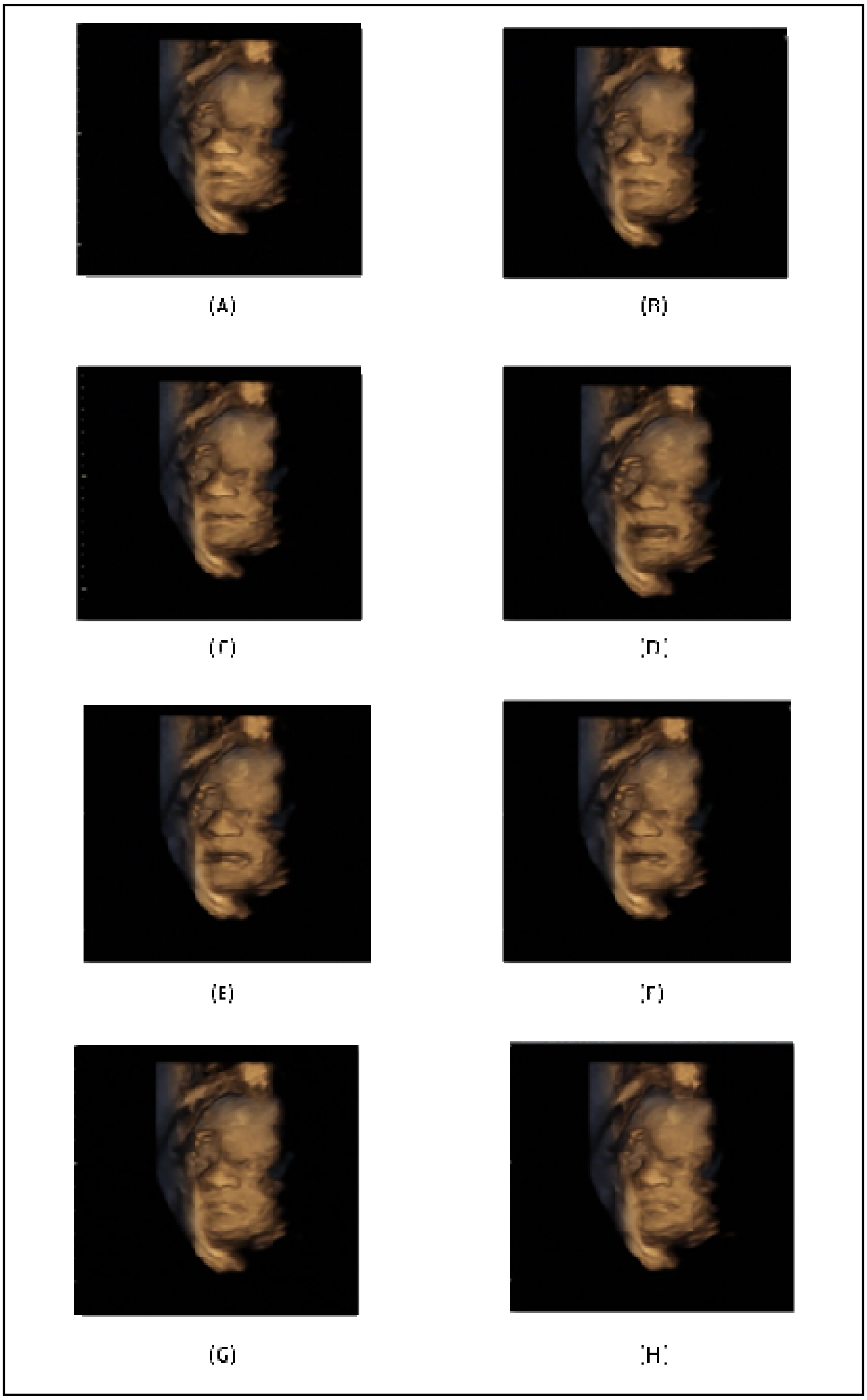


Figure 6. Distress sequence of a 24 weeks-old female fetus is observed from the on-set (Figure A) to the off-set (Figure H). Figure D shows the apex of Distress (see Appendix).

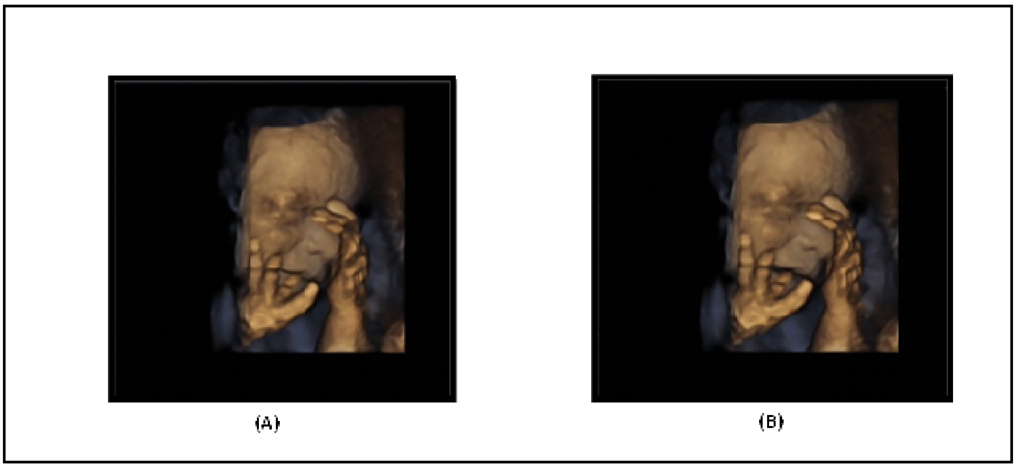


Figure 7. Hand to Face Movement and Distress sequence of a 28 weeks old male fetus. Figure (A) shows the onset and figure (B) shows the apex of Distress.

4.4. Discussion

The statistical comparisons showed significant differences between the two groups in relation to 5 complex behavioral motor patterns: *DISTRESS, STARTLE, BLINK, SWALLOWING and HAND TO FACE MOVEMENTS.*

Distress is described as a typical negative expression that anticipates, accompanies and follows cry (Dondi & Valente, 2013). As a confirmation of the greater immaturity and fragility of preterm infants, there is the increased presence of DISTRESS compared to the group of fetuses at the same gestational age.

Numerous studies investigated the characteristics of the startle spontaneous, occurring usually in form of a baby bump or tremor during sleep (Agnoli et al., 2009). This behavior occurs primarily during the quiet sleep or NREM, although it was also detected in other states (Mc- Namara, Lijowska and Thach, 2002; McNamara, Wulbrand and Thach, 1998). Korner (1969), in particular, in a study of 32 infants term aged between 45 and 88 hours of life, observed during the NREM sleep the spontaneous startle in all subjects analyzed, demonstrating considerable inter-individual stability compared to other behaviors spontaneous detected in the same children (smiles, erections and activities suction).

From 23-26 weeks of gestational age we can observe opening and closing the eyelids in utero, associated with the dopaminergic system (Einspieler et al, 2012). During the third trimester of pregnancy eye movements begin to cluster into bursts and pause, while during the last 10 weeks the percentage of time in which eye movements are absent increases. Behavioral states represent distinct condition of neural activity that change during short transitions. To define a behavioral state it is necessary a number of variables clearly meet criteria at the same time. The state concept is useful to standardize the conditions under which specific measurements of neural activity are observed. Prechtl (1999) in the study of neonatal behavior chose four variables including respiration, body movements, crying and eye movements (open vs closed). Since previous studies on preterm infants (Prechtl et al., 1999) have shown that well-developed state cycles are present few weeks before term, the question is whether the fetal behavioral states are similar to those of a preterm infants at the same age. In 1974 Prechtl defined four different heart-rate-patterns (from A to D) to describe behavioral states in combination with the presence or absence of body and eye movements. The onset of a transition is the beginning of loss coincidence between the variables, and when it ends all the variables synchronize into a new state. As extensive physiological changes occur at birth and afterwards fetal and neonatal states would not be necessarily comparable.

During prenatal life we can observe fetal swallowing that is preparatory for the neonatal ability to ingesting food and functional to the regulation of the amniotic fluid volume in utero. It is also a fundamental pattern for the development of the mandibular as well as the gastrointestinal (Einspieler et al., 2012).

The increased presence of hand to the face movements in fetuses, compared to preterm infants, respond to another factor that affects the quality and specialization of movement ability: the force of gravity. Data concerning the influence of gravity on the development of motor fetal activity are partly contradictory. The fetus' image floating or swimming in the absence of gravity cannot be considered through the entire period of pregnancy. The fetus is not in a meaningful contact with the amniotic sac walls until the end of the pregnancy, but however the sensory inputs, arising from the absence of gravity, are not entirely absent. The level of mechanical stress is necessary for fetal physiological development. Together with the muscular activity, the gravitational load causes mechanical stress. The balance forces apparently reduce the weight, as well as the effect of gravitation on the musculoskeletal system. It is clearly observable that up to 21 weeks of gestation the fetus is in a condition of neutral balance forces, after the 26 weeks is exposed to mechanical stress significantly due to gravitational forces. Therefore, fetal movements can cause the deformation of the amniotic sac of the uterus and the elastic force required to overcome the tension of the walls contributes to the total of the mechanical stresses to which the fetus is exposed. The development of muscle control in the absence of gravity is critical to normal motor development, during the first year of life. After birth, the infant is exposed to the environment, the movement against gravity begins during the first month of life, and from four months of age infants have more control to balance the strength of the extensor muscle. Proper development of the flexion and extension of the trunk is a prerequisite to the development of pelvic tilting front and rear lateral bending of the trunk, as well as the elongation. These components allow the newborn to develop the ability to shift weight, which in turn stimulates the reactions of search for balance.

Our research shows there is not only a behavioral continuity from prenatal to postnatal life, which includes facial expressions, but also that in premature infants the expression of distress is more diffused. Distress is described as a typical negative expression that anticipates, accompanies and follows cry (Dondi & Valente, 2013). As a confirmation of the greater immaturity and fragility of preterm infants, there is the increased presence of *DISTRESS* compared to the group of fetuses at the same gestational age. Another confirmation of the immaturity of preterm birth and his different responses to environmental adaptation comes from the increased presence of the startle. A preterm birth can affect the quality of the movement, as well as the newborn health. The development impact that premature birth and in particular premature birth weight may be greater during infancy, or more generally, in childhood, but may also be extended even into adolescence or adulthood (Rochat, et al., 1988). One of the premature infant's characteristic is the presence of physical activities that do not always correspond to a specific behavioral state. The undetermined behavior reflects the immaturity of the behavioral state organization, and it is possible to see the presence of tasks such as opening and closing eyes intermittently, reflecting the uncertainty or the frequent switching from a behavioral state to another one (*BLINK*).

Swallow movements are controlled by central pattern generators and by sensory feedback. The central pattern generator consists of two distinct parts: the brainstem for motor control and the part surrounding reticular formation for sensory control. Previous studies (Liley, 1972) speculated whether hunger could be the stimulus for fetal swallowing, as, actually, for fetus the amount of amniotic fluid swallowed contributes to the caloric requirement of the fetus.

This experiment is characterized by the development of a new spontaneous behavior coding scale to detect complex behavioral motor patterns (21) observed in fetuses, preterm and full-term newborns. Analysis established that these 21 behavioral motor patterns are sensitive to differences between two groups. Observations on fetal behavior permit to examine different factors that can affect prenatal development at the time of their occurrence (Segal et al, 1995). Unlike premature, it is important to emphasize that the observation of behavior before birth is also less susceptible to the influence of some variables that appear to be most salient during the neonatal period, for example, the stress of birth, gender differences, the potential effects of drugs administered during labor or immediately after birth. This research in based on the concept that the functional repertoire of movement, developed by the neural structure, needs to meet the necessary requirements for the body and the environment in which development takes place.

In sum, these differences can be attributed to difficulty of adaptation to extra-uterine environment (*DISTRESS, SWALLOW, STARTLE*) as well as to preterm immaturity response to this new environment (*BLINK*).

According to the ontogenetic perspective, the adaptive relationship with the environment is an essential factor for the survival of the human body, during every stage of development. Prechtl, therefore, argued that the spontaneous motility, as the expression of neural marker, is a fundamental interpretation of proper brain function. The observation of spontaneous motor activity is the best method to assess the ability of the CNS. All models of endogenous movement can be observed as early as 7-8 weeks of post-menstrual age, achieve development in two or three weeks later, and continue to be present for five or six months after birth. This remarkable continuity endogenously generated from prenatal life to postnatal life, is a great opportunity to identify high-risk children and fetuses, which could emerge in the development of neurological damage (Kurjak et al, 2004). Key Features of motor system development are the flow and the existence of significant variations in the complexity of the movement. The quality of each single movement includes the speed, the amplitude and strength; moreover, it seems that the assessment of the quality of postnatal movements can be an important window for the early detection of children at high risk of developmental disorders. Kurjak and colleagues (2004) have recently conducted a study with the help of 3D and 4D ultrasound confirming that there is a pattern of continuity in behavior from prenatal life to postnatal life, even if the conditions for the child after birth are very different from those of the fetus, mainly due to the gravity force. The results showed, however, a certain continuity in the behavior pre-and postnatal having no significant difference between the two groups examined in relation to 21 different categories of observation. The statistical comparisons have revealed, however, some interesting differences in the frequency of appearance of certain behaviors. We could say that although the development of the CNS is long and complex, there is a continuity that comes from life in the prenatal and postnatal continues, in different ways according to the particular conditions in which the event of the birth.

The activity of the CNS is reflected in different types of movement, and emerges from different parts of the brain. The environmental conditions can greatly affect the expression of motion in the transition from prenatal to the postnatal life. The microgravity environment intrauterine, for example, is probably a favorable condition to the fetal motor development, considering that extra-uterine environment requires a different type of adaptation and development of muscle control in response to gravity. We know that in some cases, difficulty in growing of fetal CNS may adversely affect the neuro-fetal behavior, cognition, and other functions of development. It is important to note that in the course of this experiment, it was not possible to observe all the movements described by the scale of criteria specifically designed: 1. Rooting; 2. Occipito – frontalis reflex; 3. Chin tremble; 4. Shivers; 5. Yawning; 6. Startle; 7. Sneezing; 8. Hiccups; 9. Swallow; 10. Drooling; 11. Hand to the face movements; 12. Finger-sucking; 13. Retro-flexion of the head; 14. Rotation of the head; 15. Ante-flexion of the head; 16. Blink; 17. Mouthing movements; 18. Non-rhythmic mouthing movements; 19. Tongue expulsion; 20. Smile; 21. Distress. However, it is not to be excluded that one of its possible causes can be attributed to the recordings duration, rather than the small amount of the sample.

Regarding to results, it can be argued that the concept of continuity in the development of fetal motor system is totally paradoxical. We know that the fetus is in possession of many skills, and most likely this repertoire is dependent upon post-natal life, but since the birth represents the most important change from intra-uterine to extra-uterine life, the transition from prenatal to postnatal represents also the event of major disruption to which we can assist. By closely observing the individual movement patterns present both before and after birth, we can argue that behavior is continuous, as well as the activity and the development of the central nervous system. It is completely discontinuous The occurrence of behaviors that meet requirements of dynamic integration between different systems as well as, the force of gravity, the relationship with the external environment, the stresses derived by maternal care and the resulting plasticity of CNS development in the process of adaptation to the world, that is unique for each human being.

5. Experiment II: Observing spontaneous behavior in preterm infants before and after meal

5.1. Introduction

The neuroscience describe the concept of control as the complex and hierarchical relations between the three core brain systems, brainstem, limbic, and cortical, that cooperate in the organization of behavioral output or enable consciousness (Feldman, 2009). Although the concept of regulation has not been fully defined, it is clear that it necessarily implies a systemic perspective. Therefore, researchers that have studied the early development emphasizes the role that cooperation plays in the process of adjustment of functions and influences, that arises in part from the child and in part from the outside world (Damasio , 2003). Moreover for the adjustment of these functions, it is necessary the integration of a hierarchical system that also includes the time, to synchronize the process of self-regulation and co-regulation of the different integrating systems.

The circadian rhythm is a system monitored by a biological clock located in a small region of the hypothalamus called the supra-chiasmatic nucleus. The cardiac vagal tone measures the effects of breathing on heart rate variability mediated by the parasympathetic system. From the middle of pregnancy and until the end, the biological clock and the trajectories of vagal tone are measured weekly and they cooperate in the adjustment of the sleep wake cycles (Holditch-Davis & Edwards, 1998). After a preterm birth the temporal organization of circadian rhythms cycles develops and preterm newborn exhibits changes in the duration of sleep-wake rhythms, frequencies and in percentages of the transitional states.

Recent studies suggest that probably both the duration of the different behavioral states and the alternation of sleep-wake rhythms are not fully stabilized in preterm newborn and that the different rates of development can vary, depending on the characteristics of the child and on the environment (Lehtonen & Martin, 2004). Therefore, even if the development of a behavioral state is biologically determined, for preterm infants the sleep-wake cycles can be influenced by several environmental factors and they are subject to changes (Holditch-Davis & Edwards, 1998). The environmental differences are an example of how the epigenetic mechanism may be involved in the trajectories of development (Gottlieb, 1996). In particular, nowadays, we can also assume that the adjustment of the average duration and frequency of the rhythms of sleep and wakefulness, depend on the type of medical and nursing care received by preterm infants (Thoman & Ingersoll, 1999).

Subsequent researches on the observation of behavior should be able to further specify the role that the concept of regulation plays in the development and what kind of implications are involved in the interaction between multiple systems, mediated by brain and relative to pain, appetite or to the passing of time.

In order to understand the preterm behavioral characteristics we wonder what kind of conditions can affect the development of behavioral states? What kind of behaviors and behavioral states are more exhibited?

The systematic study of spontaneous motor activity, during the early stages of development has provided important data for the early assessment of preterm and term newborns. Observing fetal movements, for example, it has been possible to note that an atypical motor pattern, characterized by monotonous, stereotyped and less fluent movements, can predict adverse neurological outcomes. Behavior follows specific trajectories and is sensitive to functional, physiological, and motivational changes (Prechtl et al., 1989; Thelen, 2000).

The objective of this study is to observe spontaneous behavior in two groups of preterm newborns, through the analysis frame-by-frame of video recordings conducted before and after the meal.

The theory of dynamical systems in the study of motor development emphasizes that changes in spontaneous motor pattern can be determined by any interaction factors, such as biomechanics growth, functional, motivational or cognitive changes (Kamm et al.,, 1990). Thanks to the studies conducted by Wolff, Korner and Prechtl we know that in full-term newborns the spontaneous behavior is modulated by behavioral states. Therefore, all the neurological and neuro-behavioral scales, commonly used in the clinical practice, describe the different behavioral states on the basis of the spontaneous behavior that the child exhibits during the examination.

Hopkins & Prechtl (1984) already hypothesized a relationship between hunger and coordination of spontaneous hand-mouth movements. These observations are based on a previous study of Rochat et al. (1988), according to which the release of sucrose on the tongue corresponded to a reduction of general movements, and an increase of movements focused on the region of mouth. They realized that the frequency of hand-mouth contact was significantly higher after the release of sucrose. The meaning of this behavior comes from the activation of a posture of suction (as at the beginning of meal), in which the arm rests on the mother's breast. In the absence of the breast the baby moves his hand to his mouth. After this study, Lew and Butterworth (1995) conducted a research to investigate the effect of hunger on the face-hand coordination or hand-to-mouth contact. They observed differences in facial contact before and after meal, and found a relationship between movements before opening the mouth and the next hand-mouth contact. In conclusion, before feeding the effect of hunger caused the anticipation of the mouth opening near the region of the hand-mouth contact. This result means that hunger is a motivating factor for the spontaneous hand-mouth contact, and that it involves the participation of the mouth. We could also assume there is a motivational threshold level for the mouth movement that anticipates the arrival of the hand (Butterworth & Lew, 1995). The appetite works as a motivational factor to the implementation of specific behaviors.

The hypothesis of the presence of changes in the distribution of specific motor patterns before and after meal is based on previous studies, that reveal a relationship between the intake of specific foods and brain function. For example, it was found that infants with a low birth weight and unusually fed by high-protein foods, had lower results on intelligence tests at school age, than those fed by less protein foods (Shulze, et al., 1995). Moreover, the intake of nutrients generates the activation of the sympathetic system, as in case of particular proteins during active sleep.

Our study focuses on behavioral differences already present in two groups of preterm infants of 36 weeks and 40 weeks of gestational age, recorded before and after meal. The goal is the observation of spontaneous behavior by a new coding scale, especially designed for the detection of behavioral categories originally described in fetuses, preterm and full-term newborns by de Vries (1982), Prechtl (1985), Kurjak et al. (2003), Einspieler et al. (2008) and Wolff (1987).

The description of the observational categories was conducted using as a starting point the Facial Action Coding System (FACS) by Ekman and Friesen (Ekman and Friesen,1978; Ekman, Friesen & Hager, 2002), with the insights and information added by Harriet Oster (Baby FACS in press). Both of these methods have shown a documented reliability in the detection of individual units of facial actions, in the first months of life as well as in preterm born infants at a very low gestational age (Dondi et al., 2008). The interest in coding facial spontaneous behavior, comes from the early ontogeny of the facial mimic muscles that develop from the 16th week of gestational age, and are directly connected to the activity of the V° and VII ° cranial nerve (Fitzgerald & Windle, 1942; Hooker, 1958; Humphrey, 1964, 1971) (See

Figure 1: Motor root of trigeminal nerve)

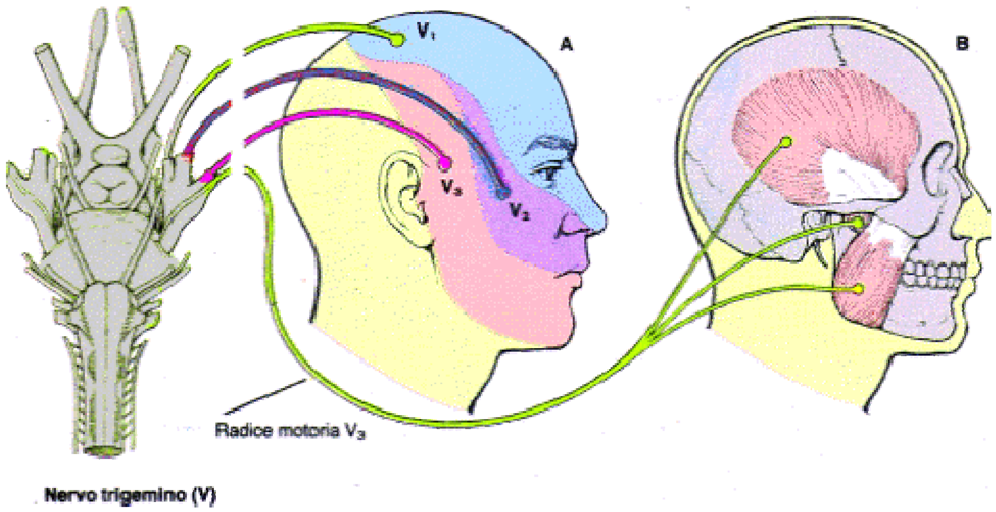


Figure 1: Motor root of trigeminal nerve (Yale University School of Medicine)

Following the descriptions coming from Baby FACS, we developed a coding scale made by 21 behavioral motor patterns described objectively and in detail, in order to ensure the reliability regardless of the subjective interpretation of individual coder. This new coding scale, based on the micro- analysis of individual muscles activity described by Harriet Oster in Baby FACS (2009), was already tested during the first experiment to compare fetuses and preterm newborns. Moreover, since it has already been demonstrated a great sensibility in detecting differences in fetal and preterm spontaneous behavior, this contribution is part of the current scientific debate on the study of behavior as a new proposal to detect also differences in preterm newborns behavior.

The value of these observations is twofold, since it opens the way for a new approach both scientific and diagnostic. The most recent studies conducted on central nervous system activity have been focused on the general movements of the body, and on the rules that regulate the so-called peripheral behaviors such as facial expressions, in order to identify additional evaluation criteria to understand the neuro-behavioral conditions of the preterm newborn (Kurjak et al., 2010).

5.2. Method

5.2.1. Participants

Then preterm and full-term neonates were examined when their post-conceptional ages were between 33 and 42 weeks (*M* = 36.90, *SD* = 3.178). Their gestational ages at birth were between 27 and 40 weeks (*M* = 33.20, *SD* = 4.59), and their weights at birth were between 980 and 3010 gr. (*M* = 1693.50, *SD* = 717.95) appropriate for gestational age (AGA; *Appropriate for Gestational Age*; Lubchenco, 1976).

None of them had serious disease at birth that involved the heart, the brain and the organs of sense. In particular, the exclusion criteria were: chromosomal abnormalities, heart disease or other congenital abnormalities, fetal infections, metabolic disorders, obvious teratogenic factors, Apgar at 5 min. under 6, AND bleeding of III° and IV° degree. The video were recorded at the Division of Neonatology and Neonatal Intensive Care Unit of the Arcispedale Sant'Anna in Ferrara. Infants were observed both while they were in the NICU and when they were in Neonatology, in pre-discharge. Written informed consent was obtained for all participants.

5.2.2. Procedure

All babies have been filmed twice, respectively before and after the first afternoon meal at 3 p.m.. Video-recordings had a duration between 20 and 40 minutes. Particular attention was paid to the position of the head, since the objective was to get a close-up of the face for the duration of the observation. Codings and data analysis were conducted at the Early Infancy Lab, Ferrara University.

5.2.3. Coding spontaneous motor behavior

The analysis of video recordings was conducted frame by frame independently by two expert coders in behavioral micro-analysis. They separately viewed the video material and, with the aid of a timer, identified in sequences videotaped the presence of the following behavioral motor patterns:*1. Rooting; 2. Occipito – frontalis reflex; 3. Chin tremble; 4. Shivers; 5. Yawning; 6. Startle; 7. Sneezing; 8. Hiccups; 9. Swallow; 10. Drooling; 11. Hand to the face movements; 12. Finger-sucking; 13. Retro-flexion of the head; 14. Rotation of the head; 15. Ante-flexion of the head; 16. Blink; 17. Mouthing movements; 18. Non-rhythmic mouthing movements; 19. Tongue expulsion; 20. Smile; 21. Distress.* Objectives aspects that ensure recognition of observational basis are reported in detail in the Appendix.

5.2.4. Reliability

We calculated the agreement between the two coders using the Cohen's Kappa coefficient, within a time window of 5 seconds. The agreement between the two observers in identifying the action was good.All disagreements were discussed and they have been solved. Statistical analyzes were conducted on the basis of a common protocol agreed between the two coders.

5.2.5. Data Analysis

Data analysis were conducted on the frequency of occurrence per minute calculated for each of the 21 behavioral motor patterns. This measure was made necessary by the different duration of the video recordings, and it was obtained by dividing, participant per participant, the total number of observations of each motor pattern into the total duration of the video recordings. According to the internal variability characterizing behavior in preterm newborns and taking into account the sample size, comparisons between the two groups (preterm *vs* full-term newborns) were performed using the non-parametric U-test by Mann-Whitney for independent samples and the non-parametric test by Friedman, for dependent samples. The newborns sample was splitted in two groups on the basis of: 1) the age of the infants at the time of testing; 2) the gestational age at birth, and 3) the birth weight. Then, three distinct series of analyses have been conducted.

5.3. Results

Age at the time of testing

The sample of newborn infants has been divided into two groups based on postconceptional age at the time of testing. In particular, Group 1 included infants between 32 and 36 weeks post-conceptional (*M* = 34.20, *SD* = 1.30); Group 2 infants of age between 38 and 42 weeks (*M* = 39.60, *SD* = 1.67). Table 2 shows for preterm and full-term newborns the mean frequencies per minute (*M*) and standard deviations (*SD*) registered for each behavioral motor pattern observed before and after meal. Regarding the sample of preterm infants, the most frequently observed behavior was *DISTRESS* (Group 1: *M* = 1.79, *SD* = 0.73; Group 2: *M* = 1.15, *SD* = 0.44). During the period of observation, none of the following categories: *Occipito-frontalis-reflex, Shiver, Sneezing* and *Head Ante-Flexion* has been coded. A first series of statistical analysis conducted with the Mann-Whitney U test and the Friedman test compared the youngest infants group with the oldest infants group both between and within groups.

TABLE 2
Behavioral motor patterns coded in Group 1 (M = 34 GA) and Group 2 (M = 39 GA) observed before and after meal (GA = Postconceptional Age at the time of testing)

		Rooting		*Chin tremble*		*Yawning*		*Startle*		*Hiccup*		*Swallow*	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
34 GA	**Before Meal**	0.00	0.02	0.00	0.00	0.10	0.11	0.16	0.22	0.00	0.01	0.01	0.02
	After Meal	0.00	0.00	0.00	0.01	0.03	0.06	0.24	0.19	0.14	0.31	0.00	0.00
39 GA	**Before Meal**	0.00	0.00	0.00	0.01	0.10	0.09	0.03	0.04	0.00	0.00	0.00	0.00
	After Meal	0.13	0.27	0.00	0.00	0.10	0.14	0.13	0.17	0.81	1.82	0.00	0.00

		Drooling		*Hand to face movement*		*Finger - Sucking*		*Head retro-flexion*		*Head rotation*	
		M	SD	M	SD	M	SD	M	SD	M	SD
34 GA	**Before Meal**	0.03	0.06	0.35	0.18	0.07	0.10	0.02	0.02	0.51	0.33
	After Meal	0.00	0.01	0.14	0.29	0.04	0.08	0.07	0.09	0.26	0.16
39 GA	**Before Meal**	0.10	0.22	0.19	0.16	0.10	0.10	0.03	0.03	0.49	0.42
	After Meal	0.02	0.04	0.35	0.53	0.03	0.05	0.02	0.04	0.24	0.38

		Blink		*Mouthing movements*		*Non-rhytmical mouthing movements*		*Tongue protrudes*		*Smile*		*Distress*	
		M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
34 GA	**Before Meal**	0.76	0.46	0.50	0.18	0.00	0.00	0.09	0.09	0.11	0.08	1.79	0.73
	After Meal	0.50	0.48	0.14	0.12	0.08	0.11	0.05	0.11	0.13	0.11	1.06	0.55
39 GA	**Before Meal**	0.54	0.36	0.34	0.21	0.03	0.04	0.11	0.17	0.16	0.08	1.15	0.44
	After Meal	0.21	0.21	0.55	0.67	0.03	0.03	0.19	0.37	0.19	0.22	0.52	0.43

Note. Behavioral motor patterns coded in Group 1 and Group 2 of preterm infants. In vertical column Means (M) and Standard Deviations (SD) refer to the frequency per minute registered for each motor pattern observed in both groups before and after meal. Statistical comparisons are found in the text.

Contrasts between groups

The statistical analysis performed by the Mann-Whitney U test, did not show a significant difference between the two groups in relation to the 21 behavioral motor patterns observed.

Contrasts within groups

The statistical analysis performed by the nonparametric Friedman test showed significant differences between the behavior exhibited before and after meal. In particular, infants exhibited a significant difference in the Mean frequency per minute of the *MOUTHING MOVEMENTS*, between the observations conducted before meal (*M* = 0.50, *SD* = 0.18) and after meal (*M* = 0.14, *SD* = 0.12) *Fr* = 5, *p* = .025, *df* = 1; (see **Errore. L'origine riferimento non è stata trovata.**). Moreover, they exhibited a significant difference in the Mean frequency per minute of the *HEAD ROTATION*, between the observations made before (*M* = 0.49, *SD* = 0.42) and after meal (*M* = 0.24 , *SD* = 0.38) *Fr* = 5, *p* = .025, *df* = 1; (see **Errore. L'origine riferimento non è stata trovata.**). Both groups (Group 1 and Group 2) showed a significant difference in the exhibition of *DISTRESS* between recordings made for Group 1 before meal (*M* = 1.79, *SD* = 0.73) and after meal (*M* = 1.06, *SD* = 0.53), *Fr* = 5, *p* = .025, *df* = 1 and the Group 2 before meal (*M* = 1.15, *SD* = 0.44) and after meal (*M* = 0.52, *SD* = 0.43) *Fr* = 5, *p* = .025, *df* = 1; (see **Errore. L'origine riferimento non è stata trovata.**).

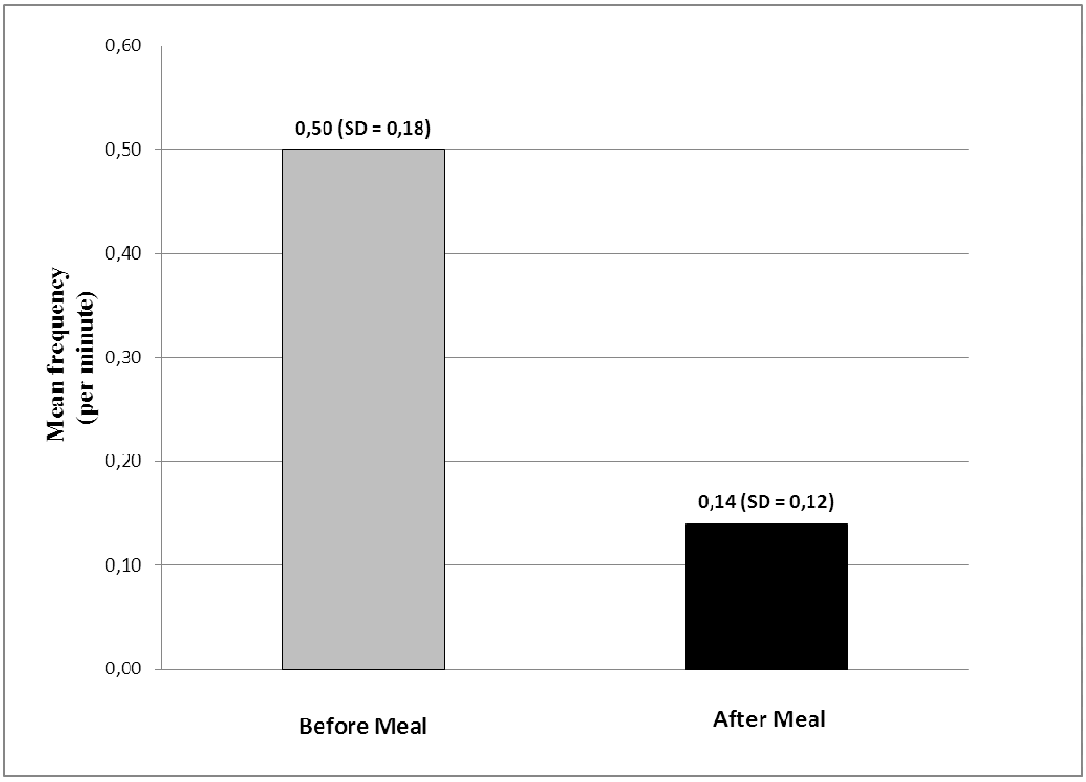


Figure 2. Graphical representation of Mouthing Movements mean frequency (per minute) of newborns at 34 weeks postconceptional at birth before and after meal; SD = Standard Deviation.

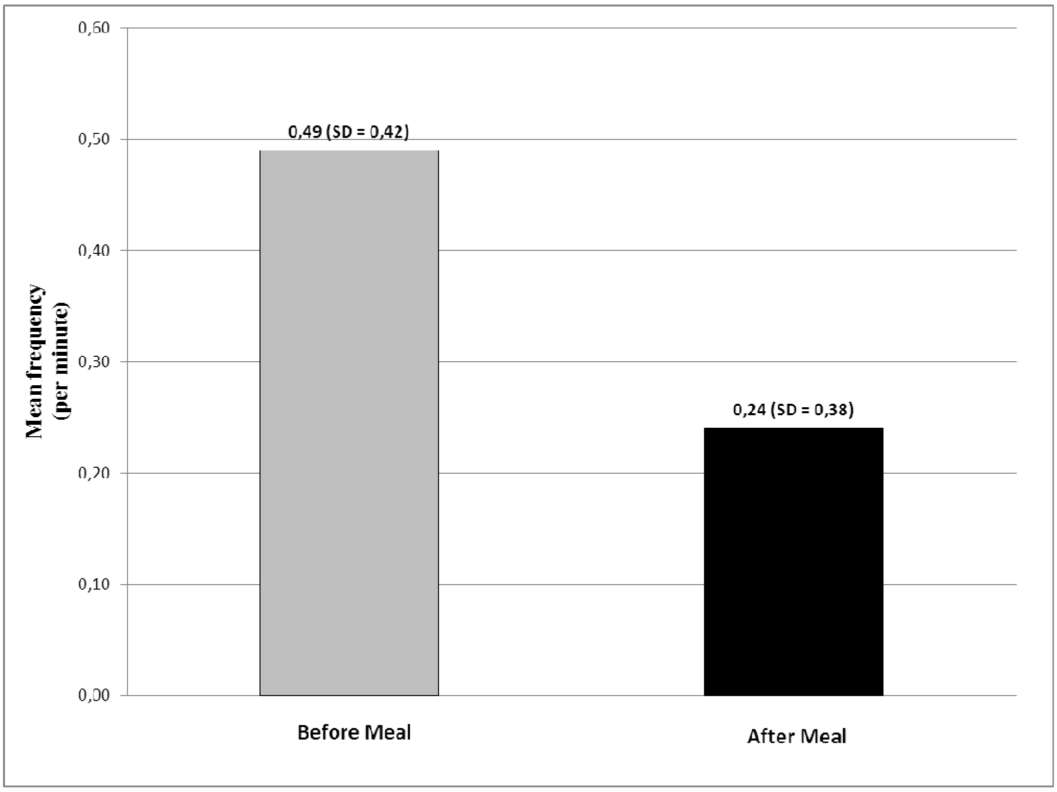


Figure 3. Graphical representation of Head Rotation mean frequency (per minute) of newborns at 39 weeks postconceptional age at the time of testing , at different birth-weight and at different birth-age before and after meal; SD = Standard Deviation.

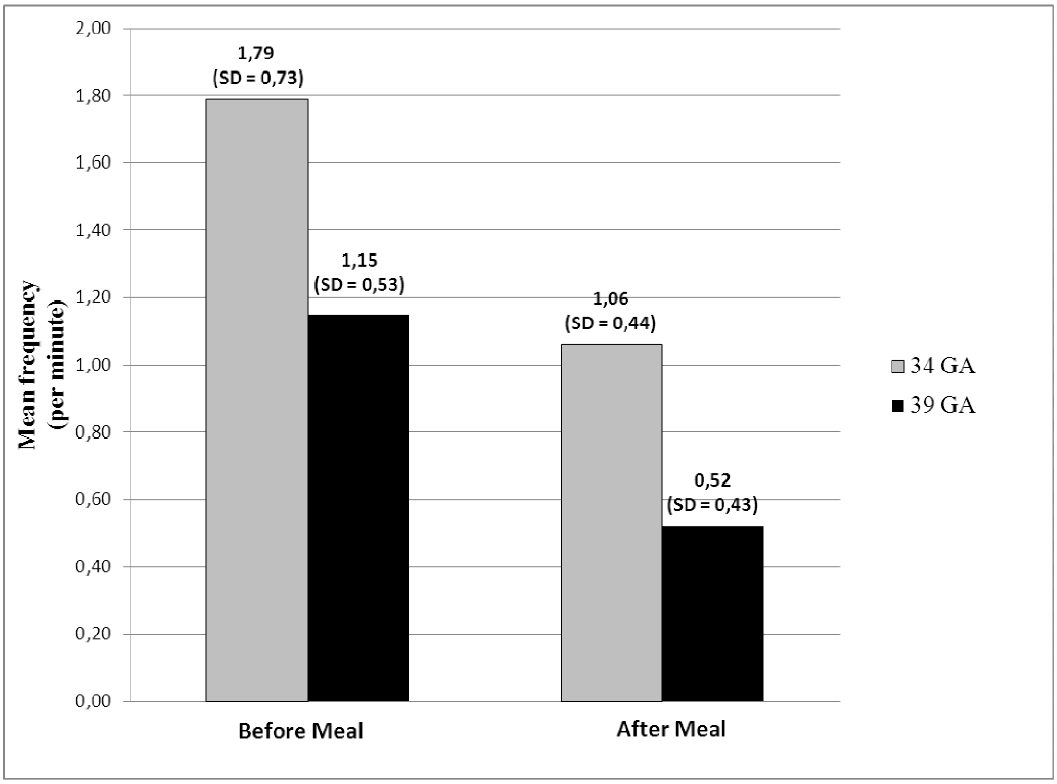


Figure 4. Graphical representation of Distress mean frequency (per minute) between newborns at different postconceptional age at the time of testing, at different birth-age and at different weight-age before and after meal; SD = Standard Deviation.

Gestational Age (GA) at birth

The sample of newborn infants has been divided into two groups based on gestational age at birth. In particular, Group 1 younger at birth (M = 31.40, SD =2.61) and Group 2 older at birth (M = 35.00, SD = 5.70). Table 2 for both groups the mean frequencies per minute (*M*) and standard deviations (*SD*) registered for each behavioral motor pattern observed before and after meal. A first series of statistical analysis conducted with the Mann-Whitney U test and the Friedman test compared the youngest infants group with the oldest infants group between and within groups.

Contrasts between groups

The statistical analysis performed by the Mann-Whitney U test, showed a significant difference between the two groups in relation to *STARTLE*. Before meal younger newborns at birth exhibited startle more frequently (*M* = 0.16, *SD* = 0.22) than the older newborns at birth (*M* = 0.03, *SD* = 0.04) *U* = 24, *p* = .010 *df* = 1.(see Figure 5)

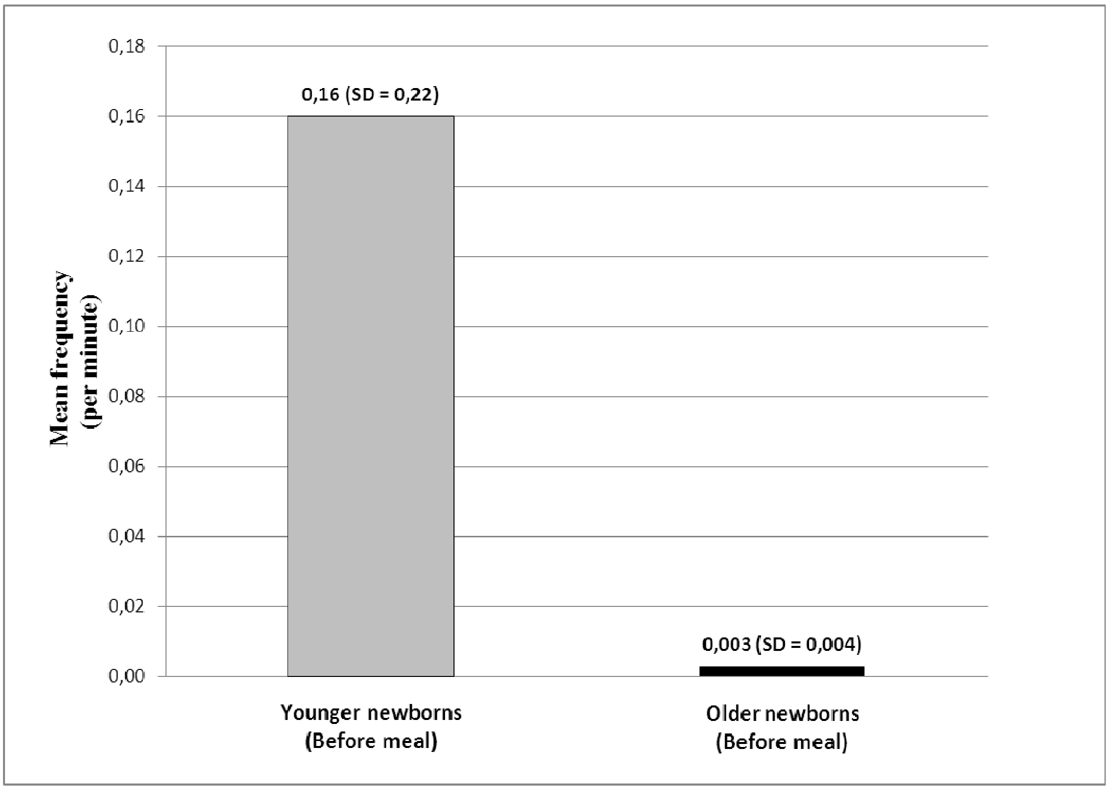


Figure 5. Graphical representation of Startle mean frequency (per minute) of newborns at different birth-age before meal; SD = Standard Deviation.

Contrasts within groups

The statistical analysis performed by the nonparametric Friedman test showed significant differences between the behavior exhibited before and after meal. In particular, the older newborns at birth exhibited a significant difference in the Mean frequency per minute of the *HEAD ROTATION*, between the observations conducted before meal (*M* = 0.49, *SD* = 0.42) and after meal (*M* = 0.24, *SD* = 0.38); (*Fr* = 4, *p* = .046, *df* = 1); see Figure 3. Both groups (Group 1 and Group 2) showed a significant difference in the exhibition of *DISTRESS* between recordings made for Group 1 before meal (*M* = 1.79, *SD* = 0.73) and after meal (*M* = 1.06, *SD* = 0.55), (*Fr* = 6, *p* = .014, *df* = 1) and the Group 2 before meal (*M* = 1.15, *SD* = 0.44) and after meal (*M* = 0.52, *SD* = 0.43), *Fr* = 6, *p* = .014, *df* = 1); see **Errore. L'origine riferimento non è stata trovata.**.

Birth-weight

The sample of newborn infants has been divided into two groups based on weight at birth. In particular, Group 1 weighed less at birth (*M* = 1417 gr., *SD* = 471.48) and Group 2 weighed more at birth (*M* = 1970 gr., *SD* = 863.92). Table 2 shows for both groups the mean frequencies per minute (*M*) and standard deviations (*SD*) registered for each behavioral motor pattern observed before and after meal. A first series of statistical analysis conducted with the Mann-Whitney U test and the Friedman test compared the infants weighed less at birth to the infants weighed more at birth between and within groups.

Contrasts between groups

The statistical analysis performed by the Mann-Whitney U test, showed a significant difference between the two groups in relation to *SMILE.* After meal newborns weighed more at birth exhibited smile more frequently (*M* = 0.19, *SD* = 0.22) than the newborns weighed less at birth (*M* = 0.13, *SD* = 0.11) *U* = 0, *p* = .009 *df* = 1.(See Figure 6).

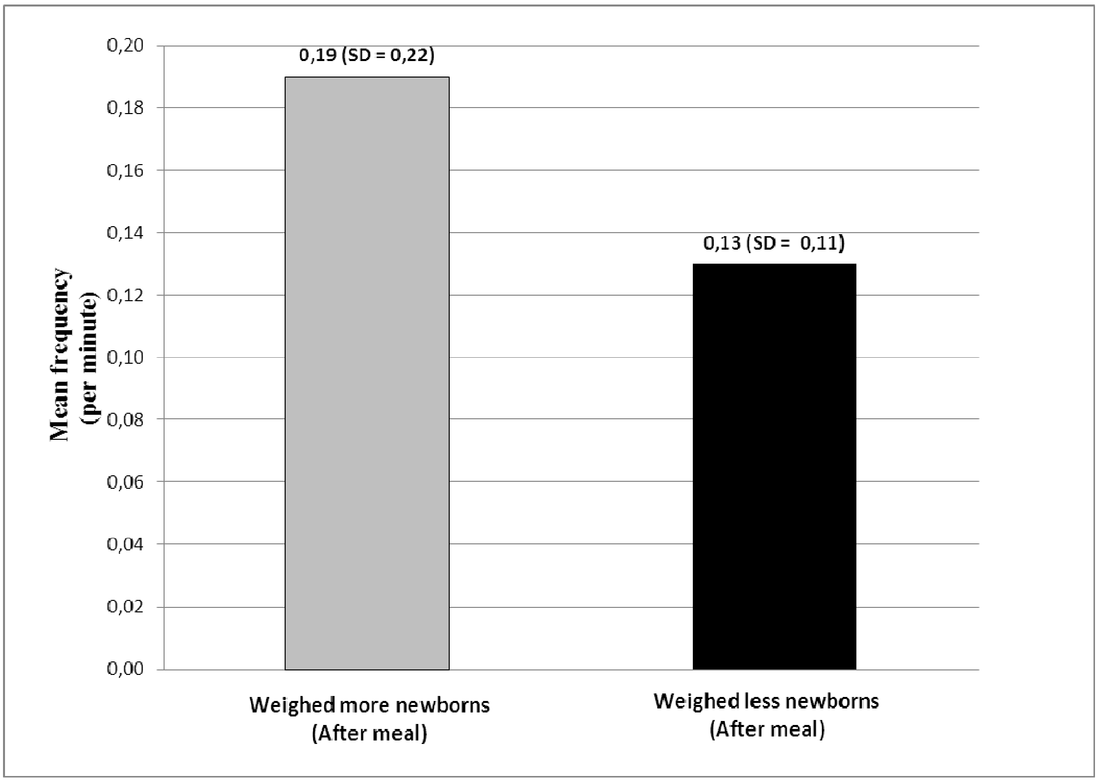


Figure 6. Graphical representation of Startle mean frequency (per minute) of newborns at different birth-weight after meal; SD = Standard Deviation.

Contrasts within groups

The statistical analysis performed by the nonparametric Friedman test showed significant differences between the behavior exhibited before and after meal. In particular, the newborns weighed less at birth exhibited a significant difference in the Mean frequency per minute of the *STARTLE* between the observations conducted before meal (*M* = 0.16, *SD* = 0.22) and after meal (*M* = 0.24, *SD* = 0.19); *Fr* = 5, *p* = .025, *df* = 1; see Figure 7. Moreover, the newborns weighed less at birth showed a significant difference of *BLINK* between the observations conducted before meal (M =0.76, SD = 0.46) and after meal (M = 0.50, SD = 0.48) *Fr* = 5, *p* = .025, *df* = 1; see Figure 8. The newborns weighed more at birth showed a significant difference of *HEAD ROTATION* between observations conducted before meal (M = 0.49, SD =0.42) and after meal (M = 0.24, SD = 0.38), *Fr* = 5, *p* = .025, *df* = 1; see Figure 3. Both groups (Group 1 and Group 2) showed a significant difference in the exhibition of *DISTRESS* between recordings made for Group 1 before meal (*M* = 1.79, *SD* = 0.73) and after meal (*M* = 1.06, *SD* = 0.55), (*Fr* = 5, *p* = .025, *df* = 1) and the Group 2 before meal (*M* = 1.15, *SD* = 0.44) and after meal (*M* = 0.52, *SD* = 0.43), *Fr* = 5, *p* = .025, *df* = 1); see **Errore. L'origine riferimento non è stata trovata.**.

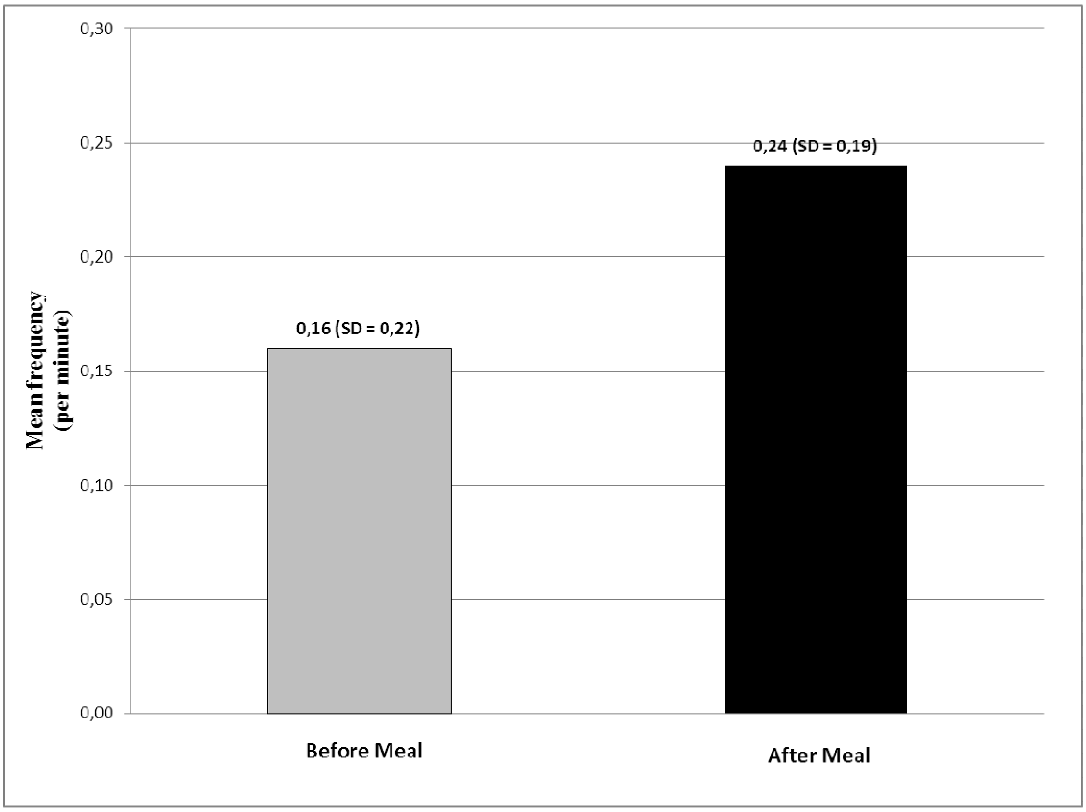


Figure 7. Graphical representation of Startle mean frequency (per minute) of newborns weighed less at the birth before and after meal; SD = Standard Deviation.

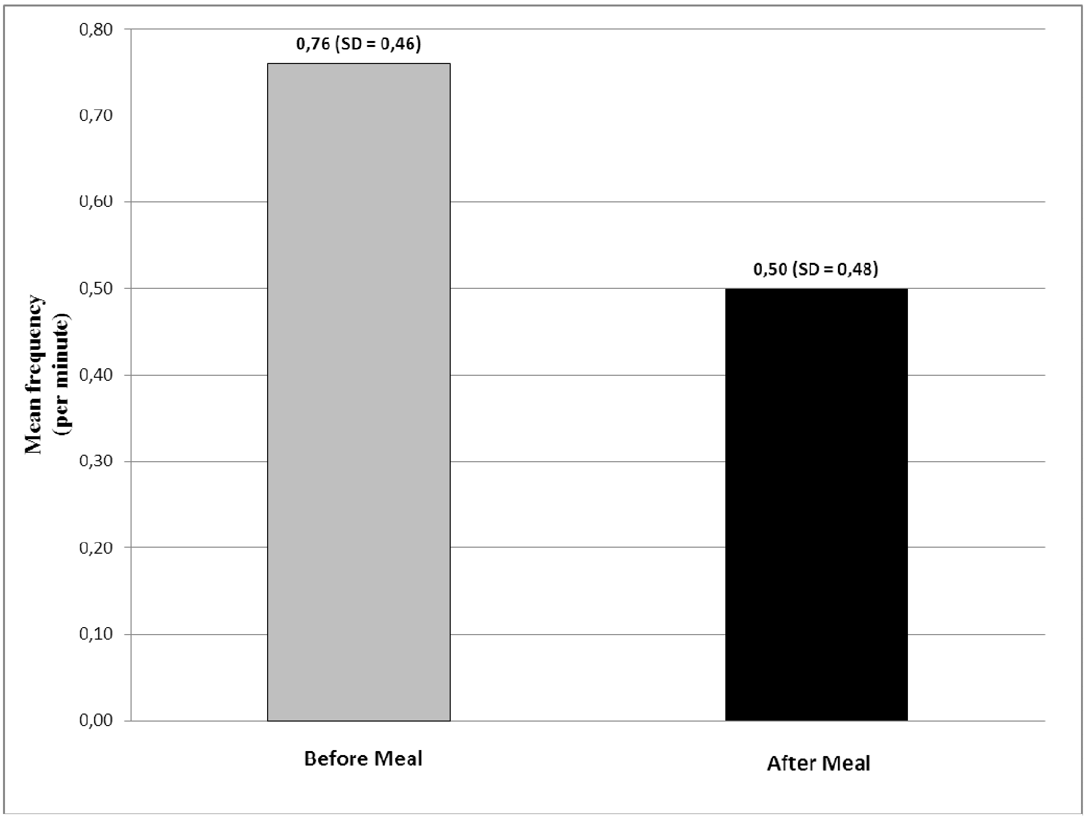


Figure 8. Graphical representation of Blink mean frequency (per minute) of newborns weighed less at the birth before and after meal; SD = Standard Deviation.

5.4. Discussion

This work is focused on the observation of spontaneous motor behavior before and after meal, in order to detect the modulation of the motor activity in response to the appetite condition as a basic need. For this purpose the new coding scale, already tested in previous experiment, was performed to detect 21 specific behavioral motor patterns.

Post-conceptional age contrasts

In post-conceptional age contrasts (Mann Whithney U-Test) observed before and after meal analysis did not show significant results. However, a possible cause of these results can be attributed to the small amount of the sample. As regard to birth-weight analysis (Man Whitney) more mature infants showed that smile was more exhibited after meal than before meal. In post conceptional age contrasts (Friedman) *DISTRESS* was more exhibited for both groups before meal and more mature newborns showed *HEAD ROTATION* more frequent before meal. *MOUTING MOVEMENT* before meal was more showed by more immature newborns.

Birth-age contrasts

In birth-age contrasts conducted by Mann Whithney U-Test the younger newborns showed more *STARTLE* before meal than the older newborns. In birth age contrasts conducted by Friedman Test the older newborns showed more frequent *HEAD ROTATION* before meal than after meal. Both groups showed more *DISTRESS* before meal than after.

Birth-weight contrasts

In birth-weight contrasts conducted by Mann Whithney U-Test newborns weighed more at birth exhibited *SMILE* more frequent after meal than those weighed less. In birth-weight contrasts conducted by Friedman Test the newborns weighed less showed more frequent *STARTLE* after meal than those weighed more at birth. Moreover, they showed more *BLINK* before meal, while the newborns weighed more at birth showed more *HEAD ROTATION* before than after meal. Both groups showed more *DISTRESS* before than after meal.

Distress is described as a typical negative expression that anticipates, accompanies and follows crying (Dondi & Valente, 2013). In preterm newborns this behavior represents the early adjustment effort to postnatal life. In this study, the difference in the observations made before and after meal could be representative of the motivational condition, or of the fulfillment of the primary feeding need.

The presence of *MOUTHING MOVEMENTS* in premature infants is usually due to the immaturity of the motor system, which is reflected in the repetition of sucking rhythmic movements (Lecanuet *et al.*,1995), and that can vary from 2 to 6. From the ontogenetic perspective the development of this behavioral pattern plays an important role on the mechanism of self-regulation and of crying inhibition (Oster, 2005).

Another confirmation of the effects of appetite on behavior of preterm newborns comes from the different distribution of the *HEAD ROTATION* before and after meal. The development of muscle control in the absence of gravity is critical for the motor development during the first year of life.

After birth the infant is exposed to the environment, the movement against gravity begins during the first month of life, and only after four months newborns have more control to balance the strength of the extensor muscle. A proper development of the trunk flexion and extension is a prerequisite to the development of the anterior and posterior pelvic tilt and the lateral flexion of the trunk, as well as the elongation. These components allow the child to develop the ability to weight shift, which stimulates the search for balance.

The principal component of a simple smile is the *zygomaticus major* muscle action (AU12). This muscle emerging from the zygomatic arch in the cheekbones and attached to the lip corners, raises the lips corners obliquely and upward toward the cheek bones. Pulling back and upward the lips corners, it creates an angular shape to the mouth. According to Baby-FACS, we can call this action "lip corner puller". In a strong AU 12, we can see infraorbital triangle pushed upward more evident, eye aperture narrowed by pushing up the cheek and the skin below the lower lid. Duchenne's smile involves contraction of *orbicularis oculi pars orbitalis* (AU6) raising the cheeks and drawing the skin below the eyes. Endogenous smile is superseded by elicited smile around 6-8 weeks after birth or at a corresponding conceptional age for preterm infants (Einspieler et al. 2012). According to Wolff (1987) the cause of spontaneous smile in sleeping is very different from social smile of six-week-old waking infants to face and voice. Behavioral states are crucial organizers of infant behavior (Wolff, 1987). Like other early spontaneous motor patterns exhibited during neonatal sleep, such as startles, mouthing, and reflexive sucks, neonatal smiling is thought to occur in the absence of recognized external or internal (visceral) stimuli (Korner, 1969;Wolff, 1987). For this reason, neonatal smiling is known as *reflexive, spontaneous,* or *endogenous smiling* (Fogel & Thelen, 1987; Sroufe, 1979, 1996;Wolff, 1987). Observational evidence suggests that newborn smiling is associated with the behavioral state of active sleep (irregular, paradoxical, or rapid eye movement [REM] sleep) and drowsiness. Early research indicated, in fact, that neonatal smiling occurred almost exclusively during REM states (Emde & Koenig, 1969) and never or very rarely while the infant was fully awake or deeply asleep (quiet sleep). For these reasons, REM endogenous smiling is considered one of the most well-circumscribed state-related behaviors found in the neonatal period (Emde & Harmon, 1972; Emde & Koenig, 1969).During early development preterm newborn behavioural states are immature although their cyclicity is clearly seen. Over preterm period temporal organization of sleeping and waking cycles develops and preterm newborns exhibit changes in sleep-wake lengths, frequencies and their percentages of transitional states. While there is an increase of bout lengths, we can observe an decrease of their frequencies. Recent researches suggest probably both lengths and frequencies of sleeping and waking states are not already set during preterm period and the developmental patterns can vary depending on infant characteristics and environmental circumstances.

Therefore, although the developmental patterns of each state is biologically determined, preterm infants sleep-wake cycle may be influenced by different interventions and it is subject to variation. These environmental differences are an example of epigenetic mechanism involving developmental trajectories (Gottlieb, 1996). In particular, nowadays we can also assume that the regulation of the mean lengths and frequencies of sleeping and waking state depends on medical and nursing care received by preterm infants.

Numerous studies investigated the characteristics of the startle spontaneous, occurring usually in form of a baby bump or tremor during sleep (Agnoli et al., 2009).This behavior occurs primarily during the quiet sleep or NREM, although it was also detected in other states (Mc- Namara et al., 2002). Korner (1969), in particular, in a study of 32 infants term aged between 45 and 88 hours of life, observed during the NREM sleep the spontaneous startle in all subjects analyzed, demonstrating considerable inter-individual stability compared to other behaviors spontaneous detected in the same children (smiles, erections and activities suction). Spontaneous startle is believed to be of endogenous nature, precisely because it occurs spontaneously and in absence of any stimulation, although this motor pattern is entirely comparable to the elicited one. Like most primitive reflexes, this behavior tends to disappear spontaneously in the first months of life (Agnoli et al., 2009).

In accordance with the ontogenetic perspective (Oster, 2005), the adaptive relationship with the environment is an essential factor for the survival of the human organism. The observation of individual movement patterns in preterm newborns confirmed that the development of behavior is related to a dynamic integration between different systems, including the motivation arising from the basic need of appetite. The plasticity of the central nervous system plays a crucial role in the research of integration between different factors that determine and influence development. In preterm newborns development, the solicitations of maternal care, the search for balance, and the satisfaction of basic needs, participate to the effort of adaptation to the world that characterizes the development of every human being from birth.

Results show that we can observe even before the end of the pregnancy behavioral motor patterns functional to nutrition and attention seeking to the caregiver (*MOUTHING MOVEMENTS, HEAD ROTATION, DISTRESS*). Moreover spontaneous behavioral patterns are already modulated by motivational conditions even for very immature infants. However we can still observe effects due to low birth weight or low gestational age at birth in behavioral patterns as regard to the frequently recorded motor patterns that seem to have more importance than post-conceptional age in differentiating groups (*SMILE, STARTLE, DISTRESS AND BLINK*).

6. Experiment III: Effects of birth-weight and gestational age on spontaneous motor behavior

6.1. Introduction

One of the most widespread question investigated by recent studies on brain development is the comparability of brain maturation in fetuses and preterm infants at equivalent age (Viola et al., 2011). We are now sharing this interest in investigating how spontaneous motor behavior can differ in preterm and full-term newborns at the same post-conceptional age.

Preterm birth is a complex condition and has come to be a challenge for public health (Muller-Nix & Ansermet, 2009), especially in industrialized societies. In recent years, modern medicine has made great successes, especially in the field of technical support vital to the fetus at less than 28 weeks of gestational age. Preterm newborn was not able to spend the last trimester of pregnancy in utero, and he is born without completing his development. It is important to note that preterm behavior is not just influenced by maturation, but it is also due to social input and to the particular environment, as well as the specific mother's expressive competence to create a dyadic relationship with his baby.

In motor system development the influence of environmental, social and maternal variables, as well as other endogenous variables resulting from the newborn individuality, cooperate to realize behavior (Castiello *et al.,* 2010). The new insights in brain maturation could provide the concept of acceleration in term of tissues microstructure and neurochemistry, supporting brain maturation after birth in healthy neonates. Viola and colleagues (2011) found that diffusion parameters in the developing brain vary with water content, cell density, formation of white matter tracts, membrane potential, and myelination. In accordance with these findings a possible explanation of the concept of acceleration in white matter development is ascribed to the environmental stimulation. In fact, comparing preterm infants explored around term with their first examination after birth, they found that metabolic results reported an increase in white matter glutamate concentration and that this elevation had been ascribed to glutamate, an amino acid that regulates the proliferation and maturation of oligodendrocyte progenitors. These studies reveal an acceleration of maturation in some cerebral regions in healthy premature neonates compared with age-matched fetuses. Brain maturational processes are concomitant with changes in white matter microstructure and with neurochemistry related to tissue injury and probable gliosis. Although further studies are required to assess the evolution of white matter anomalies, it is now recognized that intra- and extra-uterine brain maturation around term may not be equivalent. One of the most important consequence is that data from premature brain are not appropriate to understand fetal cerebral maturation *in utero*.

The aim of this study is to compare spontaneous motor behavior of eleven 40-weeks-old preterm infants to eleven 40-weeks-old full-term infants. To code newborns behavior we adopted 21 behavioral motor patterns, especially designed for the detection of behavioral categories originally described in fetuses, preterm and full-term newborns by de Vries (1982), Prechtl (1985), Kurjak et al. (2003), Einspieler et al. (2008) and Wolff (1987). The description of the complex behavioral motor patterns was conducted using as a starting point the Facial Action Coding System FACS (Facial Action Coding System) by Ekman and Friesen (Ekman and Friesen,1978; Ekman, Friesen & Hager, 2002), with the insights and information added by Harriet Oster (Baby FACS in press. This scale was already tested in two previous experiments and demonstrated a great sensibility in detecting behavioral differences between fetuses and preterm newborns, as well as in preterm newborns behavioral observations made before and after meal.

In order to provide an additional criterion to observe behavior in preterm and full-term newborns (Kurjak and Leutic, 2010), we are going to detect a wide range of behavioral motor patterns, opening the way to a new approach in studying development, both scientific and diagnostic.

We are now asking how human development can differ in full-term from preterm newborns. Recent studies, in fact, support that preterm newborns can follow different trajectories on human development and also that developmental strategies are different in preterm newborns from those born full-term (Cioni et al., 1997; Pignotti, 2006, 2008). Therefore, comparing full-term and preterm newborns at the same gestational age can add some new interesting details on this new coding scale of human motor patterns repertoire and on human behavioral development. Thanks to this experiment, we are going to open a new window to observe differences in motor behavior development between preterm and full-term babies, and to observe their different developmental trajectories.

6.2. Method

6.2.1. Participants

We examined 22 newborn infants, 11 healthy preterm neonates (8 females) with a gestational age at birth ranging between 25.3 and 32.2 weeks (*M* = 29.4, *SD* = 2.2), and 11 healthy full-term infants (8 females), with a gestational age between 39 and 41 weeks (*M* = 40, *SD* = 2). The average weight of premature infants at birth was about 920 gr. (*SD* = 186.45); while the average weight of those full-term, was about 3250 gr. (*SD* = 252) appropriate for gestational age (AGA; *Appropriate for Gestational Age*; Lubchenco, 1976) . Premature infants were tested when the post-conceptional age ranged between 37. 6 and 40.5 weeks (*M* = 39.3, *SD* = 0.98); those born full-term were tested between 4 and 42 hours after birth (*M* = 18.48, *SD* = 12.95). Preterm newborns were video-recorded at the NICU of the Annunziata Hospital of Cosenza. Full-term newborns were recorded at the maternity ward of the Pediatric Clinic of the University of Padua. None of them had serious disease at birth that involved the heart, the brain and the organs of sense. In particular, the exclusion criteria were: chromosomal abnormalities, heart disease or other congenital abnormalities, fetal infections, metabolic disorders, obvious teratogenic factors, APGAR score < 4 at 5°min., and bleeding of III° and IV° degree. Informed consent was obtained from parents of all children involved in the study.

6.2.2. Procedures

Infants were recorded while non-stimulated, between two meals, for a period between 13.45 and 30 min. (*M* = 18.07, *SD* = 5.39) for preterm newborns, and for a period between 6 min. and 12 min. (*M* = 11.45, *SD* = 1.72) for those born full-term. Video recordings were conducted by two experimenters.

Behavioral states coding. Behavioral states (quiet sleep, active sleep, alertness, active waking, crying, and drowsiness) were defined in terms of the conjunction of behavioral conditions, incorporating salient features of several well-known coding systems (Brazelton, 1983; Prechtl & Beintema, 1964; Wolff, 1987). *Quiet sleep* (regular sleep or non-REM sleep) was characterized by respiration that is regular in rhythm and constant in amplitude, firmly closed eyelids, and no movements except startles or sudden jerks. *Active sleep* (irregular, paradoxical, or REM sleep) was characterized by intermittent eye movements under closed lids, irregular respiration, and small movements smoother and more controlled than those in quiet slee*p. Alertness* (alert inactivity or quiet waking) was characterized by minimal motor activity, open eyes, and attention directed toward external stimuli. *Active waking* (alert activity) was characterized by considerable motor activity involving the limbs, trunk, face, and head, and the eyes were open. *Crying* was characterized by vigorous vocalizations; the face was contorted into a cry grimace and might be flushed bright red. In *drowsiness*, the infant was relatively inactive, the eyes occasionally opened and closed intermittently and had a dull, glazed appearance (Dondi et al., 2007). Codings and data analysis were conducted at the EARLY INFANCY LAB, of Ferrara University.

6.2.3. Coding spontaneous motor behavior in the preterm and term neonates.

The analysis of video recordings was conducted frame by frame independently by two expert coders in behavioral micro-analysis. They separately viewed the video material and, with the aid of a timer, identified in the video-recorded material sequences videotaped the presence of the following behavioral motor patterns:*1. Rooting; 2. Occipito – frontalis reflex; 3. Chin tremble; 4. Shivers; 5. Yawning; 6. Startle; 7. Sneezing; 8. Hiccups; 9. Swallow; 10. Drooling; 11. Hand to the face movements; 12. Finger-sucking; 13. Retro-flexion of the head; 14. Rotation of the head; 15. Ante-flexion of the head; 16. Blink; 17. Mouthing movements; 18. Non-rhythmic mouthing movements; 19. Tongue expulsion; 20. Smile; 21. Distress.* Objectives aspects that ensure recognition of observational basis are reported in detail in the Appendix.

6.2.4. Reliability

We calculated the agreement between the two coders using the Cohen's Kappa coefficient, within a time window of 5 seconds. The agreement between the two observers in identifying the action was good All disagreements were discussed and they have been solved. Statistical analyzes were conducted on the basis of a common protocol agreed between the two coders.

6.2.5. Analysis of data

Data analysis were conducted on the frequency of occurrence per minute calculated for each of the 21 behavioral motor patterns. This measure was made necessary by the different duration of the video recordings, and it was obtained by dividing, participant per participant, the total number of observations of each motor pattern into the total duration of video recording. According to the internal variability characterizing preterm newborns behavior and taking into account the sample size, comparisons between the two groups (preterm vs full-term newborns) were performed using the nonparametric Kruskal-Wallis Test and Mann-Whitney for independent measures. In order to identify the statistical significance it was assumed a conventional value of alpha equal to .05.

6.3. Results

Table 3 shows for preterm and full-term newborns the mean frequencies per minute registered for each behavioral motor pattern. Regarding the sample of preterm infants, the most frequently observed behavior was *DISTRESS* (*M* = 1.76, *SD* = 1.11), as well as for full-term neonates (*M* = 1.44, *SD* = 0.72). During the period of observation, none of the following categories: *OCCIPITO-FRONTALIS-REFLEX (2.), SHIVER (4.)* and *FINGER-SUCKING(12.)* has been coded.

TABLE 3
Behavioral motor patterns coded in Full-Term and Preterm newborns observed at the same gestational age

	Newborn Infants	*Rooting*		*Chin tremble*		*Yawning*		*Startle*		*Sneezing*		*Hiccup*	
	No.	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FULL-TERM INFANTS	11	0.00	0.00	0.00	0.00	0.144	0.175	0.068	0.082	0.00	0.00	0.00	0.00
PRETERMS INFANTS	11	0.008	0.019	0.013	0.29	0.071	0.122	0.049	0.074	0.005	0.015	0.492	1.584

	Swallow		*Drooling*		*Hand to face movement*		*Head retro-flexion*		*Head rotation*		*Head ante-flexion*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FULL-TERM INFANTS	0.038	0.078	0.015	0.034	0.098	0.111	0.023	0.039	0.402	0.276	0.038	0.057
PRETERMS INFANTS	0.023	0.047	0.00	0.00	0.270	0.293	0.018	0.044	0.454	0.252	0.074	0.090

	Blink		*Mouthing movements*		*Non-rhytmical mouthing movements*		*Tongue protrudes*		*Smile*		*Distress*	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
FULL-TERM INFANTS	0.462	0.448	0.697	0.310	0.136	0.215	0.308	0.379	0.280	0.348	1.765	1.114
PRETERMS INFANTS	1.393	1.087	0.719	0.213	0.183	0.230	0.377	0.330	0.415	0.358	1.438	0.729

Note. Behavioral motor patterns observed in preterm and full-term infants. In vertical column Means (M) and Standard Deviations (SD) refer to the frequency per minute of each motor pattern observed in both groups. Statistical comparisons are found in the text.

Pre-term vs. full-term infants contrasts

A first series of statistical analysis conducted by the Mann-Whitney U test compared the full-term infants group with the preterm infants group. This analysis showed significant differences with regard to the *BLINK* variable (intermittent opening and closing of the eyes; see Figure 3 and 4), *df* = 1, *U* = 26, *p* = .023. In particular, preterm newborns (*M* = 1.39, *SD* = 1.09) showed a frequency of occurrence per minute significantly higher than those born full-term (*M* = 0.46, *SD* = 0.45). No differences were detected for all the other behavioral motor patterns observed.

Gestational Age (GA) at birth

The sample of preterm infants has been reorganized and divided into two groups based on gestational age at birth. In particular, the Group 1 included preterm births < 30 weeks GA (*M* = 26.58, *SD* = 1.65); Group 2 included preterm births ≥30 weeks GA (*M* = 30.71, *SD* = 0.95) . As regards the 21 behavioral motor patterns, a series of comparisons were conducted between these two preterm infants groups and the full-term group of infants using the non-parametric test of Kruskal-Wallis. This analysis revealed statistically significant differences concerning the variable *BLINK* (*df* = 2, *H* = 8.046, *p* = .018). Further in pairs comparisons, conducted by the Mann-Whitney U test, this behavioral pattern showed a statistically significant difference concerning the group of preterm infants born over 30 weeks of gestational age, compared to full-term infants (*df* = 1, *U* = 23.5, *p* = .005). In particular, preterm infants (*M* = 1.80, *SD* = 1.14) exhibited this behavior more frequently than full-term infants (*M* = 0.46, *SD* = 0.44). (see Figure 1)

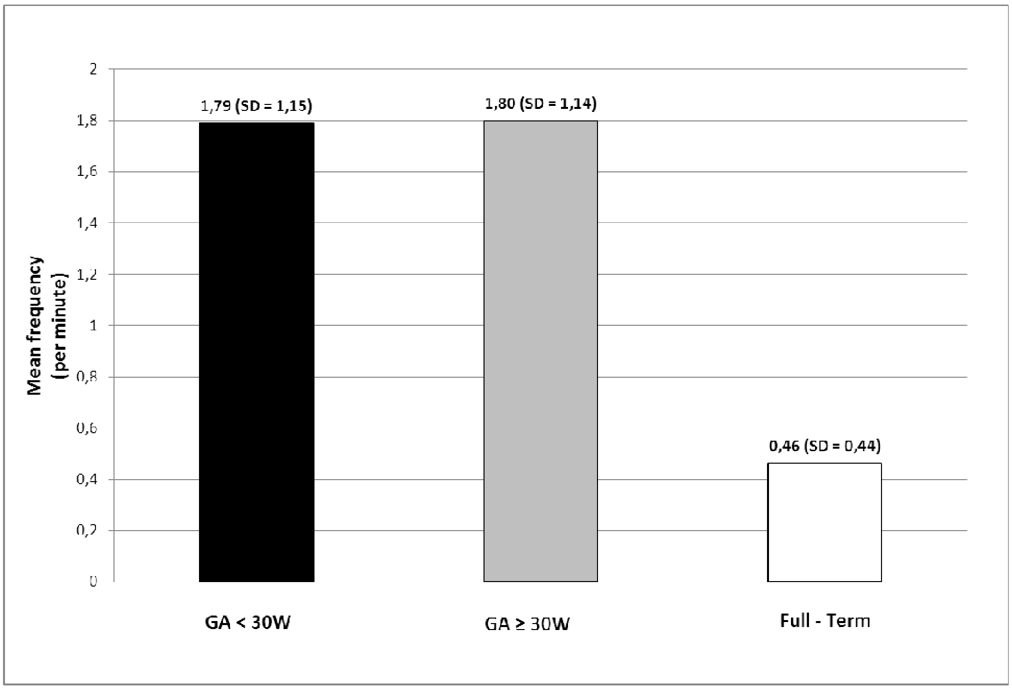


Figure 3. Graphical representation of Blink mean frequency (per minute) of infants at different gestational age at birth; GA = Gestational Age; *SD* = Standard Deviation.

Birth-weight

Following the same strategy, the sample of preterm infants was organized in two groups based on birth weight. Group 1 included the preterm infants whose birth weight was < 900 gr. (*M* = 780 gr., *SD* = 124.10); Group 2 included preterm infants whose birth weight was ≥ 900 gr. (*M* = 1088 g., *SD* = 108.03). Once again, comparing the three groups *BLINK* was the variable that showed statistically significant difference (*df* = 1, *H* = 7.635, *p* = 022) revealing, after pairwise comparisons conducted by using the Mann-Whitney U test, that the preterm infants with the lowest birth-weight (*M* = 1.97, *SD* = 1.13) showed this pattern with a frequency per minute significantly higher than the full-term infants group (*M* = 0.46, *SD* = 0.44), *df* = 1, *U* = 7.193, *p* = .007. (see Figure 2).

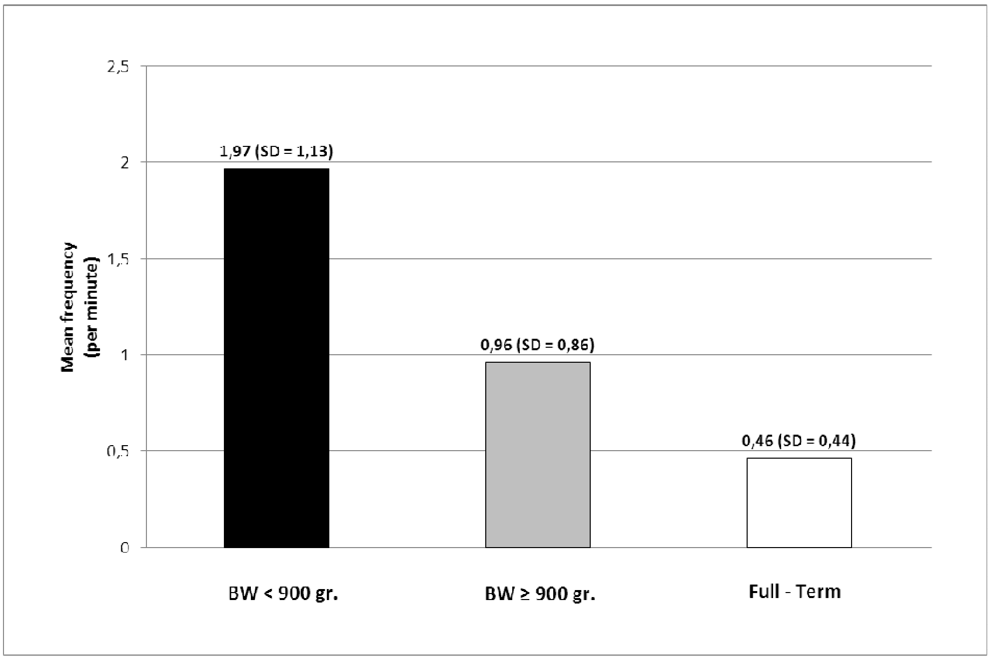
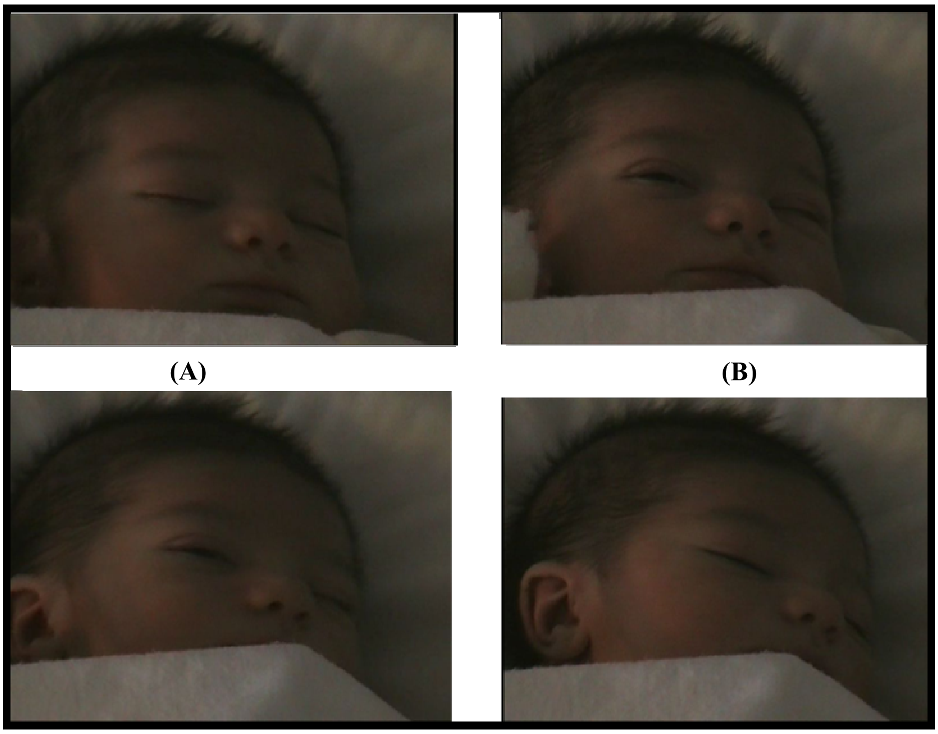


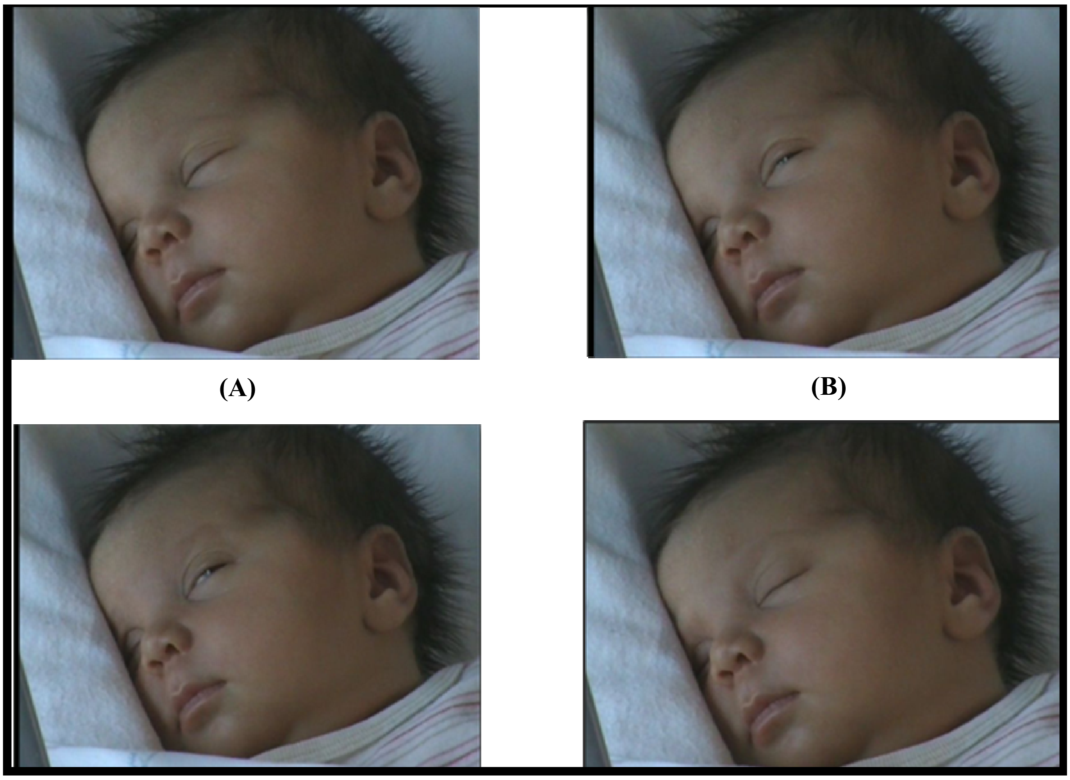
Figure 4. Graphical representation of Blink mean frequency (per minute) of infants at different birth-weight; BW = Birth-Weight; SD = Standard Deviation.



(A) (B)

(C) (D)

Figure 3. Blink of a 40 weeks-old male newborn. The sequence of Blink is observed from the on-set (A) to the off-set (D). The apex of Blink is reached in Image B.



(A) (B)

(C) (D)

Figure 4 . Blink of a 42 weeks-old female newborn. The sequence of Blink is observed from the on-set (A) to the off-set (D). The apex of blink is reached in image C.

6.4. Discussion

The aim of this experiment was to determine whether spontaneous motor behavior was different in full-term newborns from preterm newborns at the same post-conceptional age. Eleven 40-weeks-old preterm infants were compared to eleven 40-weeks-old full-term infants by the new coding scale of spontaneous complex behavioral motor patterns already tested in previous experiments. For both groups the most frequently observed behavior was *DISTRESS*. Distress involves the AU 4 (brow-knotting) and sometimes also AU 3 (Brow knitting). In AU4 we can see the co-occurrence of: "*depressor supercilii*, which lowers the middle and outer portions of the eyebrows; *corrugator supercilii*, which pulls the inner ends of the eyebrows downward and together, knitting the brows; and *procerus (depressor glabellae)*, which pulls down the inner corners of the eyebrows and "knots" the skin between the brows, just above the root of the nose." (Oster, *Baby FACS*, 2009, pg. 17)

Statistical comparisons showed significant differences between the two groups in relation to 1 complex motor pattern. In particular, full-term infants tended to exhibit more. According to Harriet Oster's Baby-FACS (2009), this action unit is called AU 45, and is coded when we can see eyes close and open very quickly, with no pause or hesitation in closed position. It could be also unilateral (involving only one eye) and the eyes must close or nearly close for a moment and then return to an open.

In the first study of human fetal eye movements conducted by Hooker (1952), contraction of the *orbicularis oculi* muscle was observed following stimulation of the upper eyelid at 10.05 weeks' gestational age. The first study conducted by sonographic scanner of fetal eye movements is by Nijhuis et al., (1982) and soon after by Birnholz (1978) with the description of the developmental course of fetal eye movements. During the third trimester of pregnancy eye movements begin to cluster into bursts and pauses. During the last 10 weeks of pregnancy they decrease. Between 36 and 38 weeks they are almost absent (Prechtl and Nijhuis, 1983). From the 23-26 weeks onwards we can observe opening and closing of the eyelid (Campbell, 2002) and repetition of blink is associated with dopaminergic system (Karson, 1982). During the last weeks of pregnancy it occurs at a frequency of 6.2 blinks per hours. Blinking can also be elicited by light perception (Del Guidice, 2010) or vibro-acoustic stimulation (Birnholz and Benacerrag, 1983). Central nervous system develops through different overlapping stages: neurogenesis, migration, synaptogenesis and myelination and its complexity reflects the complexity of cognitive, sensomotor, affective and behavioural development. Sleep and wake states appear before birth and continue through postnatal and adult life. Prechtl (1974) argued that sleep-wake states and infant behavioral states describe the same phenomena and that each state has specific properties reflecting particular central nervous system activities (Stanojevic et al.,2011). Since behavioral states are the expressions of central nervous system functions, they reflect one of the earliest forms of organized central nervous system activity and the maturation of brain. Behavioral states act as mechanism for infant's perception of internal processes and external events and a moderating mechanism for all the infant's behavior (Lehtonen & Martin, 2004).

During early development preterm newborn behavioural states are immature although their cyclicity is clearly seen. Preterm infants characteristically have a large proportion of indeterminate sleep and small amount of wakefulness. Although more studies are clearly needed, scientists recognize that optimizing the sleep cycling can improve the long-term outcome of preterm infants. Over preterm period temporal organization of sleeping and waking cycles develops and preterm newborns exhibit changes in sleep-wake lengths, frequencies and their percentages of transitional states. While there is an increase of bout lengths, we can observe an decrease of their frequencies. Recent researches suggest probably both lengths and frequencies of sleeping and waking states are not already set during preterm period and the developmental patterns can vary depending on infant characteristics and environmental circumstances (Noble & Boyd, 2012).

Therefore, although the developmental patterns of each state is biologically determined, preterm infants sleep-wake cycle may be influenced by different interventions and it is subject to variation. These environmental differences are an example of epigenetic mechanism involving developmental trajectories (Gottlieb, 1996). In particular, nowadays we can also assume that the regulation of the mean lengths and frequencies of sleeping and waking state depends on medical and nursing care received by preterm infants.

As regard to the gestational age at birth two different groups were observed: Group < 30 weeks of gestational age (*M* = 26.58, *SD* = 1.65), and a Group 2 > 30 weeks of gestational age (*M* = 30.71, *SD* = 0.95). Statistical analysis confirm the different exhibition of *BLINK*. Another confirm of the different exhibition of *BLINK* was found in a preterm infants Group compared to full-term group.

In infants human brain development is highly variable among individuals. Although several hypotheses have been put forward, the influence of genetic, epigenetic and environmental factors is still poorly understood. Previous studies on postmortem observations of fetal brains (Bos et al., 2001), demonstrated that during intrauterine life brain maturation depends on physical constraints and mechanical factors. Recently, the magnetic resonance imaging has made possible to quantify cortical development in human newborn brain. The 3D reconstructions of the inner cortical surface showed the dramatic increase in cortical sulcation with age. Since prematurity influences brain development and cortical maturation, a preterm birth may be responsible for the delay that we observed in sulci appearance. In accordance to recent findings, cortical volume and surface area of extremely preterm infants are decreased and less complex than in normal infants, and this impairment seems to increase with decreasing of gestational age at birth (Dubois et al., 2008). Both longitudinal follow-up and further correlation with the emergence of neurological functions can provide important insights into the developmental origin of brain structural abnormalities induced by genetic and early environmental factors.

A large portion of indeterminate sleep characterizes preterm behavior, but we can also distinguish an indeterminate sleep defined as a state that does not meet criteria of either active or quiet sleep (Groome et al, 1999). Sometimes indeterminate sleep is included in preterm infants active sleep and in NREM sleep of infants after term. In very immature infants, it is hard to distinguish wakefulness from arousal and the amount of crying is very small. There is an increase of crying from the 31rst week to term, with a peak in the second month of full-term infants. The premature infants had more QS (quite sleep), longer QS (quite sleep) bouts, and less AS (active sleep). The normal developmental course includes increased QS and decreased AS over age, and for this reason premature newborns seem to have more mature characteristics than full-terms. One explanation could be that postnatal age is more important than conception age for the early development of sleep.

Also as regard to the birth weight the preterm group whit lower weight showed a mean frequency per minute higher than full-term. The increased survival chances of extremely low-birth-weight (ELBW) infants (weighed ≤1000 g. at birth) has led to reflect on their behavioural outcome in childhood. Investigators noted a higher frequency of behavioural problems in such infants, also in cross-cultural comparisons. Despite cultural differences, in fact, types of behavioural problems in ELBW children were very similar and these findings suggest that biological mechanisms contribute to behavioural problems of ELBW children (Holditch-Davis, 1990). Many other studies focused on the motor outcome of extremely ELBW or very preterm (<28wks) children established the perinatal associations of developmental coordination disorder (DCD) and its cognitive and behavioural consequences. Investigators discussed that ELBW/very preterm children with developmental coordinator disorder had worse cognitive function. DCD is more common in ELBW/very preterm children, has few perinatal correlates, and is associated with poor cognitive and academic performance, as well as increased behavioural problems.

Why preterm observation is relevant for scientists and clinicians?

Observing preterm infant motor development is an interesting opportunity to understand the particular development of a human being who is still a fetus regarding his post-conceptional age but who is surviving and growing in an atypical neonatal environment. Since developmental trajectories are relative and contingent on conditions of measure performances, it could be inappropriate to evaluate the progresses of preterm infants by comparison to the developmental milestones arising from neonatal and fetal development. As regard to these research, finally we can say that it should be more appropriate to understand and to evaluate this particular development process independently from neonatal development. Preterm infants represent a source to understand the lability of the early development and to augment considerations on normal development in full-term newborns. Reciprocal influences in studies on development of full-term newborns and preterm infants should be an important exchange between theories and clinicians practice promoting health and well-being also in preterm newborns at risk.

APPENDIX

1. ***Rooting***

This reflex initiates by a tongue show (AU 19), usually tense or balled, rests in the mouth bottom. A unilateral AU 6 + 9+ 26, *orbicularis oculi pars orbitalis* + *levator labii superioris alaequi nasi* + lowering action of the mandible, appears and increases in intensity, as the head increases to turn from one side to the other. Together with the head rotation, the tongue starts to flatten on the bottom of the mouth, and the unilateral *risorius* muscle action (AU 20) appears. The apex of the root is observed when both the tongue and the lower jaw initiate slight suckle movements, the infant's head has turned flush to the side and the head moves up slightly. (Oster, 2009; Prechtl, 1958; Wolff, 1987).

2.Occipito-frontalis

"When a neonate's head falls forward, a very rapid and automatic raising of the brows usually occurs."(Oster*,* Baby FACS, 2009, pg. 79), coded as AU 95 in Baby FACS. The eyes and the brows start to raise as the head falls forward and are fully raised (AU 1+ 2), designated by the actions of the *frontalis* muscle, as the head is fully forward. The eyes are focused straight ahead and the brows may remain raised, as the head comes back to neutral.

3.Chin tremble

Most often observed before or during crying, chin tremble is: "a very rapid spasmodic movement of the chin (…). In newborns, it may occur spontaneously or may follow a startle response" (Oster*,* Baby FACS, 2009, pg. 79).

4.Shiver

This movement consists of a rapid and involuntary muscular twitching, especially observed in response to cold. Caused by muscles contraction, it is a physiological method of heat production in all animals.

5.Yawning

Yawning is a stereotyped and often repetitive behavior characterized by gaping of the mouth, accompanied by a long inspiration and a brief acme, followed by a short expiration. The expansion of the pharynx can quadruple its diameter, while the larynx opens up with maximal abduction of the vocal cords (Walusinski, 2006). Usually emerges from a relaxed face and "as the mouth opening begins to increase, the head tilts back (AU 53), the eyes droop (AU 43), and the tongue tenses, lying flat on the bottom of the mouth. AU 4 + 9 often adds in at this point, until the mouth opening reaches an apex, when AUs 20 and 38 (lateral stretching of the lips and nostril dilatation) also add in" (Oster, Baby FACS, 2009, pg. 78). One of the most characteristic yawn (AU 94) features is a relatively gradual acceleration and abrupt deceleration of its action units.

6.Startle

Provoked: "The startle response is a defensive reflex to an abrupt sensory event composed of a chained series of rapid flex or movements that cascade throughout the body" (Agnoli, Franchin, & Dondi, *Three Methodologies for Measuring the Acoustic Startle Response in Early Infancy*, 2011, pg 1; Agnoli, Dondi, Franchin & Stoppa, 2009). The startle response (Agnoli, Dondi, Mendini, & Franchin, 2007; RicciBitti & Costa, 1998; Dondi, 2001), is a defensive type innate reaction (Lang, 1995; Yeomans, Li, Scott, & Frankland, 2002), involving the sudden closing of the eyes (as a result of the blink reflex), the contraction of facial muscles, the forward movement of the head, the raising and moving forward of the shoulders, the adduction of the arms, bending the elbows, the pronation of the forearms, the closure of the hands, the movement in on the trunk and the contraction of the abdomen and the knees (Costa & Ricci Bitti, 1998, Landis & Hunt, 1936; Landis & Hunt, 1936, 1939, Yeomans & Frankland, 1996; Yeomans et al., 2002).

Spontaneous: First element to appear is the blink, followed by the head movement, while the other elements are manifested in rapid succession immediately after. Although the timing of those movements remains constant throughout the entire life (AU 96), in infants initial reaction may be followed by crying. (Oster, 2009).

7.Sneezing

Usually caused by irritation to the mucus membranes of nose or throat, sneezing is an uncontrolled, sudden and forceful air burst through the nose and the mouth. These is a relatively stereotyped action pattern, in terms of its constituent and timing. There may be individual variations characteristic for a given infant and, since they represent such tightly "packaged" behaviors, they are more easily coded as units, than in an atomistically manner (Oster, 2009). Sometimes it can involve the AU 84 "Head shake back and forth" or AU 85 "Head nod up and down". (cfr. Ekman et al., 2002).

8.Hiccup

Hiccup is a reflexive, stereotypic activity of the diaphragm (Lecanuet et al., 1995). This movement appears from 9 weeks of gestational age, consists of a jerky contraction of the diaphragm (Kurjak et al.,2010) often in series, and can lasts for up to several minutes. In utero, it is followed by isolated arm and leg movements. "Mostly repetitive short contractions of the diaphragm can also last for several minutes and can be also so forceful to move passively the fetus in his amniotic cavity" (Einspieler *et al*, 2008, pg.).

9.Swallow

This pattern initiates with two to six sucking movements, before a wide opening of the mouth and sometimes it may occur in bursts (Lecanuet et al., 1995). In utero, it is followed by low-frequency tongue movements propelling the fluid bolus in hypopharynx (Einspieler *et al*, 2008).

10.Drooling

It is coded when you can see saliva flowing outside the mouth. Usually, it may happen if the body makes too much saliva than normal. It may be accompanied by mouth opening and closing.

11.Hand to face movements

This movement involves hands and arms and ends with the contact of hand or fingers in the face region (Kurjak et al, 2003, Zoia et al, 2006).

12.Finger-sucking

This movement, involving hands and arms, is determined by the contact of finger with the mouth, lips or immediate oral region (de Vries et al, 2001; Kurjak et al, 2003; 2005; 2010, Zoia et al, 2006).

Head movements

13. Head retro-flexion: this movement, involving the neck as well as the head, is coded when the head tilts back along the horizontal axis, drawing and angular shape between 45° and 90° degrees;

14. Head rotation: when head moves from side to side along the vertical axis;

15. Head ante-flexion: when head moves forward along the horizontal axis, drawing an angular shape between 45° and 90° degrees. (de Vries et al, 1982; 1985; 2001).

17.Eye blinking

According to Harriet Oster's Baby-FACS (2009), this action unit is called AU 45, and is coded when we can see eyes close and open very quickly, with no pause or hesitation in closed position. It could be also unilateral (involving only one eye) and the eyes must close or nearly close for a moment and then return to an open.

18.Mouthing movements

This action consists of rhythmic movements involving the mandible and tongue, characterized by constant frequency and duration, until disappearance (Kurjak et al., 2003). As noted by Wolff (1987), they are typically organized in bursts of six to twelve sequential mouthing movement, followed by a rest period of four or five seconds (D'Elia et al, 2001; Einspieler et al, 2012; Nijuis et al, 1984).

19.Non-rhytmical mouthing movements

Clearly distinguished from rhythmic mouthing we can observe non-rhythmical tonguing, chewing or mouthing movements concentrated primarily in state II or during drowsiness. During the first eight hours after birth, they seem to help neonate to drain inspissated mucus (Wolff, 1987).

20.Tongue Protrudes

In AU 19 (Oster, 2009), for both adults and infants, tongue must protrude from its resting position at least as far as the inner margin of the red part of the lips. There are three different actions: when the tongue protrudes making contact with the lips AU 77, without any contact with the lips AU 19, and AU 37 for a wipe (Oster, 2009).

21.Smile

The principal component of a simple smile is the *zygomaticus major* muscle action (AU12).This muscle emerging from the zygomatic arch in the cheekbones and attached to the lip corners, raises the lips corners obliquely and upward toward the cheek bones. Pulling back and upward the lips corners, it creates an angular shape to the mouth. According to Baby-FACS, we can call this action "lip corner puller". In a strong AU 12, we can see infraorbital triangle pushed upward more evident, eye aperture narrowed by pushing up the cheek and the skin below the lower lid. Duchenne's smile involves contraction of *orbicularis oculi pars orbitalis* (AU6) raising the cheeks and drawing the skin below the eyes.

22.Distress

It involves the AU 4 (brow-knotting) and sometimes also AU 3 (Brow knitting). In AU4 we can see the co-occurrence of: "*depressor supercilii*, which lowers the middle and outer portions of the eyebrows; *corrugator supercilii*, which pulls the inner ends of the eyebrows downward and together, "knitting" the brows; and *procerus (depressor glabellae)*, which pulls down the inner corners of the eyebrows and "knots" the skin between the brows, just above the root of the nose." (Oster, Baby FACS, 2009, pg. 17). Thanks to Baby-FACS by Harriet Oster (2007), we can also distinguish AU4 from AU3 (brow-knitting), due to the isolated action of the *corrugator supercilii* muscle. AU 4 produces a rectangular muscle bulge in the area between eyebrows, the root of the nose and the lower portion of the forehead (called glabella), producing sometimes an horizontal line across the root of the nose. *Corrugator supercilii* (AU 3), the so-called "butterfly wing muscle”, produces an oblique depression or bulge running laterally upward from the inner corner of the brow toward the forehead and above the middle portion of the brow.

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