Università degli Studi di Milano

GRADUATE SCHOOL OF VETERINARY SCIENCES
FOR ANIMAL HEALTH AND FOOD SAFETY

Doctoral Program in Veterinary Clinical Sciences

Newborn calves' features in relation to the type of delivery

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Academic Year: 2011-2012

To D & D,

who make my life marvellous

Index

LIST OF ABBREVIATIONS

AGP	alpha-acid glycoprotein
APP	acute phase proteins
APR	acute phase response
BAT	brune adipose tissue
BB	belgian blue
bpm	beats per minute
CRP	c-reactive protein
CS	Caesarean section
d	days
ECS	elective Caesarean section
Fb	fibrinogen
GFR	glomerular filtration rate
GH	growth hormone
GIT	gastro-intestinal tract
h	hours
Hb	hemoglobin
Hp	haptoglobin
Ht	hematocrit
IGF	insulin-like growth factor
IUGR	intra-uterine growth retardation
m	minutes
NEFA	non-esterified fatty acids
PG	prostaglandins
PGM	prostaglandin-metabolite
PMN	polymorphonucleates
PON1	paraoxonase 1
RBC	red blood cells
RDS	respiratory distress syndrome
rpm	respirations per minute
SAA	serum amyloid A
T_3	triiodothyronine
T_4	thyroxine
TRH	thyrotrophin releasing hormone
TSH	thyroid stimulating hormone
TSR	time for sternal recumbency
TSU	time to stand up
VD	vaginal delivery
WBC	white blood cells

"Around here, however, we don't look backwards for very long.
We keep moving forward, opening up new doors and doing new things,
because we're curious…
and curiosity keeps leading us down new paths"

WD

CHAPTER 1

Foreword

1. Foreword

"The process of reproduction in cow is only successfully completed when a healthy neonatal calf is standing at it's mother's side"

(Ball & Peters – Reproduction in Cattle)

The transition from the intrauterine environment to the outside world represents a critical phase in the life of domestic mammals, in which numerous maturational and adaptational processes have to take place. In the majority of cases, these processes occur without any complications and the newborn can continue to growth and develop as a healthy neonate. Yet, a substantial number of calves do not pass this transition period uneventfully, as mirrored by neonatal morbidity and perinatal mortality figures reported from dairy farms in Europe and the USA (Meyer *et al*, 2001; Berglund *et al*, 2003; Steinbock *et al*, 2003).

It has been recognized for several decades that such problems are especially associated with calves born from heifers, because dystocia, or at least prolonged calvings, are more common in these dams (Smidt & Huth, 1979; Mee, 2008*a*; Zaborski *et al*, 2009; Gundelach *et al*, 2009). However in more recent years an increase in perinatal mortality figures has been reported in dairy calves and, quite typically, this appears not to be associated with an increase in the rate of dystocia (Mee, 2006; Mee, 2008*b*). These perinatal losses not only represent a tremendous economic loss but also mirror an important welfare problem on cattle farms.

In order to reduce the losses occurring during the perinatal and neonatal period, management of the parturient cow and newborn calf have to be addressed critically (Mee, 2008*c*).

Because farmers are usually the first who have to recognize the onset and progress of calving, standard calving management protocols and recommendations for intervention, or for refraining from intervention, have to be implemented together with the help of veterinarians. Apart from management, critical observation and/or investigation of the calf during and immediately after calving belongs to the responsibility of the veterinarian. This is important considering that several problems, encountered during the first days after delivery, can originate from the calving period itself.

In a crude analysis for fetal and neonatal outcomes related to the type of delivery in the human specie (Villar *et al*, 2007) the highest rates of neonatal morbidity and mortality were seen in the elective caesarean group, while fetal death rates were similar in the three groups (vaginal delivery – elective caesarean section – intrapartum caesarean section). The rates of preterm delivery were 7% for vaginal deliveries, 12% for elective caesarean, and 9% for intrapartum caesarean. Elective caesarean delivery could increase neonatal morbidity and mortality because lack of labour affects the physiological process for initiation of respiration. Caesarean delivery is known to be associated with respiratory distress syndrome and transient tachypnoea possibly mediated by the lower release of catecholamine and prostaglandins, as well as the lack of the mechanical compression of the lungs during labour needed to facilitate postnatal lung adaptation.

Moreover, several studies on babies (Gasparoni *et al*, 1992; Gronlund *et al*, 1992; Herson *et al*, 1992; Hasan *et al*, 1993; Usmani *et al*, 1993; Steinborn *et al*, 1999; Redźko *et al*, 2005; Yektaei-Karin *et al*, 2007) demonstrated that the type of delivery can deeply influence the adaptational process in the newborn, modifying the immune response, the oxygenation status and the developing endocrine axis; the influence of delivery is not only immediate but extended in time, possibly affecting also the future health status of the subject.

Aims of the thesis

The main focus of this experimental thesis is on the effects of two different type of delivery on the condition of the calf at birth and during the neonatal period; possible differences in clinical features and biochemical, metabolic, hormonal, hematological and inflammatory profiles, will be described and discussed in details.

The experimental trial was conducted in two different European countries, Italy and Belgium. Holstein Friesian calves from spontaneous parturitions and Belgian Blue (BB) calves from Caesarean section were enrolled, and data collected from the two groups were compared.

Results were divided in different manuscript due to several data available.

The main limitation of this experimental trial lays on the enrollment of two different breeds, the Holstein Friesian and the Belgian Blue cattle breed. The difference in breed (and attitude) represents an important confounding factor that can deeply influence results.

Best choice would have been to compare calves from different parturition within the same breed and attitude, but different options were not available. In Belgium, where the cost of a caesarean section is very low and the value of the calf relatively high, 95–99.9% of the double muscled BB cows are delivered by caesarean section (Kolkman *et al*, 2007). For the same but opposite reason (high cost of caesarean section and low value of calf) Holstein Friesian calves in Italy are delivered by spontaneous parturition or, at the most, by forced extraction; most of the time, when a difficult parturition is dealing, forced extraction is the first choice, followed by a fetotomy or, lastly, by a caesarean section. Therefore, it was not possible to find a sufficient number of Holstein Friesian calves delivered by Caesarean section and a sufficient number of Belgian Blue calves delivered spontaneously. Since data regarding this comparison are missing, or they refer to different cattle breeds together, it was assumed that this research could be of some values in clarifying at least some aspects of impact of delivery on the newborn calf.

This thesis is based on the following original articles (I-II) and on data published in congress proceedings (III)

I. **Comparative study on 15-ketodihydro-$PGF_{2\alpha}$ plasma concentrations in newborn horses, donkeys and calves**.

Reproduction in Domestic Animals 2011, 47(1):82-86

II. **Mode of delivery is associated with different haematological profiles in the newborn calf.**

Theriogenology 2011
doi:10.1016/j.theriogenology.2011.09.010

III. **Preliminary data about paraoxonase (pon1) in newborn calves: age related variation and comparison between healthy and sick animals**

Proceedings of 8° European Colloquium on Acute Phase Proteins
Helsinki, 24-27 august 2010

CHAPTER 2

Neonatal adaptation: general aspects

2. Neonatal adaptation: general aspects

The expression "*neonatal adaptation*" refers to a complex of changes which occurs in the neonate immediately after the expulsion from the birth channel, and that makes the newborn suitable for the new life.

The major changes already take place during the first minutes after birth, but they slowly go through the first hours, with stabilization between 24 and 48 hours after birth (Pescetto *et al*, 1989). It is not possible to identify an exact limit between the end of the adaptational phase and the subsequent phase of neonatal development, since a gradual change from one to the other exists.

As for all newborns, also survival of the calf and its subsequent health conditions require a perfect transition from fetal to extra-uterine life; this means that, firstly, all organs and systems have to mature during the final stage of pregnancy.

Later on, within a relative short period of time from birth (minutes/hours) major physiological occur, as separation from the umbilical circulation, closure of circulatory shunts, inflation of air and consequent expansion of the lungs, thermoregulatory adaptations and active movements, standing and suckling.

2.1 Cardiocirculatory system

The adaptational processes of the cardiocirculatory system result from the break of the placental circle, the opening of the pulmonary circulation and the closure of some typical fetal ways; these changes respectively occur immediately, within few minutes or during the first hours/days after birth.

The resulting changes in blood flow, which are dramatically important during the first hours of life to allow the neonatal survival, are also essential to facilitate the initiation of pulmonary gas exchange.

2.1.1 Maturation

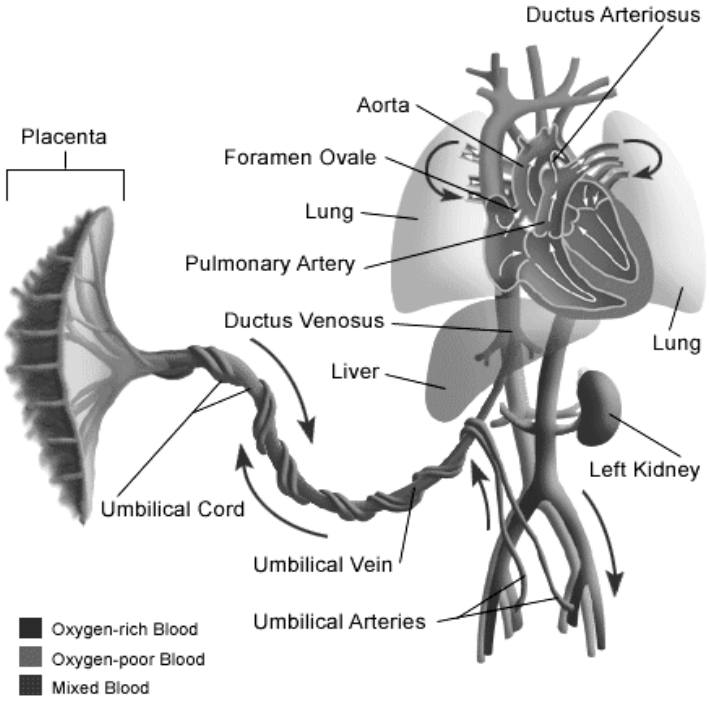
During the fetal period, pulmonary and systemic circulation are still "in parallel" thanks to the presence of special communications called *shunt* :
- intracardiac shunt = *foramen ovalis*, communication between the right atrium and left atrium;
- venous shunt = *ductus venosus of Aranzio*, communication between the umbilical vein and inferior vena cava;
- arterial shunt = *ductus arteriosus of Botallo*, communication between the aorta and pulmonary artery.

The placental oxygenated blood, coming from the umbilical vein (*figure 1*) passes through the ductus venosus into the inferior vena cava, entering the heart in the right atrium; here, the superior vena cava carries blood from the venous effluent upper body, but these two blood currents do not mix themselves, thanks to the presence of the Eustachian valve. The venous blood coming from the superior vena cava enters the right ventricle, and passes into the pulmonary artery, which supplies the inactive lung. From the lung, through the duct of Botallo, the blood reaches the descending aorta, where arterial and venous blood are mixed and directed to the peripheral circulation.

The oxygen-rich blood that reaches the right atrium from the inferior vena cava (and therefore from the umbilical vein) goes through the foramen ovalis into the left atrium, then into the left ventricle, and finally into the aorta, reaching brain and heart circulation. The blood characterized by low O_2 content and high CO_2 coming from peripheral blood and from brain and heart finally gathers in the umbilical artery, being delivered to the placenta.

Therefore, during the prenatal period, two vascular systems can be recognized: the placental and the somatic, functioning in parallel with a single engine represented by the fetal heart.

Fig. 1 - Schematic representation of fetal circulation



At birth, fetal circulation is interrupted by clamping of the umbilical cord. The consequent accumulation of CO_2 in the newborn blood represents the stimulus to the onset of breathing: after the first breath, the lungs expand and the resistance of the pulmonary vascular bed decreases.

Following lacing cord blood, there is an increase in systemic vascular resistance and left atrial pressure. When the pressure of the left atrium exceeds the one in the right atrium, the foramen ovalis closes; when the systemic blood pressure exceeds the pulmonary artery pressure, the flow through the ductus arteriosus is reversed (from right-left to left-right), increasing pulmonary blood flow. These changes lead to the transformation of the fetal circulation to an adult type of circulation, a phenomenon that takes place gradually during the first hours of life. The functional closure of the ductus arteriosus is enhanced by biochemical stimuli including rises in arterial PO_2, while the definitive anatomical closure occurs after few days of life.

The functional closure of the *foramen ovalis* is generated from the different pressure between left atrium and right atrium, while the anatomical closure is caused by adhesion and conglutination of the *tunica intima*.
Failure of closure or incomplete closure of the foramen ovalis possibly lead to the right-left or left-right shunts in the neonatal circulation, with serious and dangerous abnormalities in the cardiac output.

The functional closure of the *ductus venosus of Aranzio* should immediately follow the interruption of the umbilical circulation, although the exact mechanism is not fully understood. A definitive closure of this ductus normally occurs within the first week of life.

While the closure of the *foramen ovalis* and the *ductus venosus* are mainly regulated by pressure stimuli, the functional patency of the *ductus arteriosus of Botall*o is hormonally determined and controlled. The main endocrines responsible for this control mechanism are represented by prostaglandins, while prenatal and the immediate postnatal surges of thyroid hormones, T_3 and T_4 (*chapter 2.1.2*) have a significant impact on cardiovascular contractile and metabolic function.

2.1.2 Endocrine Regulation

Prostaglandins

Prostaglandins (PG) were firstly discovered and isolated from human semen in the 1930s by Ulf von Euler of Sweden. Thinking they had come from the prostate gland, he named them prostaglandins (Von Euler, 1935). Early studies on prostaglandins carried out by Euler and others found that these substances were capable of both lowering blood pressure and inducing the contraction of uterine tissue. Later efforts revealed much more, like the fact that that PG can be found in many tissues since they are not restricted to specific organs.

Prostaglandins are unsaturated carboxylic acids, consisting of a 20 carbon skeleton that also contains a five member ring. They are biochemically synthesized from a fatty acid, the arachidonic acid.

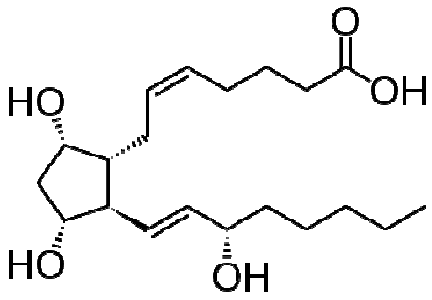
Since their structural similarities to the C-20 polyunsaturated fatty acids, the eicosanoid acids, they belong to a subclass of lipids known as the *eicosanoids*. Prostaglandins vary from one to another for subtle differences in their chemical structures, for instance exhibiting different side-chain substitutions. These small variations are probably responsible for the immense diversity of effects of prostaglandins. In general, PG, similarly to hormones, act by stimulating target cells into action; however, they differ from hormones since they can act locally, near their site of synthesis, and they are rapidly metabolized. Moreover, the same prostaglandins can act differently in different tissues.

Eicosanoids, including prostanoids, are not stored within cells, but are synthesized in response to hormonal stimuli. The first step is the release of the substrate fatty acid, such as arachidonic acid, from the cellular phospholipids, by the action of the enzyme phospholipase A_2. Next, the free acids are acted upon by one of two related enzymes, cyclooxygenase-1 and cyclooxygenase-2 (COX-1 and COX-2, respectively), also called prostaglandin endoperoxide H synthases-1 and -2 (PGHS-1 and PGHS-2, respectively).

Both enzymes catalyze the same two reactions at different sites. For example, a cyclooxygenase reaction followed by a peroxidase reaction leads the formation of prostaglandin PGG_2 and then, successively, prostaglandin PGH_2. PGH_2 is an unstable intermediate from which all other prostanoids are derived by a variety of different enzymatic reactions.

The most common stereochemical form of $PGF_{2\alpha}$ (*figure 2*) is synthesized by via two routes. It can be produced directly from PGH_2 by the action of prostaglandin H-endoperoxide reductase, using NADPH. Alternatively, it can be synthesized via PGE_2 by the action of an enzyme prostaglandin E 9-ketoreductase. A second of the four stereochemical forms of $PGF_{2\alpha}$, 9α,11β-$PGF_{2\alpha}$, is formed from PGD_2 by reduction of the keto group in position 9 by a PGD 11-ketoreductase.

Fig. 2 – Biochemical structure of prostaglandin $F_{2\alpha}$



Analysis of prostanoids is not a simple task, because they occur at such low levels in tissues and because of their high reactivity. If biological roles of $PGF_{2\alpha}$ should be studied, the best parameter to evaluate is 15-ketodihydro-$PGF_{2\alpha}$ (PGM). It represents its initial metabolite, with longer half-life in the circulation and higher concentrations. Moreover, it is less sensitive to methods of sample collection and handling procedures (Granström & Kindahl, 1982).

Prostanoids are ubiquitous lipids that coordinate a multitude of physiologic and pathologic processes, either within the cells in which they are formed or in closely adjacent cells in response to specific stimuli. Approximately 12 different prostaglandins have been identified, each of which has different kind of activities and effects on various tissues. Under normal physiologic conditions, they have essentially homeostatic functions such as in the cytoprotection of gastric mucosa, renal physiology, gestation and parturition; they are also implicated in pathological conditions, such as inflammation, cardiovascular disease and cancer. Several prostaglandins also play an integral role in the maturational events during fetal and neonatal life (Pace-Asciak, 1978).

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PGE_2, together with PGI_2, represents the most important dilator system of the ductus arteriosus, through an inhibitory effect on ductal smooth muscle (Smith, 1998; Schneider & Moore, 2006). In detail, PGE_2 increases the sensitivity of the ductus to vasodilators, while decreases its sensitivity to several vasoconstrictors (Smith, 1998).

Though several studies have been done regarding the role of prostaglandins in human neonates, only few studies have reported the circulating prostaglandin levels in human babies. First data concerning newborn plasma prostaglandins date back to 1970s, when Siegler *et al* (1977) evaluated PGE umbilical concentrations immediately after parturition and PGE plasma concentrations from 2-3 days of life until adolescence. They found higher circulating levels of PGE in samples obtained by umbilical plasma compared to the ones from newborn babies; by 48 to 72 hours of age however, PGE plasma concentrations had fallen to significantly lower levels than those in adults, while an increasing trend was observed until adolescence. The authors suggested that the low PGE levels at birth could be related to an immature renal function (Siegler *et al*, 1977).

The closure of the ductus in newborns is usually complete within 48 hours after birth in humans, and it is due to the contraction of its smooth muscle, triggered by the increase in oxygen tension and to the decline of PGE_2 levels (Smith, 1998; Yokoyama *et al*, 2006). PGE_2 and PGI_2 levels probably fall because of the functioning metabolism of the lungs and the elimination of placental source (Schneider & Moore, 2006).

Lucas and Mitchell (1978) found that plasma levels of PGE, PGF and PGFM are higher in infants with patent ductus arteriosus compared to normal neonates.

Mitchell *et al* (1978) affirmed that the levels of circulating prostaglandins (PGE, PGF, PGM) during the first month of life are greater than those in normal adults; concentrations of both $PGF_{2\alpha}$ and PGM are greater in umbilical plasma than maternal plasma. Moreover, they are both significantly increased in umbilical plasma after the onset of labor due to fetal placental unit production (Saeed *et al*, 2003). Human neonates born at term exhibit a rapid decrease in the circulating levels of PGE, but not PGF and PGM.

A similar pattern was also measured in pre-term infants, who exhibit by 5 to 8 weeks of life lower PGE levels than adults and PGF and PGM levels approximately three-folds greater than in adults. Six days after delivery, no significant differences were present between neonates born at term and those born prematurely (Mitchell *et al*, 1978) but the literature does not give information about prostaglandin levels in preterm infants during the first six days of life.

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A recent study investigated PGM plasma profile in newborn horse foals (Panzani *et al*, 2009). In spontaneous foals, the plasma PGM concentration significantly increased at 20 and 30 minutes after parturition, then consistently declined reaching low levels (<100 pmol/L) at 10 days after birth. The presence of high $PGF_{2\alpha}$ levels in the days after foaling could be explained by their role in completing organ maturation: when compared to human infants, in whom PG remain high for several weeks after birth, newborn foals exhibit low levels by 10 days after birth, probably because of a faster completion of development.

Thyroid Hormones

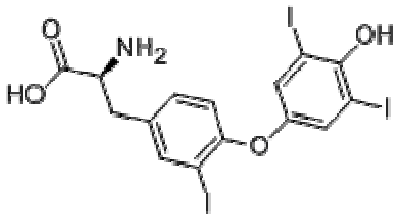
Thyroid-stimulating hormone (TSH or thyrotrophin) is a peptide hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland. Its role is the regulation of the endocrine function of the thyroid gland.

TSH production is controlled by a Thyrotrophin Releasing Hormone (TRH), which is synthesized in the hypothalamus and transported to the anterior pituitary gland, where it increases TSH production and release.

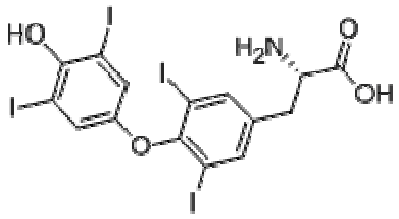
Somatostatin, also produced by the hypothalamus, has an opposite effect, decreasing or inhibiting TSH release. TSH stimulates the thyroid gland to secrete the hormones thyroxine (T_4) and triiodothyronine (T_3). The levels of T_3 and T_4 in the blood have an additional effect on the pituitary release of TSH. When the levels of these hormones are low, the production of TSH is increased; on the opposite, when levels of T_3 and T_4 are high, then TSH production is decreased. This effect creates a regulatory negative feedback loop.

The thyroid hormones T_3 and T_4 (*figure 3*) are tyrosine-based hormones and an important component in their synthesis is iodine. T_4 represents the major form of thyroid hormone in the blood. The ratio T_4/T_3 released in the blood is roughly 20 to 1. Thyroxine is converted to the more active T_3 (three to four times more potent than T_4) within cells by deiodinases (Ogilvy-Stuart, 2002). These hormones are then further processed by decarboxylation and deiodination to produce iodothyronamine and thyronamine.

Fig. 3 – Biochemical structures of T_3 and T_4



Triiodothyronine



Thyroxine

Thyroid hormones regulate multiple cardiovascular functions through different mechanisms; these mechanisms all undergo transition during the perinatal period (Breall *et al*, 1984).

The possibility that the postnatal increase in cardiac output can be related to thyroid activity is entertained, because plasma TSH, as well as T_3 and T_4 concentrations, have been shown to increase dramatically after birth. Serum thyroid-stimulated hormone concentrations increase within 30 min, and T_3 concentrations increase immediately and within several hours have risen 3- to 6 fold (Nathanielsz *et al*, 1973; Fisher *et al*, 1977). It has been clearly demonstrated that in lambs thyroidectomized before birth, T_3 is not detectable in fetal plasma and there is no increase in concentrations after delivery; in these lambs, cardiac output is considerably lower than in non-thyroidectomized lambs, as well as heart rate and arterial blood pressure.

Increased plasma thyroid hormone concentrations are associated with increased myocardial contractility (Buccino *et al*, 1967; Skelton *et al*, 1970) and there are several possible mechanisms that have to be considered. It has been suggested that thyroid hormone has a direct effect on the myocardium (Levey & Epstein, 1969) or that it is largely dependent on altered cardiac myosin ATPase activity (Morkin, 1979).

Increased contractility of the hyperthyroid heart could in part be accounted for by appearance of a new species of myosin with a greater actin-activated ATPase activity (Fink & Morkin, 1977).

Another possible mechanism is through its effect on NaK-ATPase activity (Philipson & Eldman, 1977), or through regulation of energy conservation in mitochondria (Werner & Nauman, 1968). Others proposed that thyroid hormones may indirectly influence myocardial performance through the sympathetic-adrenal system. Several studies have indicated that excess thyroid hormones increase the number of myocardial beta adrenergic receptors (Williams *et al*, 1977; Whitsett *et al*, 1982), thereby facilitating myocardial response to the increased circulating catecholamine concentrations.

The postnatal cardiovascular adaptations are dependent on normal prenatal thyroid function and not on the postnatal increase in thyroid activity. Thyroid activity increases progressively during late gestation, but within a few days prior to birth there is a marked increase in plasma T_3 concentrations. It is possible that this late prenatal rise in plasma T_3 concentrations increases myocardial beta adrenergic receptor numbers or responsiveness, so that there is greater myocardial response to sympathetic-adrenal stimulation.

Thyroid has been reported also to increase myocardial mass by inducing hypertrophy (Breall *et al*, 1984) and this could influence the ability of the heart to increase cardiac output. However, the hypertrophy could be secondary to the increased work to which the heart is subjected.

As consequence of these functions, hyperthyroidism produces a marked decrease in the systemic vascular resistance, which facilitates an increase in cardiac output and augments peripheral blood flow (Klein & Ojamaa, 2001). T_3-induced peripheral vasodilatation occurs through smooth muscle and endothelium-dependent vasorelaxation (McAllister *et al*, 2005). Responses to T_3 vary according to vascular bed with greater vasodilatation in large conductance vessels such as aorta.

The literature appears to lack studies evaluating the relationship between systemic vascular resistance and thyroid hormone in perinatal models. The relationship between pulmonary vascular resistance and thyroid levels is directly pertinent to right ventricular function during transition to extra uterine life. Multiple reports exist in the adult literature showing an association between hyperthyroidism and pulmonary hypertension (Armigliato *et al*, 2006; Siu *et al*, 2007). Reversal of hyperthyroidism generally lowers the pulmonary artery pressure, suggesting that the relationship does not represent an association of two inflammatory diseases, but instead is related directly to chronic elevation in thyroid hormones levels.

2.1.3 Current knowledge about the newborn calf

Despite the knowledge about the role of prostaglandins during parturition in cattle and the research on prostaglandin in newborns of human species, very few data are present in literature about PG levels in the newborn calf.

Kornmatitsuk *et al* (2004) investigated $PGF_{2\alpha}$ plasma concentrations in 17 calves at birth and 1 hour after birth; calves were divided in groups according to the index of stillbirth. Plasma levels of PG-metabolite (PGM) were very high at birth and decreased significantly within 1 h in all groups. In a weak calf (purple mucous membranes, unwillingness to lift the head and making no attempt to escape from external stimuli), lower levels of PGM were found compared with the average levels of all groups. The authors hypothesized that the levels of PG-metabolite decreased rapidly within 1 h after birth due to the rapid metabolism, thus attributing neonatal PGM concentrations to maternal or placental source.

As already said, concentrations of both $PGF_{2\alpha}$ and PGM are greater in umbilical plasma than maternal plasma, and they both significantly increase in umbilical plasma after the onset of labor due to fetal placental unit production (Saeed *et al*, 2003), but it is difficult to distinguish between maternal/placental or fetal source.

It is more likely that plasma PG concentrations in the newborn result from both maternal and the own fetal production.

More investigations are necessary to determine $PGF_{2\alpha}$ plasma profile in healthy calves at birth and during the neonatal period; relationships with type of birth (spontaneous parturition versus caesarean section) or with clinical conditions at birth should be examined.

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Some data about thyroid hormones levels in calves are available from literature; T_4 and T_3 levels are high at birth and increase after colostrum intake, remaining slightly elevated until 32 hours after birth (Stojic *et al*, 2002). The decrease starts a little at 48 hours of age, with higher values in female than in male calves (Steinhardt *et al*, 1995). At 7 days of age plasma thyroid hormones levels are much lower than at birth (Jovanovic *et al*, 1982; Ronge & Blum, 1988; Stojic *et al,* 2002). Grunberg *et al* (1998) found that T_4 and T_3 concentrations in blood serum of calves were beyond maternal levels, increasing significantly in the first 24 hours with strong individual specificity, and reaching levels of adult animals at the end of the three-month-period; for T_4 and T_3 a strong influence of maternal levels on the hormone levels of the calves could be ascertained.

Although the levels of thyroid hormones were individually specific, strong correlations were found between the thyroid hormone values of the cow and those of the calf, and between T_3 and heart rate of the calves (Steinhardt *et al*, 1995).

2.2 Respiratory system

The key event in the transition to extra uterine life to is the beginning of respiratory movements, with inflation of the lungs and subsequent independent oxygenation; these processes already start during gestation, since they require an anatomical and functional maturation of the lungs and of the hormonal axis.

2.2.1 Maturation

Fetal breathing movements (FBM), representing contractions of the intercostals muscles and of the diaphragm, do occur already during the last months of pregnancy (unpublished data). The benefits to the fetus of FBM are thought to include exercising the diaphragm in preparation for birth and promoting growth of the fluid-filled fetal lungs, but they are obviously not involved in gas exchange (Thorburn, 1995), which takes place through the placenta. The alveolar air spaces of potential fetal lungs are thus partially relaxed by a fluid secreted by lung tissue. During vaginal delivery, thorax is compressed and a certain amount of this fluid is evacuated through the larynx, while most is absorbed through the alveolar walls in the initial stages of ventilation. The absorption of the fluid involves both adrenergic receptor of lung epithelial and thyroid hormones.

Towards the end of fetal development, the pneumocytes type II produce the *surfactant*. The word "surfactant" was created from the term "surface active agent," which describes its function in the lungs (Bleul, 2009). Surfactant is a combination of 90% lipids and 10% proteins: the lipid portion is primarily phospholipids, the critical component of surfactant to reduce surface tension in the lungs. Type-II pneumocytes produce, store, secrete and reabsorb phospholipids (Zimmermann *et al*, 2005) which form a monolayer in the alveoli and distal bronchioles.

Type II pneumocytes also produce surfactant proteins (SP): four types of SP have been described for humans, cattle, and a few other species (Takahashi *et al*, 1990; Zimmermann *et al*, 2005).

SP-A is the most common type in mature neonates: it regulates the secretion of phospholipids and their inclusion in the surfactant monolayer, along with reabsorption into type II pneumocytes. Together with SP-D it also plays a important role in pulmonary immunodefense, since it can bind pathogens and promote their removal. The SP-B and SP-C enhance rapid inclusion of phospholipids in the air-fluid interface of the monolayer and have a key function in surfactant metabolism.

The presence of a good quantity and quality of surfactant is crucial for the onset and maintenance of air breathing, since it is responsible for the stability of the alveolar surface, preventing their collapse upon expiration by lowering surface tension. Surfactant promotes the expansion and stabilization of initial pulmonary alveolar air sacs, which remain partially inflated after exhalation, allowing the reinsufflation to happen with less effort. The surfactant reduces the effort needed to overcome the high surface tension present in the cells not inflated.

During vaginal birth, but less so during Caesarean section delivery, the thoracic cage is compressed; this produces an ejection of tracheal fluid via the airways. The recoil of the chest wall causes a passive inspiration and establishes an air-liquid interface. The first active breath is made slightly easier by the fact that some fetal lung fluid is retained in the alveoli and smaller airway, thus requiring less distending pressure than a totally collapsed lung. In addition, in near-term and term newborns, surfactant produced by type II pneumocytes decreases alveolar surface tension, which prevents the lungs from total collapse at the lower transpulmonary pressures that occur in the subsequent breaths.

The stimulus for the first active inspiration is debatable but is likely to be a multifactor set of events including change in temperature, light, noise, gravity, hypercapnea, sudden change in PaO_2. At birth in fact, the first breath is usually preceded by a physiological apnoea; this lasts few seconds, and it is solved thanks to many stimulus from different sources. With the breaking of the umbilical cord asphyxia rapidly develops, associated with an increase in blood carbon dioxide tension. This increase in pCO_2 is the primary stimulus that starts the breathing and lung inflation; other stimuli are represented by cold temperature and tactile sensations.

In addition to the mechanical effects of the first breath, the pulmonary circulation that parallels lung development and is maintained at high pulmonary vascular resistance during fetal life must also transition from a fetal to a newborn circulation. The latter is characterized by a gradual lowering of the pulmonary vascular resistance and closure of the foramen ovalis and ductus arteriosus within few minutes from birth, and a change from a parallel circulation to one in series. The resultant effect is ultimately to match ventilation to perfusion for the most efficient oxygen extraction and delivery, and for carbon dioxide removal.

2.2.2 Endocrine regulation

Prostaglandins

Newborn mammals often suffer of hypoxia during parturition or develop hypoxemia secondary to respiratory distress (Tyler *et al*, 1975). During fetal life and the transition to extra-uterine air breathing, arachidonic acid metabolites play an important role in regulating pulmonary vascular tone and blood flow (Tyler *et al*, 1975; Heymann, 1999).

Above all, PGI_2 modulates tone maintaining vascular resistance relatively constant (Heymann, 1999). Other prostaglandins, as PGD_2, act in the newborn causing pulmonary vasodilatation and then, at 12-15 days after birth, produce pulmonary vasoconstriction (Heymann, 1999).

Hypoxia increases pulmonary and systemic arterial pressures and pulmonary vascular resistance: the PGE levels are higher in infants with chronic or acute hypoxia compared to controls (Reznichenko *et al*, 1993). $PGF_{2\alpha}$ content is decreased in infants with chronic hypoxia while it is increased with acute hypoxia (Reznichenko *et al*, 1993).

Thyroid hormones

Infants affected by respiratory distress syndrome, requiring ventilatory support, present lower T_4 levels compared to controls during the first 48 hours of life; possibly a depressed metabolic rate represents an adaptive response to illness (Borges *et al*, 1985; Ogilvy-Stuart, 2002).

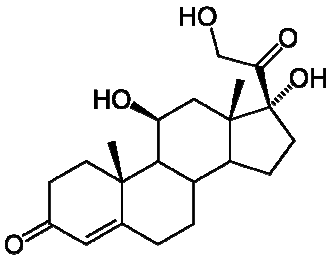
A recent study found significantly lower levels of TSH, T_4 and T_3 between 18 and 24 h of life in asphyxiated newborns compared to controls (Pereira & Procianoy, 2003); this suggests central hypothyroidism secondary to asphyxia.

Cortisol

Cortisol is one of the major stimulus for lung maturation and surfactant production and release (Fowden, 1995); it is synthesized from cholesterol (*figure 4*) and the synthesis takes place in the *zona fasciculata* of the cortex of the adrenal glands. The adrenal cortex also produces aldosterone (in the *zona glomerulosa*) and some sex hormones (in the *zona reticularis*).

The synthesis of cortisol in the adrenal gland is stimulated by the anterior lobe of the pituitary gland with adrenocorticotropic hormone (ACTH). The production of ACTH is in turn stimulated by corticotrophin-releasing hormone (CRH), released by the hypothalamus. ACTH increases the concentration of cholesterol in the inner mitochondrial and then cholesterol is converted to pregnenolone, catalyzed by cytochrome P450 SCC.

Fig. 4 - Biochemical structure of cortisol



In normal release, cortisol (like other glucocorticoid agents) has widespread actions which help in restoring homeostasis after stress. Cortisol has anti-inflammatory effects by reducing histamine secretion and stabilizing lysosomal membranes. It also inhibits phospholipase by producing a substance known as lipocortin, a suppressor of phospholipase, and thus reduces the availability of free arachidonic acid (Buckingham *et al*, 2006). Consequently, it reduces the possibility to produce prostaglandins.

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In many species, including the large farm animals, the secretion of glucocorticoid hormones from the adrenal cortex increases markedly during the final days of gestation. This prenatal increase in fetal glucocorticoid secretion plays an important role in the cascade of endocrine events leading to parturition, and it is known to stimulate essential maturational events in the lungs, liver,

kidney and gastrointestinal tract in preparation for postnatal life (Nathanielsz, 1998; Challis *et al*, 2000).

The effects of glucocorticoids on the prenatal development of organ function is highly dose-, age-, birth- and species-dependent (Sangild *et al*, 2000); regardless of these limitations, provision of glucocorticoids remains an obvious potential to improve the viability of weak and immature newborns, particularly those born by caesarean section. Hence, treatment of pregnant women with synthetic glucocorticoids has been used routinely during the last decades as a means to enhance pulmonary maturation in premature newborn infants (Bolt *et al*, 2001). In the late 1960s, Liggins (1968, 1969) and Liggins & Howie (1972) were among the first to propose a positive effect of glucocorticoids on fetal maturation in lambs, and reported benefits of prenatal corticosteroids given to mothers at risk of preterm delivery. Since then, many studies reported the clinical benefits of prenatal corticosteroids for prevention of respiratory distress syndrome.

The exact mechanism of glucocorticoid-induced lung maturation is still unknown, but recent advances in molecular genetics have increased the knowledge about the regulation of pulmonary development and surfactant production (McCormick & Mendelson, 1994; Ballard *et al*, 1997; Reichardt *et al*, 1998).

Glucocorticoids, and specifically cortisol, appear to have, besides the stimulation of surfactant, additional effects on pulmonary maturation (Roel *et al*, 2001). Several mechanisms have been proposed to explain the stimulatory effects of glucocorticoids on pulmonary maturation.

The effects of glucocorticoids on structural lung growth and development (Schittny *et al*, 1998; Whitsett & Stahlman, 1998), on antioxidant enzymes (Saugstad, 1998), on lung tissue growth factors (Saugstad, 1998; Jaskoll *et al*, 1996), on inflammatory mediators (Vyas & Kotecha, 1997) and on the regulation of pulmonary absorption (Zhou *et al*, 1996) have been implicated in playing a role in pulmonary maturation, in conjunction with stimulation of surfactant synthesis (Gross, 1990).

During the first few hours of life, high cortisol levels represent a normal response to the stressors associated with labor, birth and transition (Elverson & Wilson, 2005). The perinatal cortisol concentrations represent the maximum levels observed throughout the entire life span.

Many sick preterm infants can exhibit signs of adrenal insufficiency; since the activation of the hypothalamic-pituitary-adrenal axis is fundamental to maintain homeostasis in response to stress, a low secretory capacity of the adrenal cortex causes a decreased stress response during acute illness in preterm newborns, leading to an increase morbidity in these infants (Bolt *et al*, 2002; Watterberg, 2004). Serum cortisone concentrations are greater than serum cortisol levels during the first 2 months after birth, while cortisol levels are higher than cortisone levels after 2 months of age.

However, in premature infants, serum cortisone concentrations are greater than serum cortisol levels even after the first 2 months, and total concentrations of cortisone and cortisol are equal to those in controls (Fujitaka *et al*, 1997). Cortisone is predominant in premature infants older than 32 weeks of equivalent gestational age, but cortisol is higher than cortisone from equivalent gestational age 24 to 31 weeks (Fujitaka *et al*, 1997).

These findings suggest that premature infants are able to secrete glucocorticoids like at term newborns. Moreover, the fetal zone of the cortex, associated with a predominance of cortisone, remained functional in premature newborns for a longer time than in control (Fujitaka *et al*, 1997).

In a study by Scott and Watterberg (1995) plasma cortisol concentrations in 120 premature infants (gestational age 24-36 weeks) were evaluated. An inverse relationship between gestational age and cortisol concentrations was found, with the youngest infants having the highest cortisol values. Illness had a significant negative effect, above all ventilatory support pattern or "use of surfactant"; in fact, cortisol values were lower in infants with the highest ventilatory requirements or that received surfactant compared with levels observed in infants who did not have these requirements (Scott & Watterberg, 1995).

The postnatal pattern in plasma cortisol concentrations therefore depends on gestational age (Scott & Watterberg, 1995). Cortisol values increases in ill infants more than 27 weeks gestational age from day 2 to day 6, although cortisol concentrations decreased in healthy infants. In contrast, cortisol concentrations decreases from day 2 to day 6 in both well and ill infants that were less than or equal to 27 weeks (Scott & Watterberg, 1995). The elevated steroid precursors in preterm infants and low cortisol levels in stressed sick preterm infants may indicate an immaturity of adrenal enzyme activity and inadequate adrenal reserve for stress (Lee *et al*, 1989).

Stressed at term infants with respiratory distress, instead, present significantly increased basal concentrations of cortisol compared to normal newborns (Das *et al*, 2002; Soliman *et al*, 2004). Asphyxiated newborn infants had a significantly higher serum level of cortisol than the control group. Mean serum cortisol levels collected 12-18 hours after birth were similar between both groups. These findings suggest that elevated serum levels of cortisol are related to birth asphyxia, and that the majority full-term newborns have adequate adrenal cortical function in response to stress: therefore, they do not need any corticosteroid supplementation in routine practice (Soliman *et al*, 2004).

There is today much evidence that labour and vaginal delivery have beneficial short- and long-term effects on neonatal respiratory adaptation to extra uterine life (Halliday, 1999). Indeed, the increased rates of elective caesarean section (ECS), a procedure long believed to be less distressing for the fetus, have been associated with a higher risk of iatrogenic respiratory distress syndrome (RDS) for the neonate (Zanardo *et al*, 2004; Gerten *et al*, 2005).

In animal and human studies, decreased secretion and deposition of surfactant in the alveolar space, and decreased levels of catecholamines and stress hormones have been found to occur in individuals with RDS.

When comparing the endocrine response between newborn babies born by ECS and babies born by vaginal delivery (Zanardo *et al*, 2006), blood levels of cortisol were found to be significantly lower in the ECS.

These findings lend support to the suggestion that some clinical cardiorespiratory consequences of ECS might be initiated and enhanced by the reduced production at birth of stress hormones and cortisol (Gerten *et al*, 2005). Reduced blood concentrations of neuroendocrine markers provide additional insight in the pathophysiologic mechanism that brings a poor immune-neuroendocrine response at birth into the cascade of events that can lead to altered postnatal adaptation and iatrogenic respiratory distress syndrome (Zanardo *et al*, 2006).

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In domestic animals, some authors found higher cortisol levels in fetal lambs affected by hypoxemia as compared to controls (Gardner *et al*, 2001, 2002).

In foals, since fetal cortisol increases late in gestation, cardiovascular and endocrine function maturation could be delayed, and the cortisol increase during the early postnatal life to continue these maturational events (O'Connor *et al*, 2005). In a recent study (Panzani *et al*, 2009) cortisol levels in foals appeared high at birth and declined to basal levels within 3 hours after parturition. This seems in agreement with other studies (Rossdale *et al*, 1982; Silver *et al*, 1991) where, in full-term foals, high cortisol levels were detected at birth (67 ng/mL), followed by a further increase by 2 hours after parturition (141 ng/mL) (Silver *et al*, 1991). In that study, premature foals exhibited significantly lower cortisol levels (17 to 27 ng/mL). Moreover, no postnatal rise in cortisol levels was observed in premature foals.

2.2.3 Current knowledge about the newborn calf

Experiments in which the bovine fetus has been surgically provided with indwelling catheters in its circulation, have demonstrated that values of PO_2 and PCO_2 in fetal blood are relatively stable during late gestation (Comline *et al*, 1974; Taverne *et al*, 1988). This relative stability is mediated by a uterine blood flow. It is important to realize that the circulation through the pregnant uterus is not auto-regulated and thus blood flow will follow any change in perfusion pressure. The clinical implication of this becomes evident when an acute bleeding occurs, for example after rupture of the broad ligament of the pregnant horn. This will cause an abrupt fall in perfusion pressure and only when the fetus is delivered immediately from the uterus by an emergency caesarean section, it will has a chance to survive hypoxia and anoxia.

During parturition, fetal oxygenation can become endangered by the occurrence of rhythmic contractions of the uterus. Because blood vessels to the maternal side of the placentomes pass the myometrium, contractions will increase vascular resistance and impede uterine blood flow. When intrauterine pressure exceeds the blood pressure in the umbilical veins (for example when the dam makes abdominal straining movements during the expulsive stage), also the flow of oxygenated blood towards the fetus becomes temporarily hampered or interrupted (Bleul *et al*, 2007*a*). The possible negative effect of elevated intrauterine pressure for circulation becomes especially evident during transvaginal obstetrical procedures, such as repositions or extraction. These will not only elicit prolonged abdominal straining by the dam but will also enhance (Ferguson reflex) additional release of oxytocin. Once the myometrium becomes over-stimulated and relaxation intervals between individual contractions have disappeared, the decreased uterine blood flow becomes a serious threat for the calf. This implies that such obstetrical measures have to be performed quickly and should not be continued when no real progress is obtained. It is also one of the reasons why the application of exogenous oxytocin should never be considered as long as the calf is still within the birth canal.

At the end of gestation there is a steep rise in plasma cortisol levels in the bovine fetus. Since cortisol, which is produced and secreted by the fetal adrenals, also forms the fetal trigger for the onset of calving (Comline *et al*, 1974; Hunter *et al*, 1977), there is an efficient synchronization between the control of parturition and fetal lung maturation.

Because synthetic corticosteroids can pass the placental barrier, they can reach the fetal circulation when applied to the mother and in this way they can accelerate lung maturation (Houfflin-Debarge *et al*, 2005) and surfactant production (Zaremba *et al*, 1997). Calves that are born before some 90% of the normal gestation length in fact usually suffer from respiratory problems after birth and this is most likely associated with inadequate synthesis of surfactant by their lungs (Bleul, 2009).

In this way the use of corticosteroids for induction of premature calving can reduce the incidence of respiratory distress syndrome in the neonatal calves. Anyway, little is known about the physiological effects of prenatal glucocorticoid treatment on premature newborn calves. Some studies report the normal plasma cortisol profile in the newborn calves. Cortisol concentrations, which appear high at birth, tend to decrease during the first week of life and to decline markedly after feed intake (Lee *et al*, 1995; Hadorn *et al*, 1997; Hammon & Blum 1998).

Neonatal calves born by caesarean section seem to be more predisposed to develop a respiratory distress syndrome, and therefore a respiratory acidosis, during the first hours of their life (Cambier *et al*, 2000).

Moreover, some authors (Danlois *et al,* 2000; Danlois *et al*, 2003) found that belgian double-muscled calves, who usually undergo a caesarean section, present some characteristics that can explain their higher sensitivity to hypoxia; they present a lower blood oxygen equilibrium curve (OEC) compared to dairy calves, and few protein C content in the surfactant.

In a study from Massip (1980), blood samples were taken from 24 calves, six born normally, 11 by caesarean section and seven after difficult delivery; mean values for plasma cortisol concentrations did not differ much with the three types of delivery, although cortisol levels were highest after dystocia.

A more recent study (Hoyer *et al*, 1990) determined plasma glucocorticoid concentrations for 6 days in 47 newborn calves that had been subjected to various obstetrical procedures at term. Concentrations of glucocorticoids were uniformly high at birth (70 to 103 ng/ml), but increasing degrees of acidosis were accompanied by increasing glucocorticoid concentrations in plasma.

Plasma glucocorticoid concentrations decreased sharply during the first 6 hours after delivery and reached a plateau at 48 hours after birth (14 to 21 ng/ml). The latter was taken as an indication that adaptation had been achieved. Again, calves subjected to severe pulling had higher glucocorticoid concentrations at birth (110.4 ng/ml) than calves requiring no assistance (88.3 ng/ml), calves requiring only slight assistance (83.8 ng/ml), or calves that had been delivered by caesarean section (82.9 ng/ml).

Whether this higher incidence of RDS in calves from caesarean section is someway due to genetic differences or to different levels of cortisol induced by different mode of delivery is still unknown; therefore, more investigations on plasma cortisol pattern in newborn calves are necessary.

2.3 The kidney

2.3.1 Maturation

A basic knowledge of the principles of nephrogenesis is indispensable for a good understanding of neonatal renal physiology (Drukker & Guignard, 2002).

▪ Glomerular function

Creatinine clearance remains a widely used clinical tool for evaluating renal glomerular function. Many years ago, creatinine was chosen for clinical clearance determination because:
- serum creatinine can be easily measured in the laboratory
- serum creatinine is constant in adults because it depends on total muscle mass
- creatinine is totally filtered at the glomerulus, not reabsorbed by the renal tubule, and only slightly secreted by the tubular cells.

To measure GFR for research purposes, the clearance of exogenously infused inulin is used. In the neonate, as in all very young children, the creatinine clearance is cumbersome and is unreliable unless a bladder catheterization is used to assure an accurate, timed urine collection. Such an invasive procedure is not indicated for routine use; therefore, it is customary to follow renal function in newborn babies on the basis of repeated serum creatinine levels.

Unfortunately, serum creatinine levels in the first 3 weeks of life are not reliable. At birth, serum creatinine is high, reflecting maternal levels. During the first weeks of life, the highest levels are observed in the most premature infants (Bueva & Guignard 1994). In term neonates, serum creatinine decreases rapidly to reach stable neonatal levels by 1 to 2 weeks of age. In very premature infants, there is a transient increase in serum creatinine with a peak on day 4 (Gallini *et al*, 2000) followed by a progressive decline toward normal neonatal values by 3 to 4 weeks of life.

This decline is probably caused by the tubular reabsorption of creatinine, as observed in newborn rabbits before the completion of nephrogenesis (Matos *et al*, 1998). The authors speculate that this transient phenomenon is the consequence of passive back-diffusion of creatinine across leaky tubules (Guignard & Drukker, 1999).

▪ Tubular function

Many renal tubular secretory and absorptive functions aside from those of creatinine are affected by postnatal maturational changes. However, most endocrine, secretory, and absorptive tubular processes, although immature, are relatively well developed at birth. Additional developmental changes are observed in the activity of vasoactive substances, the dopamine system, and a variety of enzymes such as alkaline phosphatase, glucose-6- phosphatase, 5nucleotidase and tubular cell Na+/K+- adenosine triphosphatase activity. All these proteins are important for the regulation of postnatal renal function (diuresis and tubular handling of water and sodium) and blood pressure. The delineation of the molecular basis of the various tubular-epithelial transport mechanisms and associated genetic defects has shed new light on a number of rare pediatric renal disorders (Zelikovic, 2001).

Of the many developmental aspects of tubular function, the most interest is a recent finding related to the specific acid-base status of the newborn. Plasma bicarbonate (HCO_3^-) concentrations depend on the renal HCO_3^- threshold, which is low in the term newborn and even lower in premature or very low birth weight (< 1,300 g) infants. The low renal HCO_3^- threshold may be caused by the physiologic volume expansion of the premature newborn and by the relative immaturity of tubular transport mechanisms.

Three Na^+/HCO_3^- co transporters and approximately 14 zinc-metallo-enzymes known as carbonic anhydrases (CAs) govern the proximal tubular reabsorption of HCO_3^-. Winkler *et al* (2001) recently described the maturational aspects of CA-IV that facilitate renal acidification by catalyzing the dehydration

of H_2CO_3 in the luminal membrane of the tubule. During the first 2 weeks of life, expression of CA-IV mRNA and protein in neonatal rabbits was approximately 20% of that found in the adult animal and subsequently increased more or less with other enzymes. The maturation of CA-IV occurred in a centrifugal pattern, implying that the development starts in the medullar region (physiologically, the oldest part of the kidney) and later proceeds toward the cortex. As the CA system matures, the excretion of an endogenous or exogenous acid load is not impaired. Therefore, very low birth weight babies generally thrive despite the physiologic acidemia.

▪ Sodium and water metabolism

Water and electrolyte homeostasis in the term and preterm infant differs in many ways from that of the mature person. The fluid balance of the neonate is characterized by rapid changes in the distribution of water between the intracellular and extracellular fluid compartments. Thus, term and premature infants undergo, respectively, approximately a 10% or a 15% postnatal loss of extracellular water and perhaps also of intracellular water, mainly via the kidneys. This diuresis is accompanied by sodium loss, accounting in part for the renal sodium-losing state after birth. Premature babies lose more sodium than term infants and almost inevitably will temporarily need oral sodium supplementation. The high fractional excretion of sodium (FENa) falls within days, at least in the term neonate, followed by renal sodium conservation during the next 2 to 3 postnatal weeks. This process is delayed in premature infants. The postnatal change in FENa was studied by Gallini *et al* (2000) in 83 preterm neonates with a gestational age of less than 32 weeks: FENa correlated negatively with postnatal age, whereas the creatinine clearance, calculated from serum creatinine values, showed the expected increase. The authors summarized their findings by stating, "Glomerular function shows a progression directly correlated to gestational age and postnatal age, whereas tubular function correlates inversely to the same

parameters." These data show that immediately after birth, all babies are in a negative sodium balance, especially premature infants.

Throughout the remainder of early infancy, the kidney of the newborn infant is in a sodium-conserving state; a positive sodium balance in the neonate is a prerequisite for growth and development. The avid incorporation of sodium in the tissues is apparently the cause of late hyponatremia, seen in rapidly growing very low birth weight infants at 4 to 6 weeks of postnatal age. This late hyponatremia is not caused primarily by excessive loss of body sodium or by conservation of free water but by an increased need of sodium for growth.

It is well established that infants cannot efficiently excrete a hypertonic saline load compared with adults, even when the maximal attainable FENa is almost comparable with that of the mature person. In a recent summary of renal sodium metabolism during early postnatal development, Chevalier (2001) explains the latter finding on the basis of experiments in rat pups as "a fine interplay between the developing brain, heart, thyroid, adrenals and the sympathetic nervous system." His main point, however, is a clinical one. Because the neonate needs sodium for adequate growth and the dietary supply of sodium (breast milk or neonatal formula) is limited, nature "elicits an adaptive anti-natriuretic renal tubular response." The neonatal kidney is thus rather well equipped to deal with the rapidly changing conditions of extra uterine life.

2.3.2 Endocrine regulation

Role of prostaglandin

The newborn kidney's main physiologic limitation is its very low glomerular filtration rate (GFR), maintained by a delicate balance between vasoconstrictor and vasodilator renal forces. These forces recruit maximal attainable filtration pressure in the face of minimal renal blood supply resulting from a combination of a low mean arterial blood pressure and a high intrarenal vascular resistance.

The low GFR of the newborn kidney, although sufficient for growth and development under normal conditions, limits the postnatal renal functional adaptation to endogenous and exogenous stress (Toth-Heyn *et al*, 2000). Such stress may take the form of anoxia, sepsis, or exposure to nephrotoxic medications (all causing renal hypoperfusion). This stress becomes even more problematic when these factors occur in combination, which is not rare. Vasoactive forms of nonsteroidal anti-inflammatory drugs (NSAIDs) can induce renal hypoperfusion resulting in generally reversible, oliguric acute renal failure.

This adverse renal effect of cyclooxygenase inhibition appears to be specific for the term and particularly the premature newborn (Chamaa *et al*, 2000; Drukker *et al*, 2001). The mechanism of action of these drugs is abolishing the vasodilator action of *prostaglandins*, which maintain an effective neonatal GFR.

PGE and PGF are biosynthesized in the fetal and neonatal kidney medulla, and this could be related to their role of control on the hemodynamic and fluid and electrolyte homeostasis (Friedman & Demers, 1980). High urinary excretion of prostaglandins during the newborn period and some distinct changes with advancing age suggest that these prostanoids might play a specific role during child development (Leonhardt *et al*, 1992).

PGE_2 main roles regard the renal function, contributing to kidney development, regulating perfusion and glomerular filtration rate and controlling water and electrolyte balance (Antonucci *et al*, 2007). Moreover, PGE_2 counteracts the vasocostrictive effects of high levels of different mediators, like angiotensin II, that are typical of the transitional period from fetal to extra uterine life (Antonucci *et al*, 2007). Also in pathological conditions, like congenital or acquired nephropathies, PGE_2 plays important pathophysiologic role (Antonucci *et al*, 2007). In full term infants, mean urinary PGE_2 is significantly lower compared to preterm babies, while $PGF_{2\alpha}$ is significantly higher.

The decrease of the $PGE_2/PGF_{2\alpha}$ ratio is usually accompanied by an increase in blood pressure and an increased urinary osmolarity due to the acquired concentrating capacity of the kidney (Csaba *et al*, 1979; Joppich *et al*, 1979; Antonucci *et al*, 2007).

These are the reasons for, in health, the prostaglandin levels in the fetus and the newborn are very high. When prostaglandin synthesis is inhibited, the vasoconstrictor state of the newborn kidney is unopposed. These observations are of great clinical importance because NSAIDs are prescribed during pregnancy for the prevention or management of toxemia, polyhydramnios, and premature birth. These drugs easily pass the placenta, so the fetus is readily exposed to their toxic effects.

Postnatally, recurrent boluses of NSAIDs are infused to promote the pharmacologic closure of a hemodynamic significant patent ductus arteriosus.

2.3.3 Current knowledge about the newborn calf

Some authors (Sommardahl *et al*, 1997; Hammon & Blum, 1998; Wiest & Klee, 1998; Steinhardt & Thielscher, 1999; Kuhne *et al*, 2000) investigated the biochemical profile of newborn calves, mostly in relation with different nutrition strategies.

Anyway no data are available regarding influence of parturition on biochemical pattern in the newborns of this specie.

2.4 Thermoregulation

2.4.1 Maturation

Unlike poikilotherms, whose body temperature can vary substantially with the external environment, mammals are homeotherms and must generate heat to maintain a body temperature that varies only within a relatively small range (normal variance of only 0.3%).

During development, the core body temperature of the fetus is closely correlated with the mother, and as such, it will normally remain a consistent and approximate 1°C above that of the mother. After birth however, the newborn is exposed to an environment that is often substantially cooler and it is subject to the basic mechanisms through which it will start to lose heat: these processes are evaporative heat loss (which is a function of humidity) conduction (direct transfer of heat from newborn to contact surface), convection (loss of heat to cooler surrounding air) and radiation (indirect transfer of heat to nearby lower temperature objects). To avoid substantial heat loss as the result of these mechanisms, the core body temperature in mammals is maintained through regulatory processes that include vasoconstriction, and shivering and nonshivering thermogenesis. However, the extent to which newborn can control thermoregulation to maintain an optimal core body temperature is limited, relative to adults. Although shivering thermogenesis is quantitatively the most important mechanism in adults, for newborns the primary mechanism is through chemical thermogenesis, in the absence of muscular contraction and shivering (Stern, 1980).

Production of heat to maintain homeothermy in the neonate is dependent on shivering thermogenesis in the muscle and nonshivering thermogenesis in brown adipose tissue (BAT). BAT is a specialized organ whose thermogenic capacity is attributed to a unique uncoupling protein (UCP) located in the mitochondria.

The UCP in BAT "uncouples" mitochondrial respiration from oxidative phosphorylation (synthesis of adenosine triphosphate, ATP) thereby using energy generated to produce heat.

It is estimated that approximately 40 to 50% of the thermogenic response during summit metabolism is attributed to nonshivering thermogenesis with the balance (approximately 50 to 60%) attributed to shivering thermogenesis.

The two types of adipose tissue are white and BAT. The primary function of white adipose tissue is storage and release of fatty acids for use as an energy source, while that of BAT is generation of heat through nonshivering thermogenesis. The major anatomic location of BAT is around the kidneys, and it appears to be similar in lambs, babies and calves. BAT is extensively vascularised and brown adipocytes and the blood vessels are highly innervated by the sympathetic nervous system. Even though BAT accounts for only 1.5-2% of body weight in newborn lambs, it can account for 40 to 50% of maximal thermogenesis during cold exposure. NE stimulation of BAT thermogenesis activate hormone-sensitive lipase which activates lipolysis to provide free fatty acids for mitochondria respiration. Thus, NE release during sympathetic stimulation plays a critical role in the activation of BAT thermogenesis during cold exposure.

Beyond the fact that physiological and behavioural responses are relatively immature in the term newborn, the limited ability of newborns to maintain core body temperature is particularly compromised among babies born prematurely or those with low birth weight. These infants have limited vasoconstriction capability compared with term infants (Knobel *et al*, 2009) greater surface-to-mass ratios, and preterm and/or low birth weight babies have lower brown fat deposits (Aherne & Hull, 1966) which are essential for nonshivering thermogenesis.

Heat loss through evaporation of the amniotic fluid from the newborn skin is the most important mechanism. In addition, specific newborn care practices can contribute substantially to the loss of heat, especially in the first hours of life; birthing in an insufficiently warmed room, placing the newborn uncovered on the ground, floor, or other surface while awaiting delivery of the placenta, insufficient or delayed drying or wrapping of the neonate after birth, immediate bathing with cool or cold water and delayed drying, and delayed breastfeeding, are all practices that might increase the risk of heat loss.

The heat loss occurs immediately after birth; continuous recording of body temperature in a variety of settings has demonstrated the initial drop is normally followed by a subsequent increase, which may depend on birth weight, ambient temperature, and proximity to the mother (Ellis *et al*, 1996). The initial loss of heat can be quite large; for example in the first 10 to 20 minutes after birth it is not uncommon for a baby to lose 2 to 4°C, with further subsequent losses possible in the presence of the above practices. As a consequence of both the factors surrounding the immediate care of newborns and the risk these pose for heat loss, and the innate features of the neonate, it is of little surprise that hypothermia is a common phenomenon in low resources settings, including among infants born in facilities and in the community.

2.4.2 Endocrine regulation

Successful transition to extra uterine life requires that the fetus, previously poikilothermic, with the placenta subserving most of its metabolic needs, becomes homeothermic and self-sustaining. Crucial integration of a variety of neural and endocrine events is a prerequisite for this transition. The coordinated actions of adrenal cortical, adrenal medullar and thyroid hormones facilitate the transition to air breathing, neonatal cardiovascular adaptation, glucose homeostasis, thermogenesis, and gut maturation.

Thyroid hormones

Thyroid hormones have a major role in energy, nutrients and inorganic ions metabolism, in thermogenesis and stimulation of growth (Ogilvy-Stuart, 2002).

Warm-blooded species are unique, in that TSH increases oxygen consumption and stimulates enzymes, such as the mitochondrial 3-phosphate glycerol dehydrogenase and lipogenic enzymes (Weirich *et al*, 1987), which may be accessory to their thermogenic effect.

That TSH increases the rate of aerobic metabolism and heat production has been known for over a century, but there is still not a clear understanding of the physiological role of the thyroidal secretion.

At birth, TSH is often normal or slightly elevated, and healthy full-term newborns present normal free T_4 levels and low T_3, due to an immature T_4 deiodination system. During the first 24 hours of life, TSH consistently increases so that a rapid rise of T_3, and subsequently of T_4 levels, can be observed (Glinoer *et al*, 1992). Until day 3 or 4 of age TSH decreases by T_4 feedback inhibition (Kratsch & Pulzer, 2008). Increased serum T_4 binding protein can also be found in newborns, because of the high maternal estrogen impregnation during gestation (Glinoer *et al*, 1992).

Both in newborns babies at term and in preterm infants, there is a surge of TSH within 30 minutes after birth, with a subsequent release of T_4 and T_3. In at term babies they rise to above normal levels and T_4 remain higher compared to adults until 6 months of age, while T_3 gradually reach infants levels between 2 and 112 weeks from parturition. Premature babies instead present an immature thyroid axis. For this reason the production of hypothalamic TRH is reduced, the immature thyroid gland does not respond to TSH and the follicular cells of the thyroid are not able to organify iodine. Finally they also have a low capacity to convert T_4 into T_3. For this reason, preterm babies show lower T_4 levels compared to at term neonates and these levels correlate with gestational age and birth weight (van Wassenaer *et al*, 1993).

TSH and T_3 levels are normal to low and free T_4 levels are lower than normal, while TRH is high. The site of immaturity is mainly represented by the hypothalamus, since responses of TSH and T_4 to TRH are normal. Also thyroid binding proteins are reduced (Ogilvy-Stuart, 2002).

Hypothyroxinaemia of premature newborns is physiological (Ogilvy-Stuart, 2002; Kratsch & Pulzer, 2008) while a severe hypothyroxinemia has been associated with increase mortality and morbidity and a consequent negative outcome (Ogilvy-Stuart, 2002; Fisher, 2007).

The majority of studies on babies have found that vaginal deliveries result in higher average TSH cord levels (Franklin *et al*, 1985; Miyamoto *et al*, 1991; Lao & Lee, 2002; Chan *et al*, 2001a; Chan *et al*, 2001b). This may be related to the observation the stress during delivery seems to be associated with elevated TSH in cord blood (Copeland *et al*, 2002; Therani *et al*, 2003). However, not all studies that have examined delivery mode have produced consistent findings.

Some report that TSH in cord blood is not influenced by mode of delivery (Erenberg, 1978; Ericsson *et al*, 1987; Fuse *et al*, 1991) and one concluded that deliveries via caesarean section result in higher TSH measured in neonatal bloodspots (McElduff *et al*, 2005). In a few studies, the researchers were able to classify the deliveries further, dividing caesarean section into elective C-section and emergent C-section, and dividing vaginal deliveries into spontaneous vaginal deliveries and instrumental vaginal deliveries. Some of these authors (Bird *et al*, 1996; Tehrani *et al*, 2003; Herbstman *et al*, 2008) concluded that babies born via elective C-sections had higher average T_3 and TSH levels, and lower T_4 concentrations (Bird *et al*, 1996). Two other studies reported that instrumental vaginal deliveries had higher cord blood TSH values than spontaneous vaginal deliveries (Miyamoto *et al*, 1991; Chan *et al*, 2001*a*; Chan *et al*, 2001*b*). Herbstman *et al* (2008) detected the highest average cord blood TSH levels among "assisted" vaginal births, consistent with the notion that stress during vaginal delivery may lead to higher TSH levels in cord blood.

Prior studies examining the duration of labour have reported inconsistent results; one found no association between labour duration and cord TSH or free T_4 (Fuse *et al*, 1991) and others found that longer duration of labour (particularly the second stage) was associated with increased cord TSH levels (Miyamoto *et al*, 1991; Lao *et al*, 1992). Other factors marking a stressful delivery have been linked to higher cord TSH levels, including malpresentation (Chan *et al*, 2001*a*).

It is possible that factors such as delivery mode, labour, and labour duration are potential confounding factors for thyroid hormone status; however, there are no clear examples of this in literature. Among these factors, delivery mode has a strong and consistent relationship to thyroid hormone status and should be considered in research studies.

The functional status of the thyroid gland in the perinatal period has been subject to many investigations; all authors agree that newborn domestic animals are born with high peripheral levels of thyroid hormones (Davicco *et al*, 1982; Fisher *et al,* 1966; Hernandez *et al*, 1972; Jovanovic *et al*, 1982).

In a study conducted on neonatal pigs, mean free T_4 and T_3 were found to be significantly raised during the first 3 days of life, with the highest values between 0 and 12 hours after birth. Later, until the end of the first week, thyroid hormone levels decreased, while during the 2nd and 3rd week remained to steady and relatively low levels (Nowak, 1983).

A study found high concentrations of T_3 at birth in full-term foals (4.3 ng/mL), rising to a maximum during the following 2 hours (8.4 ng/mL) (Silver *et al*, 1991). During the same period, premature foals present significantly lower levels (2.9 and 5.4 ng/mL, respectively). This study underlined also a relationship between T3 and cortisol, suggesting that prematurity is associated with low concentrations of both hormones (Silver *et al*, 1991).

2.4.3 Current knowledge about the newborn calf

At parturition the calf moves from the controlled, warm uterine environment to the often-times hostile external environment. This transition necessitates many physiological actions to maintain normal body temperature (homeothermy) especially in seasonal environments typical of cattle production in northern regions. Climatic conditions affect neonatal survival and at low environmental temperatures mortality increases (Azzam *et al*, 1993).

Himms-Hagen (1990) and Carstens (1994) reviewed thermal regulatory physiology in the newborn calf. The ability of the neonate to maintain normal core body temperature is a function of its ability to produce enough heat to balance the loss of heat by evaporative and nonevaporative heat losses. Nonevaporative heat loss involves flow of heat across temperature gradients from the metabolic heat sources in the animal to the environment by radiation, convection, and conduction.

Evaporative heat loss occurs as water evaporates from the skin and respiratory tract surfaces; these losses are generally considered minimal except during wet weather and the immediate postnatal period when amniotic fluid is evaporated from the skin and respiratory tract of the neonate. The cold lethal limit is the critical ambient temperature below which the calf is unable to generate sufficient heat to offset heat loss, is no longer able to maintain thermal balance and hypothermia begins. Prolonged periods of exposure below the cold lethal limit will obviously result in death.

The normal temperature for a newborn calf will be around 39.5ºC, around 0.8 ºC above the body temperature of the mother. There is usually a drop in the body temperature of the calf during the first few hours and this decrease appeared more pronounced in non-vital calves when they were placed in a quite cold environment (Vemorel *et al*, 1984). The temperature drop is mainly caused by evaporation of fluids from the skin and the respiratory tract.

When left with the dam, the calf is licked intensively, especially during the first few hours (Edwards & Broom, 1982). This licking will make the calf dry more quickly, thereby reducing the loss of heat. So, when the calf is separated from the mother immediately after birth, it is important to dry the skin by rubbing it intensively.

In a normal calf both shivering and non-shivering thermogenic mechanisms take place to adapt to the extra uterine environment (Carstens, 1994). This adaptation can also be facilitated by management measures, such as the use of infrared heaters. A recent study showed the beneficial effects of external heating (for the first 24 hours after birth) on respiratory, circulatory and hematologic parameters and on rectal temperature of normal calves (Uystepruyst *et al*, 2002*b*).

A prepartum temperature drop in the dam just preceding parturition is common to essentially all mammalian species studied (Lammoglia *et al*, 1996); this endocrine control of the prepartum temperature drop appears to be associated with PGM and T_3 changes, but involvement of progesterone, estrogens and cortisol cannot be rule out. It is interesting to speculate what the physiological effects of this temperature change might be.

Laburn *et al* (1994) reviewed the literature and found that body temperature of the latter fetus is about higher than that of the dam. The feto-maternal temperature gradient is established before the end of gestation and reflects the balance between rate of heat production by the fetus and fetal heat loss, which occurs mainly via the utero-placental circulation.

Decrease blood flow might be expected to compromise fetal heat loss resulting in a rise in the feto-maternal temperature gradient, which could be potentially dangerous to the fetus. Uterine blood flow declines during labour resulting in an increase in the feto-maternal temperature gradient; in addition, muscular activity during labour increases maternal temperature. All these factors could potentially result in increased fetal temperatures with possible damaging consequences, especially during prolonged parturitions.

There could also be a possibility that this fetal temperature rise may also be a mechanism preparing the fetus for the temperature transition from uterine life to the outside world. High rectal temperatures in calves at birth have been reported, and this heat dissipation could be part of the thermogenic adaption mechanism for the neonate. In addition, it is interesting to speculate what effects hyperthermia during prolonged parturition might have on calf vigour and survival.

For data about basal thyroid hormones levels in calves see chapter *2.1.3*.

Regarding the influence of parturition, Vermorel *et al* (1983) found lower thyroid hormones plasma levels in calves born after dystocia than in eutocial calves, hypothesizing that this difference could explain the lower heat production and the drop in rectal temperature registered in these subjects.

An influence of the course of parturition on the T_3 level and the T_4/T_3 ratio in the first 24 hours was disclosed (Grunberg *et al*, 1998), but never deeply investigated. These hormones show correlations to the pH and pCO_2 in the neonatal calves, and they are also subjected to the effects of breed, surrounding temperatures and type of husbandry (Grunberg *et al*, 1998).

2.5 Growth

2.5.1 Maturation

Most of the maturational and growth processes going on in the newborn already start during gestation, and are consequent upon a close relation between maternal and fetal endocrine regulation.

2.5.2 Endocrine Regulation

During pregnancy, the placenta is an important endocrine organ. It produces numerous hormones, including estrogens and progesterone, lactogen and GH; some of these hormones play a role in the regulation of fetal growth (Murphy *et al*, 2006).

There are little data to suggest a direct role for estrogens and progesterone in fetal growth regulation, but some studies have demonstrated correlations between the concentrations of these hormones and birth weight or placental weight (Mucci *et al*, 2003; Mucci *et al*, 2004).

Placental lactogen promote early embryonic growth (Karabulut *et al*, 2001) and are thought to exert its influence on the fetus by stimulating production of other hormones such as IGF-I and insulin (Handwerger & Freemark, 2000).

Insulin-like Growth Factors

The insulin-like growth factors, IGF-I and IGF-II, have a key role in regulating both fetal and placental growth throughout gestation and postnatal development; alterations in the IGF axis are associated with fetal growth restriction in both animal models and human studies.

They are polypeptides with a sequence similar to insulin (Rinderknecht & Humbel, 1978) and they have mitogenic properties, inducing somatic cell growth and proliferation (Zapf *et al*, 1978; Ashton & Spencer, 1983); they also have the ability to influence the transport of glucose and amino acids across the placenta. IGF-I and IGF-II are important for embryonic development but, postnatally, IGF-I is the predominant growth factor regulating growth.

In many species, both the IGF-I and IGF-II genes are expressed in fetal tissues from the earliest stage of pre-implantation development to the final phase of tissue maturation just before birth. Most IGFs in the fetal circulation originates from fetal tissues that express IGFs and their binding proteins, which allow the fetus to adjust local levels of growth factors, thereby modulating cellular growth and differentiation in an autocrine or paracrine manner.

Fetal serum concentrations of IGF-I and IGF-II increase significantly with advancing gestation, with the greatest rise in IGF-I (Bang *et al*, 1994). These circulating fetal IGFs are likely to be derived predominantly from the fetal tissues, and may be modulated by the placenta (Han *et al*, 1996). The concentration of IGFs in the fetus are positively correlated to birth weight in a number of species including humans, primates, sheep, pigs, rabbits and rodents (Daughaday *et al*, 1982; Gluckman & Butler, 1983; Lee *et al*, 1993; Kind *et al*, 1995; Ong *et al*, 2000). Despite some differences in results obtained from various studies, it is clear that the IGF axis has a crucial role to play in modulating fetal growth during human pregnancy.

Both IGFs are detected in the fetal circulation from early in gestation but plasma concentrations of IGF-II are 3–10 fold higher than those of IGF-I during late gestation in all species studies. Plasma IGF-I levels increase rapidly after birth, primarily as a result of the onset of growth hormone stimulated IGF-I production by the liver. There is, therefore, a shift in IGF predominance from IGF-II before birth to IGF-I after birth, which has led to the concept that IGF-II is the IGF primarily responsible for fetal growth (Jones & Clemmons, 1995).

Gluckman & Butler (1983) found that plasma concentrations of IGF-I measured were lower in the newborn lamb than in the adult sheep during the first 2 days after birth: IGF-I values rose 3-7 days after birth and within 60 days they had fallen to adult values. In contrast, IGF-II levels were higher in the fetus than in the adult and showed a fall starting several days before birth (Gluckman & Butler 1983). By 12 h after birth, IGF-II concentrations were similar to the adult and showed no subsequent postnatal change. These results demonstrate that IGF-I and IGF-II are not secreted in parallel in the perinatal lamb and that major changes in the regulation of both IGF-I and IGF-II are close in relationship to birth. It is suggested that the high fetal IGF-II concentrations are maintained by a stimulus withdrawn before birth. The postnatal rise in IGF-I may be related to the increase in hepatic somatogenic receptors at this age.

Thyroid hormones

Thyroid hormones are necessary for normal growth in children and young animals, as evidenced by the growth-retardation observed in thyroid deficiency (Setian, 2007). The growth-promoting effect of thyroid hormones is intimately intertwined with that of growth hormone.

In the human infant, as in the mouse, the increase in circulating T_3 levels associated with parturition normally triggers maturation of tissue and organ functions essential to postnatal metabolism and homeostasis (e.g. pulmonary, hepatic, intestinal and cardiac functions and brown fat thermogenesis). Thyroid hormone-stimulated maturation of vision and hearing appears to be triggered by the local expression of deiodinases mediating local T_3 production (Fisher, 2008).

Congenital hypothyroidism is one of the most common causes of neurodevelopmental retardation in human infants.

Non Esterified Fatty Acids

Non-esterified ("free" or unsaturated) fatty acids (NEFAs) are the major component of triglycerides (the fat stores in the body), which consist of three fatty acids linked to a glycerol backbone. Hydrolysis of stored triglycerides (fat) in adipose tissue by hormone sensitive lipase liberates NEFAs and glycerol. Hormone sensitive lipase (which is found within the cytosol of adipocytes) is stimulated by various hormones, including glucagon (which is released from α-cells in pancreatic islets in response to low glucose). NEFAs can be used as an energy source by many tissues, including skeletal muscle and hepatocytes. In hepatocytes, their fate differs depending on energy needs, hormone balance and substrate availability; i.e. they can be used for energy production, re-packaged into triglycerides and exported as very low density lipoproteins (VLDL), stored within the liver or converted to ketones.

Essential fatty acids and their long-chain polyenes are indispensable for development and health. During pregnancy, fatty acids are required for changes in fetal tissue composition, particularly that of the brain and adipose tissue (Jumpsen *et al*, 1997). They are also of specific significance during postnatal period, when several processes of maturation and adaptation take place.

Normoglycaemia is an essential factor for the newborn that must adapt to extra uterine life (Barsnick *et al*, 2011; Sperling, 1994). In order to maintain normal levels of hepatic glucose production, the infant must have adequate stores of glycogen and gluconeogenic precursors, like fatty acids, appropriate concentrations of the hepatic enzymes required for gluconeogenesis and glycogenolysis, and a normally functioning endocrine system. The absence of any of these requirements leads to a disruption of glucose homeostasis that usually results in neonatal hypoglycaemia (Barsnick *et al*, 2011; Sperling, 2004; McGowan, 1999). Newborns are highly dependent on glucose intake, and because their carbohydrate stores are limited, during illness these are quickly depleted, leading to mobilization of fat depots (Barsnick *et al*, 2011). For this reason free fatty acids can be a marker for lipolysis.

Plasma levels of total free fatty acids in normal infants are highest at day 1 and decreased rapidly thereafter. Their content of polyunsaturated fatty acids (PUFA), fatty acids susceptible to oxidation, is lowest at day 1 and then increased (Behrman & Harris, 1974; Hara *et al*, 1999), providing strong evidence that oxidative stress occurs as a physiological condition at birth at the initial stage of neonate life. At birth, the newborn must switch abruptly from a state of net glucose uptake and glycogen synthesis to one of independent glucose production and homeostasis.

Similar changes in free fatty acids levels and compositions were observed in infants with asphyxia (Hara *et al*, 1999; Labadaridis *et al*, 2007). The essential fatty acid, linoleic acid, was found to be significantly higher in IUGR placentae compared with those from appropriately grown fetuses, which may have implications for fetal brain development (Pardi *et al*, 2002). Moreover premature infants soon after birth have higher long-chain PUFA levels than term neonates (Labadaridis *et al*, 2007).

2.5.3 Current knowledge about the newborn calf

The somatotropic axis of neonatal calves is basically functioning, although it is not yet fully mature (Hammon & Blum, 1997). During the postnatal period insulin-like growth factor (IGF)-I becomes more important than IGF-II (Butler & LeRoith, 2001). The somatotropic axis and especially IGFs, besides insulin, is involved in GIT development and especially in proliferation and maturation of enterocytes (Laburthe *et al,* 1988; Schober *et al,* 1990; Odle *et al,* 1996; MacDonald, 1999; Menard *et al,* 1999).

It has been demonstrated that IGF-II and insulin are involved in the mechanisms governing the differentiation of intestinal epithelium while IGF-I is mostly associated with crypt cell proliferation

The effects of IGFs and insulin depend, at least in part, on receptor number and affinity, which may be involved in GIT development in pre-term calves. Because calves are born relatively mature compared with other species such as rats, mice and humans, differences with other species with respect to IGF circulating levels receptor numbers and thus of GIT responses to ingested food components can be expected; therefore specific studies in calves are justified and needed.

A study from Georgiev *et al* (2003) found that IGF-I receptor and IGF-II receptor expressions in intestinal mucosa in calves were different at different intestinal sites and were variably affected by age, but no significantly affected by differences in nutrition. Receptor densities were selectively associated with intestinal mucosa growth.

Regarding IGF-I plasma concentrations, there are conflicting results. Egli & Blum (1998) found that IGF-I concentrations increased from birth to 7 days of life in suckling calves, which was in contrast with previous studies on breeding calves (Hadorn *et al*, 1997; Hammon & Blum, 1997,1998). Differences in feeding intensity, but also in the growth hormone status, may have been responsible for this. Sparks *et al* (2003) found no significant correlation between birth weight and serum IGF-I concentration across sexes and within sexes; in contrast, Breier *et al* (1988) found a significant correlation between IGF-I concentration and birth weight in newborn male Friesian calves. Moreover, it was found that fetal BW to is strongly correlated with serum IGF-I concentrations. Reasons for these discrepancies are unclear.

Whether breed type, mode of delivery and (or) nutritional status of the dam affects neonatal IGF-I levels at birth requires further study.

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For data about cortisol and thyroid hormones levels in calves see chapter *2.1.3*.

Studies made on calves showed high NEFA concentrations after birth, followed by a rapid decline after the first meal (Ronge & Blum, 1988; Vermorel *et al*, 1989; Hadorn *et al*, 1997; Hammon & Blum, 1998)

Vermorel *et al* (1983) found a reduced mobilization of body lipids in dystocial calves in comparison with eutocial calves, reflected by lower levels of plasma fatty acids; therefore a difficult birth may have an influence also on energy metabolism.

2.6 Immune defences

After birth, newborns go through a period of rapid growth and development, and adapt to various physiological functions. Exposure to the new environment and foreign antigens requires the establishment of appropriate defence responses. The neonate is immunocompetent, but the adaptive immune system is immature (Kovarik & Siegrist, 1998; Morein *et al*, 2002). Functional immaturity of neonatal lymphocytes during the first weeks of life has been reported (Nagahata *et al*, 1991); thus, non-specific defence mechanisms response may be important for the adaptation to the extra uterine life, such as the acute-phase response (*APR*).

The term "APR" was introduced by Abernathy & Avery in 1941 to indicate a series of non-specific biochemical defence mechanisms occurring in the host after a tissue injury or an infection. Two categories of defence mechanisms can in fact be distinguished: the specific and the non-specific immunity. The first category is known as the immunological response, including antigen presentation, antibody generation and B- and T-cell activity. The second category consists of physical barriers such as skin, mucus and gastric acidity, and of biochemical barriers divided in local and systemic reactions. The systemic reaction includes changes in plasma concentration of acute-phase proteins (*APPs*; Baumann & Gauldie, 1994), which play a role in the defence response of the host (Vogels *et al*, 1993). Acute-phase proteins are mainly produced by hepatocytes, following receptor activation by cytokines such as interleukin-I (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Monitoring the blood concentrations of APPs can therefore provide information on the progression of the inflammatory reaction (Kent, 1992).

Nowadays, about 30 proteins have been described as acute-phase proteins. Some of these proteins increase whereas others decrease in plasma concentration following disturbance of the physiologic homeostasis; they are therefore

respectively called positive acute-phase proteins and negative acute-phase proteins. An other classification is based on the change in concentration during the APR: class A includes proteins increasing 20-1000 fold during APR, class B consists of proteins rising 2-5 fold, class C increase 30-60% in concentration, class D do not change in concentration, and class E proteins decrease in concentration.

Characterization of changes in concentrations of APPs after birth could elucidate the role of the inflammatory response in the adaptational mechanisms of the newborn.

Possible age effect on the concentrations of APPs also complicates the use of APPs as response markers in newborn animals and therefore specific references range according to the age are necessary.

2.6.1 Acute phase proteins after birth

Adaptation of neonatal animals to extra uterine life is a complicated physiological process involving many different mechanisms; APR is one of the essential mechanisms to regain homeostasis. The host-protective properties of circulating APPs and APP production in response to pathogens are even more important in early life, when the immune system is functionally immature. Consequently, it can be hypothesized that initiation of inflammatory response, reflected as changes in APP concentrations, would be seen in newborns.

Possible factors affecting the concentrations of APPs after birth include:

1. Immaturity of synthesis capacity of APP of the newborn liver
2. APP stimulation by birth trauma or stress
3. APP stimulation by intake of colostrum
4. Introduction to the extra uterine environment

1. Immaturity of synthesis capacity of APP of the newborn liver

Immaturity of the neonatal liver to mount an APP response to an inflammatory stimulus could affect APP concentrations in neonatal animals.
In humans, serum amyloid A (SAA) and alpha-acid glycoprotein (AGP) have been reported to be lower at birth and to increase progressively to normal adult concentrations by 6 months of age (Kanakoudi *et al*, 1995; Brunn *et al*, 1998). Also low Hp concentrations are common in newborn infants. This has been related to immaturity of the liver to produce Hp in a situation where Hp consumption is increased because of haemolysis of fetal erythrocytes (Dobryszycka, 1997). Studies in laboratory animals indicate that APP gene expression in hepatocytes is age-dependent. Newborn rats had lower APP mRNA expression than adults (Schwarzenberg *et al*, 1991), reaching adult levels by day 7–19.

2. APP stimulation by birth trauma or stress

Physical trauma or stress during parturition may induce a rise in systemic APP concentrations of the neonate. Lipopolysaccharide binding protein (LBP) concentrations, by contrast, have not been reported to be affected by labour in humans (Behrendt *et al*, 2004).

Concentrations of systemic C-reactive protein (CRP) in infants at birth were negatively associated with the Apgar score used to assess the fitness of the baby and positively associated with a birth complication, namely rupture of membranes for 18 h or longer (Chiesa *et al*, 2001). However, these associations were no longer significant when CRP was measured at 24 and 48 h after birth (Chiesa *et al*, 2001).

Babies delivered by Caesarean section also had lower peak CRP values at 48 h than vaginally born babies, but by the end of the first week concentrations decreased to baseline levels (Ishibashi *et al*, 2002). Results from an animal study (Richter, 1974) also support theories of the effect of birth trauma and/or

colostrum on systemic APP concentrations of the newborn; piglets born by Caesarean section and deprived of colostrum had only temporal and low elevation of serum Hp compared with conventionally reared piglets.

Higher SAA concentrations in two calves needing forceful extraction were found by Orro (2008). As overall only three calves needed forceful extraction and the highest systemic SAA concentrations were noted during 7-14 days of life in most calves, trauma from the birth process probably had only a minor effect on age-dependent changes in SAA.

High endogenous cortisol values of newborn calves (Hammon *et al*, 2002) may also influence APP production by stimulating some APPs and inhibiting others (Smith & McDonald, 1992; Wan *et al*, 1995).

3. APP stimulation by intake of colostrum

Colostrum contains high quantities of inflammatory mediators (Munoz *et al*, 1990; Bocci *et al*, 1993); therefore, both a direct transfer of APPs from colostrum to newborns and an induction of APR in the newborn may potentially occur.

At least a partial effect of colostral pig-MAP (APP in pigs) on the elevation of piglet pig-MAP serum concentrations after birth has been proposed (Martin *et al*, 2005). Despite the lack of evidence of a direct transfer of APPs, free pro-inflammatory cytokines present in colostrum (Sordillo *et al*, 1991; Goto *et al*, 1997; Hagiwara *et al*, 2000) may have crossed the neonatal intestine and stimulated hepatic production of APPs.

After colostrum intake, systemic concentrations of pro-inflammatory cytokines in calves increase, with the highest concentrations occurring on the first day of life (Yamanaka *et al*, 2003*a*). Concentrations then gradually decrease and are undetectable around 3–4 weeks of life. Possibly, free pro-inflammatory cytokines from the colostrum directly stimulate the production of APPs or trigger cytokine production of the newborn (Bessler *et al*, 1996; Hagiwara *et al*,

2001; Yamanaka *et al*, 2003*b*). Colostrum also contains cytokine antagonists and soluble cytokine receptors (Buescher & Malinowska, 1996; Hagiwara *et al*, 2000). However, concentrations of these factors are not probably sufficient to markedly interfere with the biological effects of colostral cytokines (Yamanaka *et al*, 2001; Yamanaka *et al*, 2003*b*).

Schroedl *et al* (2003) found that bovine colostrum contained high levels of C-reactive protein (CRP), and calves had elevated systemic CRP concentrations after colostrum consumption. Although CRP is a major APP in many species (e.g. pigs, dogs and humans), it is a constitutive protein in cattle, and blood concentrations do not change markedly during inflammation (Maudsley *et al*, 1987). Schroedl *et al* (2003) concluded that transfer of CRP from colostrum was the reason for elevated serum concentrations of CRP in newborn calves, and higher CRP levels contribute to protection against infections.

A short-lived (up to one week) elevation of systemic SAA concentration immediately after birth has been described in some species, e.g. in pigs (Llamas Moya *et al*, 2007), horses (Nunokawa *et al*, 1993; Stoneham *et al*, 2001; Duggan *et al*, 2007) and humans (Marchini *et al*, 2000). McDonald *et al* (2001) have reported high concentrations of mammary-associated SAA in the colostrum of healthy cows and they suggested a possible role for mammary SAA in supporting the welfare of calves. Human mammary-associated SAA was shown to have a primarily protective effect on the gastrointestinal tract of neonates by stimulating mucin production and reducing adherence of pathogens (Larson *et al*, 2003). Potential transfer of SAA from colostrum to the circulation of newborns has not been investigated.

Hp serum concentrations changes seem to be relatively different between pigs and cattle. Pigs have a high constitutive Hp concentrations and a relatively small rise during inflammation (Eckersall *et al*, 1996), whereas cattle have a low basal levels of Hp (Eckersall & Conner, 1988) and a relatively higher increase during APR. Another interesting difference between calves and piglets should be noted. In piglets, a 50% drop in overall IgG concentrations occurs within the second week of life (Martin *et al*, 2005; Sorrells *et al*, 2006). In neonatal dairy and

beef calves, a considerably smaller (approximately 20-30%) relative decrease in total immunoglobulin during the first weeks of life has been described (Rajala & Castren, 1995; Suh *et al*, 2003). Although speculative, this may support the different hepatic regulation of Hp production between calves and piglets. The background of a rapidly decreasing passive immunity and the simultaneous protective function of increasing levels of Hp (Eaton *et al*, 1982; Dobryszycka, 1997) may play a role in the intrinsic defence mechanisms of newborn piglets.

In conclusion, some patterns of systemic APP response in different species are comparable, although these species live in very different environmental conditions. This suggests that the changes in APPs described here are not merely caused by some coincidental disease-related mechanisms, but may result from the multiple overlapping factors discussed above; in general, they reflect the physiological adaptation of newborns to extra uterine life.

5. Introduction to the extra uterine environment

Transient changes in APP hepatic gene expression seen in neonatal laboratory animals (Glibetic *et al*, 1992) or temporal changes in APP concentrations in newborn piglets (Martin *et al*, 2005) may reflect the adaptation mechanisms necessary for extra uterine life, as suggested by the authors. Some studies suggest that concentrations of APPs may be influenced by the presence of subclinical disease processes. However, the question of a possible effect of exposure to the extra uterine environment and predisposing pathogens on APP response in newborn animals has not yet been thoroughly addressed.

2.6.2 Current knowledge about the newborn calf

Few studies on the concentrations of APP in bovine calves after birth are available, and many conflicting results are evident. The concentrations of APPs in dairy calves have been seen to change within the first 3 weeks of life, stabilizing thereafter. The relative changes were biggest in the concentrations of SAA and AGP.

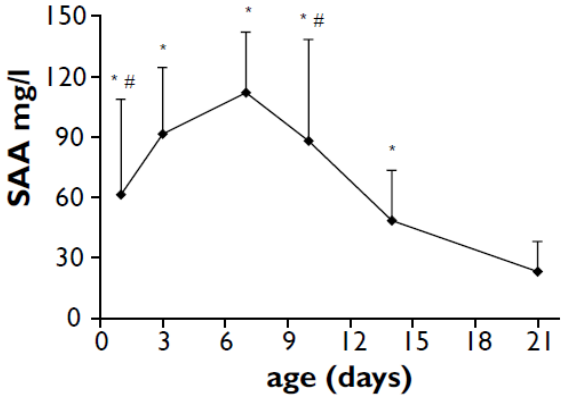
- Serum Amyloid A

SAA is a high-density lipoprotein (HDL)-associated apolipoprotein and a major APP in many animal species including ruminants. This multifunctional protein is involved in such immunological processes as cholesterol transport and immunomodulation (Urieli-Shoval *et al*, 2000).

Alsemgeest *et al* (1993) reported no changes in SAA concentrations in 4 cannulated foetuses before and after birth; in another study, the same authors found very low SAA concentrations in calves sampled within 10 minutes after parturition (Alsemgeest *et al*, 1995). The mean SAA concentration was independent of weight, degree of acidosis, sex and type of obstetrical help. In the group of diseased calves, the mean SAA concentration was significantly higher than in healthy newborn calves.

Orro (2008, *figure 5*) also registered low SAA concentrations in calves within a few hours of birth, but the concentrations then increased rapidly, suggesting the presence of some external stimulatory factor for SAA at birth or soon after.

Fig. 5 – Mean (±SD) SAA concentrations in calves for the first 21 days after birth



(*Taken from Orro, 2008*)

- Fibrinogen

Fibrinogen (Fb) is a constitutive plasma protein that behaves as an APP in most species, including birds (Jain, 1993; Petersen *et al*, 2004) and cattle (Conner *et al*, 1988). It increases in various inflammatory diseases of cattle, such as peritonitis, endocarditis, pericarditis, pneumonia, mastitis, enteritis and nephritis (McSherry *et al*, 1970; Sutton & Hobman, 1975). During the last decades, Fb, together with Hp, has probably been the APP most commonly used as a marker of host inflammatory response in research of cattle. Fb, factor I of the coagulation system, is the circulating precursor of fibrin. This plasma protein plays an important role in haemostasis and thrombosis by its interaction with thrombin, factor XIII, plasminogen, glycoprotein IIb/IIIa and endothelial cells (Jain, 1993).

A temporal elevation of mean Fb concentration during the first 2 weeks after birth was found in calves, although the rise was relatively small and concentrations did not exceed the reference limit (Knowles *et al*, 2000). Very similar transient and relatively small increases in Fb concentrations during the first 2 weeks of life in calves have been reported earlier (Gentry *et al*, 1994).

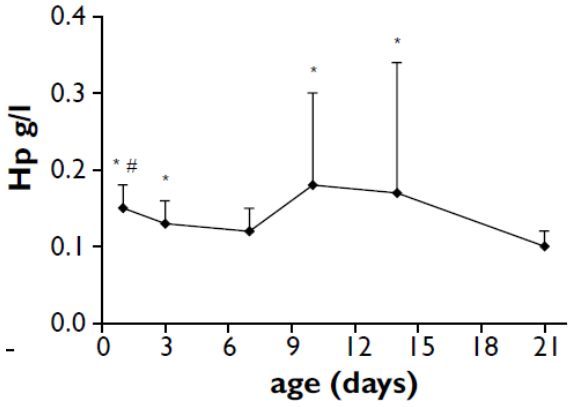
- Haptoglobin

Haptoglobin (Hp) is a glycoprotein with hemoglobin-binding capacity. It has been shown to be a major APP in domestic ruminants (Eckersall & Conner, 1988; Skinner & Roberts, 1994) and is frequently used in studies evaluating bovine inflammatory conditions (Alsemgeest *et al*, 1994; Humblet *et al*, 2004). In addition to numerous other biological functions, Hp is involved in host defence responses to infection and inflammation (Dobryszycka, 1997).

Knowles *et al* (2000) reported considerable fluctuation and some very high Hp concentrations during the first 2 weeks of life in a group of 14 calves. In the study by Schroedl *et al* (2003), Hp concentrations in newborn calves did not differ among samples obtained at birth, at 1 day of age and at 10 days of age.

Orro (2008, *figure 6*) reported higher mean Hp concentrations within 3 days of birth; after a small decrease, the concentrations stayed relatively stabile. This difference of Hp from SAA may be explained by different regulatory mechanisms for hepatic synthesis (Alsemgeest *et al*, 1996); a decrease of Hp during the early stages of inflammatory response, exactly opposite to the other APPs (Fb, CRP and SAA), has been described in cattle (Arthington *et al*, 2003). The decrease of Hp values during the first week of life could also be related to the increased consumption of Hp due to haemolysis of fetal red cells and to functional immaturity of the neonatal liver to compensate (Dobryszycka, 1997).

Fig. 6 - Mean (±SD) Hp concentrations in calves for the first 21 days after birth

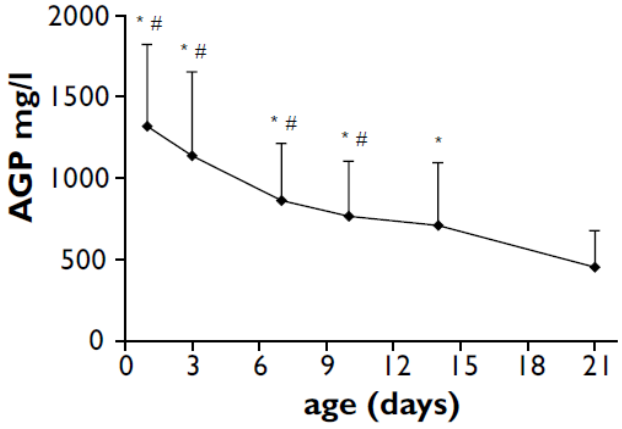


(*Taken from Orro, 2008*)

- Alpha$_1$-acid glycoprotein

A rise of AGP already in the fetal stages has been reported in calves, the highest concentrations being reached at birth, followed by a decrease during the first 3 weeks of life to adult levels (Stone & Maurer, 1987; Orro, 2008) (*figure 7*). A different AGP isoform in neonatal calves compared with the isoform present in adults has been described. These studies indicate that neonatal AGP is probably fetally regulated and a high serum concentration of AGP after birth is not necessarily a sign of the activation of APR by some external stimulus.

Fig. 7 - Mean (±SD) AGP concentrations in newborn calves for the first 21 days after birth



(*Taken from Orro, 2008*)

- Paraoxonase-1

Around 1950's dr. Mazur discovered that some organophosporus compounds like Parathion, a potent insecticide and acaricide, could be hydrolyzed by natural enzymes; consequently to this ability, the new family of enzymes was named *Paraoxonase*. It consists of 3 main enzymes, PON1, PON2 and PON3, expressed in the liver and excreted in the blood associated with high-density lipoproteins (HDL) particles.

PON1 is the most investigated member of this family; it is a calcium-dependent ester hydrolase that catalyzes the hydrolysis of many xenobiotics (Ferrè *et al*, 2002). Since the compounds that can be hydrolysed by PON1 are nonphysiological substrates, these activities are not likely to be the physiological functions of PON1 (van Himbergen *et al*, 2006). Recent researches found out that hydrolytic action towards aromatic esters and lactones is the native activity of PON1. The role is vivo is not clearly understood, but PON1 is thought to attenuate the oxidation of the low-density lipoproteins and prevent the oxidation of the HDL, thus having a key role in the prevention of the atherosclerosis process. Moreover, Paraoxonase is considered a negative APP (James & Deakin, 2004); its concentration is strongly reduced after LPS challenge (Feingold *et al*, 1998). Oxidative stress affects PON activity, and there is an inverse relationship between lipid peroxidation and PON (Aviram & Rosenblat, 2004).

A study on cows (Bionaz *et al*, 2007) showed that PON1 concentrations are negatively correlated with Hp and positively correlated with albumin, confirming its role as a negative acute-phase protein.

No data about PON1 concentrations and age related variations in the newborn calf are nowadays available.

2.7 Hematologic profile

There is no time in life when physiology changes more rapidly than in the neonatal period. The blood is very much affected by the transition from the intrauterine to the extra uterine environment; during this time, the normal range becomes a moving target, making it difficult to distinguish many abnormalities from physiologic variations. The use of blood biochemical and hematological measurements as an aid to diagnosing disease continues to increase as the ease with which samples can be analyzed improves with automation. Diagnoses can be made, or aided by, a comparison of the values of variables measured in a clinical case with those found in the general, healthy population.

In human medicine, normal haematological values for the fetus are now available due to the use of umbilical-cord blood sampling. In veterinary medicine instead, reference ranges for blood biochemical and hematological variables usually apply only to adult animals, and they can be misleading if applied to young animals because there are often large changes in the values of the variables associated with the normal process of growth.

2.7.1 Maturation

Neonatal erythropoiesis differs significantly from that in older children and adults. The birthing process with the rapid changes in oxygen concentration precipitates drastic changes in the erythroid system of the newborn. To understand neonatal erythropoiesis, one needs to understand the ontogeny of erythropoiesis, from the embryo through the fetus to the newborn.

The current hypothesis of hematopoiesis is that there is a pleuripotent hematopoietic stem cell that gives rise to all hematopoietic lineages. As stem cells differentiate, they lose their ability for self-renewal. Proliferation, differentiation, and survival of erythroid progenitor are dependent on the hormone erythropoietin. In utero hematopoiesis is primarily erythroid.

The blood and bone marrow of fetuses and newborns is rich in stem cells as well as erythroid progenitors (Wyrsch *et al*, 1999) and their response to erythropoietin is normal (Shannon *et al*, 1987). However, at different stages of embryonic and fetal development, erythroid cells behave differently. Embryonic erythropoiesis gives rise to large nucleated red cells endowed with embryonic hemoglobin. Through gestation, erythropoiesis switches from this primitive erythropoiesis to definitive or mature erythropoiesis with smaller, anuclear cells that contain adult-type hemoglobin. Although in utero hematopoiesis is predominantly erythropoietic, macrophages, megakaryocytes, and platelets have been identified in the yolk sac (Palis & Yoder, 2001). Progenitor cells of all lineages can be grown from yolk-sac cells.

▪ Red blood cells

Ninety-five percent of the protein in the erythrocyte is hemoglobin. Hemoglobin is a tetramer of two pairs of, usually unlike, globin polypeptide chains, each associated with a heme group. Developmentally, there are embryonic, fetal, and adult hemoglobin. The transition from fetal Hb to adult Hb begins during gestation and continues postnatally.

The remaining proteins in the erythrocyte protect and sustain viability of the erythrocyte. Maintaining the viability of the erythrocyte is critical, as free hemoglobin is catabolized and excreted renally within minutes. Therefore, the function of the red blood cell is to protect hemoglobin and the function of hemoglobin is to transport oxygen from the lungs to the tissues and to facilitate the return of carbon dioxide.

Neonatal erythrocyte membranes differ from adult membranes in several ways that make them more resistant to osmotic lysis. At the time of birth, some cells have increased osmotic fragility, but they are selectively destroyed within the first few days. Newborn erythrocytes are less filterable, especially in the presence of acidosis and hypoxemia (Gross & Hathaway, 1972; Tillman *et al*,

1977). They also have lower intracellular and membrane viscosity, with a more tightly linked cytoskeleton (Landaw *et al,* 1982).

Newborn erythrocytes have diminished glutathione peroxidase, rendering them more vulnerable to hydrogen peroxide-induced oxidant injury. Additionally, newborns have less capacity for handling singlet oxygen and superoxide radicals (Carrell *et al*, 1975). Superoxide dismutase converts superoxide radicals to hydrogen peroxide. Its level varies widely between newborns; this could result in accumulation of the superoxide radical. Free radicals are detoxified by antioxidants; however, if superoxide dismutase levels are increased, the hydrogen peroxide presented to reduced glutathione may not be detoxified adequately (Stockman, 1977). When an imbalance occurs between enzymes involved in production and detoxification of free radicals and oxidative intermediates, oxidant-induced injury may result.

At birth with exposure to higher concentrations of oxygen, the newborn undergoes critical changes in erythropoiesis. The birthing process itself also presents specific and serious challenges to erythropoiesis. The newborn can undergo rapid changes in hemoglobin concentration, making the evaluation of anemia difficult. In addition to the increased oxygen content of the blood in the extra uterine environment, changes in the hemoglobin itself contribute to the physiologic anemia of infancy and the anemia of prematurity.

▪ White blood cells

Hematopoiesis in the fetus is initiated in the yolk sac, with the formation of "blood islands" from primitive blood progenitor cells. Later, the fetal liver becomes the major site of blood-cell production, which then extends to include the spleen. The earliest components of marrow-based blood production appear and continue to become more prominent over the subsequent months leading up to term, while splenic and hepatic hematopoiesis diminishes. During this time, pluripotent hematopoietic stem cells are present in the circulation (Nathan 1989), presumably in transit from the hepatic and splenic hematopoiesis sites to

the marrow to populate the latter for subsequent blood-cell production during extra uterine life.

The proliferative potential of the cells in the circulation at the time of term birth is actually greater than that of adult bone-marrow cells (Liu *et al*, 1999), suggesting that the myeloid blood-cell production capabilities of the neonate should be normal. However, at the time of delivery and during the neonatal period, myeloid cell production and kinetics differ from those of adults. The greatest differences are the smaller size and lesser mobilization of the mature PMN storage pool in the neonate (Cairo, 1989). In the infant, the storage pool comprises approximately twice the number of PMNs as are in the circulation, while in adults, the storage pool is approximately ten times the circulating PMN number.

The functional capabilities of PMNs from normal adults are complex and highly integrated. Following production of mature PMNs in the marrow, they are transported into the circulation, where they segregate into either the circulating pool or the marginated pool of cells within the circulation. Normally, these pools contain approximately the same numbers of cells and there is free exchange of cells between them. Marginated-pool PMNs are associated loosely with vessel walls and can be readily mobilized into the circulating pool by adrenergic stimuli. This process, called demargination, is a component of the "fight-or-flight" response to threat, providing a rapid increase in the numbers of circulating PMNs capable of participating in any inflammatory responses to injury that might occur. Neonatal PMN morphology is not grossly different from that of PMNs from older children and adults. However, numerous in vitro functional abnormalities exist in these cells, and although no single abnormality would be considered severe in its degree of effect (relative to recognized PMN dysfunction syndromes), when these abnormalities occur together, they result in significantly altered PMN-mediated host defences in the neonate. Most studies of neonatal PMN chemotactic responses have reported lower responses than are usually observed in older children and adults (Anderson *et al*, 1981; Sacchi *et al*, 1982).

Some authors found normal chemotaxis responses at birth while decreased neonatal PMN chemotaxis was observed by the sixth postpartum day and persisted to at least six months.

Compared with neonatal PMNs, mononuclear phagocytes of newborn infants have been examined less extensively. Deformability characteristics of neonatal monocytes are not different from those of adult. In general, monocytes perform the same phagocytic cell functions as PMNs (adherence, chemotaxis, phagocytosis, respiratory burst activity, microbial killing, etc.), but cell for cell, they are quantitatively less capable than PMNs. The greatest difference between monocytes and PMNs is the stage of differentiation at which they enter the circulation from the marrow. At this point, the monocyte is a precursor cell with the potential for further differentiation into a long-lived macrophage/histiocyte once it exits the circulation, while the PMN is a terminally differentiated cell that enters the tissues and dies after it completes its functions.

2.7.2 Influence of parturition

Studies on newborn babies (Gasparoni *et al*, 1992; Herson *et al*, 1992; Steinborn *et al*, 1999; Marchini *et al*, 2000) demonstrated the early capacity of the neonate to mount a powerful inflammatory response already during the process of labour. This increase and redistribution of immune cells at birth could be an adaptive response designed to enhance the vigilance at the major defence barriers of the body. The observation that phagocytosis and microbicidal activity in ill or stressed neonates is deficient because of lower opsonic activity in the plasma of infants prompted examination of whether stress altered other neonatal PMN functional capabilities.

Interest in whether the mode of delivery (Caesarean section with or without labour versus labour with vaginal delivery) might contribute to the stress of the infant and, therefore, lead to alteration of neonatal PMN function led to a series of studies.

Labour process normally leads to leukocytosis, which is selective for neutrophils, monocytes and natural killer cells (Gasparoni *et al*, 1992; Herson *et al*, 1992; Hasan *et al*, 1993; Thilaganathan *et al*, 1994) but differences were found according to the type of delivery. Chemotaxis responses of Caesarean-delivered infants were better than those from vaginally delivered infants (Gasparoni *et al*, 1992). Infants exposed to labour followed by either Caesarean section or vaginal delivery had higher rates of resting oxygen consumption than infants born by Caesarean section without labour (Frazier *et al*, 1982). PMNs from infants delivered by spontaneous vaginal delivery had greater adherence to glass than PMNs obtained from infants born by Caesarean section (Kinoshita *et al*, 1991). These observations are consistent with labour-induced stress altering neonatal PMN functions. However, a fourth study (Usmani *et al*, 1993) was unable to find any effect of labour or mode of delivery on PMN function in healthy term neonates.

Several studies (Usmani *et al*, 1993; Redźko *et al*, 2005) compared haematological values among three groups of newborn babies: born vaginally, by elective caesarean section or by caesarean section after labour. The results indicated that the mode of delivery influences not only the white blood cells count, but also haemoglobin (Hb) and hematocrit (Ht) levels, red blood cell distribution width (RDW), platelets count, and the number of nucleated red blood cells (Redźko *et al*, 2005). Babies who were born by caesarean section after labour had the highest Ht and Hb levels (Redźko *et al*, 2005) while babies born by elective caesarean section had the lowest Ht and Hb levels and the lowest leukocyte and neutrophil counts (Gronlund *et al*, 1999; Redźko *et al*, 2005). Oka *et al*. (2007) demonstrated that a transient increase of blood Hb concentration in the human newborn can be induced also by hypoxic conditions, as a balancing reaction through spleen contraction. Ghosh *et al* (2003) found higher Hb level and red blood cell count in newborn babies with evidence of perinatal asphyxia compared to healthy babies.

Since stress and temporary hypoxia in babies are more frequent and prolonged in vaginal delivery compared to caesarean section, and even more in caesarean section after labour, it was speculated that different stress and oxygenation levels could be the reason for the differences in the haematological pattern (Hasan *et al*, 1993). Elevated white blood cell counts in neonates at term have also been associated with both metabolic and respiratory acidosis (Redźko *et al*, 2005).

2.7.3 Current knowledge about the newborn calf

Some studies investigated the haematological profile of healthy newborn calves (Benjamin, 1984; Schalm, 1984; Kerr, 1988; Radostits *et al*, 1994; Kaneko *et al*, 1997), but never related it with the birth process or other perinatal factors. Specific haematological and biochemical reference ranges according both to the age of the calf and the type of delivery, could promote the ability of clinicians for more accurate interpretations of disorders.

Contrary to what happens in the human neonates, neonatal calves born by caesarean section seem to be more predisposed to develop a respiratory distress syndrome, and therefore a respiratory acidosis, during the first hours of their life (Cambier *et al*, 2000). Moreover, some authors (Gustin *et al*, 1997; Cambier *et al*, 2000) found that double-muscled calves, which are usually born by caesarean section, present a lower blood oxygen equilibrium curve (OEC) compared to dairy calves, and this probably contributes to the higher sensitivity of double-muscled calves to hypoxia. Since this difference between breeds disappears after the first month of life (Gustin *et al*, 1997), it could be due to the different type of birth.

CHAPTER 3

The Caesarean section

3. The Caesarean section

The goals of the caesarean section are preservation of the dam and calf and the future reproductive efficiency of the dam. The outcome of the caesarean section is a self-fulfilling prophecy and numerous variables may affect the successful outcome of this procedure.

The first Caesarean section (CS) in cattle was reported in 1813 by Morange; many veterinarians then tried different techniques in different species during the following decades. First CSs were performed on recumbent animals on the right flank, then on the left flank, and finally on standing animals. Use of antibiotics decreased mortality rate after CS, which became a treatment of choice for most indications of dystocia, except for dead calves that were delivered by fetotomy.

In performing a Caesarean section in cattle, case selection is the most important and often overlooked variable; in addition, skin preparation, surgical technique, calf viability at the time of surgery, and exteriorizing the uterus can affect outcome. Minimizing excessive adhesion formation is equally important because it may affect reproductive efficiency adversely. Good surgical technique, including gentle tissue handling, appropriate suture materials and patterns, and adequate infolding of the uterine incision to prevent leakage, combined with antibiotics and anti-inflammatory when indicated can help minimize detrimental adhesions that may affect adversely the future reproductive efficiency of the cow (Newman & Anderson, 2005).

Caesarean section is potentially indicated in cases of dystocia when a calf cannot be delivered by fetal mutation and extraction. The decision on whether to perform a fetotomy or a caesarean section is dictated by whether the calf is alive, the availability of operating space in the cow's pelvis, whether the cervix is open, access to restraint facilities, perceived value of the cow, perceived value of the calf, and the importance of future cow fertility.

Perhaps the most important factor in choosing between these two procedures is the specific preference and expertise of the practitioner (Schultz *et al*, 2008). Other reasons for dystocia can be: immaturity of the heifer (Newman & Anderson, 2005), incomplete dilation of the birth canal, irreducible uterine torsion (Schonfelder & Sobiraj, 2006), rupture of the uterine wall before calving, relative foetal oversize and deformities of the calf (Vandeplassche, 1974; Cattell & Dobson, 1990; Dawson & Murray, 1992). Risk factors for CS that were identified in dairy cows are: a single male calf, a long gestation period and young age at first calving (Barkema *et* al, 1992).

Whereas in dairy cows a CS is only performed when all other attempts to deliver the calf per vaginam have failed, for veterinarians working with Belgian Blue (BB) breed has become a first choice approach at parturition (Kolkman *et al*, 2007). This beef breed is characterized by an extreme muscular hypertrophy which generates an incompatibility between the size of the pelvis (too narrow) of the cow and her calf (oversized) (Swatland & Kieffer, 1974). Consequently, BB calves are almost routinely born by CS: only 5–10% is born per vaginam, a relatively oversized calf being the most important indication to perform a CS in BB cows. In some countries, this has caused an aversion towards this breed, basing on the general belief that delivery per CS causes more pain and discomfort than delivery per vaginam. A recent study performed by Kolkman *et al* (2010) tried to assess differences in pain perception in cows calving per vaginam vs cows delivering by caesarean section; this study on the results of the showed some significant short-term behavioural differences between BB cows that calve naturally and those that deliver by CS, although the differences are subtle and of short duration.

Whether the Caesarean section has an impact also on the neonate or not is still a matter of debate. Basing on human studies, the type of delivery deeply influences the conditions of the newborn, being related to different stress stimulus, different time necessary for birth and different physical efforts.

Whether calves born by caesarean section could experience smaller or greater difficulty adjusting to extra uterine life is still to verify.

CHAPTER 4

Clinical aspects of the newborn calf

4. Clinical aspects of the newborn calf

Once a calf has been born, it is crucial to differentiate as soon as possible between those calves that need critical care and/or treatment, and those that will most likely survive without human interference.

4.1 The Apgar score

In human obstetrics the so-called Apgar numerical scoring system is routinely used to judge the clinical condition of the newborn baby (Apgar, 1953). This score is based on heart rate, respiratory rate, muscle tone, reflex irritability and skin color. Similarly, many veterinary clinical researchers tried to classify the vitality status of newborn calves. Mülling (1977) introduced a kind of modified Apgar score for calves and this system was based on muscle tone, spontaneous movements, reflex irritability, respiration and the color of mucous membranes. This scoring was subsequently used by several investigators (Held, 1983; Schulz & Vollhardt, 1983). Also several other methods have been proposed, based on both reflexes and respiration (Amman *et al*, 1974), muscle tone and cardiac activity (Szenci, 1982) or jugular blood pH values (Eigenmann *et al*, 1981; Szenci & Taverne, 1988). Many other spontaneous vital functions, such as time from birth till head-righting, till sternal recumbency, till first obvious efforts to stand, till the calf is standing up, till the first suckling, or a combination of attitude, vital signs, feeding behavior and locomotion, have also been applied for judging of newborn calves (Schuijt & Taverne, 1994).

Apart from the conclusion that there is very little conformity in clinical judging, the validity (in terms of sensitivity, specificity and predictive values) of these different methods has not been analyzed. Any method for judging the vitality should be simple, as objective as possible, quick, cheap and practicable by everyone who attends bovine deliveries, including the farmer.

In this thesis, a modified Apgar score for bovine (see *Palmer J*) was measured within 5 min of birth.
Scores of 2, 1, or 0 were assigned in relation to:

- heart rate and rhythm (≥100 bpm and regular rhythm, irregular rhythm or <100 bpm, or absent)
- respiratory rate and rhythm (≥30 rpm and regular rhythm, irregular rhythm or <30 rpm, or absent)
- body tone (sternal/active, hypotonic, or atonic)
- colour of the mucous membranes of eyes and mouth (pink, pale, or hyperemic/cyanotic)
- response to nasal and ear stimulation (avoidance of stimulation, grimace or weak response, or absent response)

An Apgar index ≥7 was considered as normal.

4.2 Time to sternal recumbency

The time taken by a newborn calf to attain sternal recumbency (TSR) might meet most of the criteria of a good tool. It is a primary activity and reflects the combined functioning of the circulation and the locomotion and nervous systems.

In a large study, including 219 calves born either on the farm or at the university obstetrical clinic, the TSR was recorded for diagnosing of vitality. Calves were defined as vital if they received routine care without medical treatment and survived seven days from birth without any symptoms of illness. Those calves which did not fulfill these conditions were categorized as non-vital ones. Analysis took also place in conjunction with the type of delivery: calves either delivered spontaneously, by caesarean section, by normal traction (power of one male person), or by forced extraction (Schuijt & Taverne, 1994).

The mean (±SD) TSR values of the calves born by forced extraction were significantly higher than the ones of the other three delivery groups. In addition, acid-base balance in jugular blood, collected at several fixed times after birth was measured. Mean pH and base-excess of vital calves at 10 minutes after birth were significantly higher and lower, respectively, than those of non-vital calves. A TSR of at least 15 minutes had a predictive value of 84% for non-vitality, while a pH value ≤6.9 in a sample taken at 10 minutes after birth had a predictive value of 68% for non-vitality.

So the recording of the TSR appears to be a valuable, additional tool, which can be used both by the farmer and the obstetrician.

4.3 Temperature

The normal temperature for a newborn calf should be around 39.5°C, some 0.8 °C above the body temperature of the mother. There is usually a drop in the body temperature of the calf during the first few hours and this decrease appeared more pronounced in non-vital calves when they were placed in an environment of 10 °C (Vemorel *et al*, 1984). The temperature drop is mainly caused by evaporation of fluids from the skin and the respiratory tract. When left with the dam, the calf is licked intensively, especially during the first few hours (Edwards & Broom, 1982). This licking will make the calf dry more quickly, thereby reducing the loss of heat. So, when the calf is separated from the mother immediately after birth, it is important to dry the skin by rubbing it intensively.

In a normal calf both shivering and non-shivering thermogenic mechanisms take place to adapt to the extra uterine environment. (Carstens, 1994). Yet this adaptation can also be facilitated by management measures, such as the use of infrared heaters. A recent study (Uystepruyst *et al*, 2002*a*) showed the beneficial effects of external heating (during the first 24 hrs after birth) on respiratory, circulatory and hematologic parameters and on rectal temperature of calves.

4.4 Blood gas and acid-base profile

Blood gas and acid-base analysis remain valuable tools of bovine perinatology, because they can give the clinician a better understanding of the causes of hypoxia and acidosis.

All calves suffer from some degree of respiratory acidosis at birth. The duration of calving and the duration and force of extraction during delivery will affect their condition at birth and their chances for survival after birth. Calves born after a prolonged calving have increased respiratory and metabolic acidosis (Szenci, 1982; Schuijt & Taverne, 1994). A study with 56 newborn calves born from heifers clearly illustrated that different types of delivery affect the plasma pH, base-excess and glucose levels in jugular samples taken at 1, 10 and 30 min after birth (Chan *et al*, 1993). By contrast, several selected clinical enzymes and electrolyte concentrations in these samples were not affected by obstetrical procedures.

Given the observations that even a normal undisturbed calving will result in a rather mild combined respiratory-metabolic acidosis of a calf at birth, the question can be raised how quickly the condition is normalized again. As already mentioned, the degree of acidosis usually becomes slightly more severe during the first 10-15 minutes after birth. At this time lactic acid appears rapidly in the circulation, once oxygenation increases with the onset of respiration. In healthy calves that were born after a normal gestation length, blood gases and acid-base balance in jugular blood samples usually reach normal, stable values within the following 6-10 hours (Walser & Maurer-Schweizer, 1979). This return to physiological levels is significantly delayed in depressed calves. Carbon dioxide tension and oxygenation may not even reach standard values at 24 hours after birth in these calves, depending on the severity of the acidosis and the care and/or treatment they received.

The etymological meaning of the term "asphyxia" is "pulseless", but clinically it represents a disturbed exchange of respiratory gases in either the placenta (during gestation or parturition) or in the lungs after birth. Biochemically, asphyxia is characterized by a lack of oxygen in combination with acidemia. Birth asphyxia is the major cause of perinatal mortality.

When calves become asphyxiated while they are still in utero, breathing can already have been initiated utero. Once they are born, respiration is usually disturbed and a primary apnoea occurs because of the depressing effect of hypoxia on the respiratory centre in the brain. Intrauterine gasping may have caused excessive aspiration of fetal fluids into the respiratory tract. To enable the passage of air to the lungs during the first extra-uterine inspirations, clearing of the airways is the first action to be undertaken in asphyxiated calves when a heart beat is still present. Lung fluid is usually partly squeezed out and absorbed during a vaginal delivery. The increased release of catecholamines from the adrenals of the calf, will stimulate this absorption. The first inspirations also stimulate fluid absorption across the alveolar walls (Humphreys *et al*, 1967). The presence of aspirated meconium may hamper this process; clearing of the nose and mouth by hand or by suctioning can be followed by a short period of suspending the calf on its hind legs, so that gravity will support the discharge of lung fluid. However, a recent study with calves delivered by caesarean section has clearly demonstrated that only suspension for a period less than 90 seconds had beneficial effects on respiratory performance of the calves (Uystepruyst *et al*, 2002*b*).

Asphyxiated calves very often show apnoea. One has to be careful with the clinical interpretation of absence of inspiration during the very first seconds after delivery, because in case of a very rapid and uneventful delivery, the blood gas values of the newborn can still be so good that it will take some time before the respiratory centre is triggered. When respiration does not start spontaneously, it has to be stimulated.

The transition to a cold environment represents a vigorous stimulus for the onset of breathing, and the first breaths sometimes take place before the calf has been completely expelled. Breathing movements can in fact already be stimulated in the unborn sheep fetus when it is cooled in utero (Thorburn, 1995). Most farmers pour cold water over the head of the calf when calves do not initiate regular breathing themselves, although beneficial effects of this treatment can be questioned (Uystepruyst *et al*, 2002*a*). Intensive rubbing of the snout and chest wall will also stimulate the onset of breathing.

There are realistic possibilities nowadays to measure the acid-base balance in blood samples from asphyxiated calves under farm conditions. Sometimes it can be difficult to obtain a jugular blood sample in such calves because of a rather low venous pressure. Recently a technique has been evaluated to collect arterial samples from neonatal calves from the caudal auricular artery (Bleul *et al*, 2007*b*). Such samples have the advantage that not only the metabolic part of the acidosis can be estimated but also the respiratory component. On the basis of such measurements and the clinical investigation of the calf, it can be decided to treat the acidosis with intravascular application of a buffer. Intravenous bicarbonate ($NaHCO_3$) is the most frequently used therapy (Amman *et al*, 1974; Walser & Maurer-Schweizer, 1979; Bleul *et al*, 2005); the bicarbonate anion reacts with a hydrogen ion to form carbon dioxide and water. The effectiveness of this therapy has recently been confirmed in neonatal calves with birth asphyxia (Bleul *et al*, 2005).

4.5 Prematurity

Premature calves can be born spontaneously (the incidence is not well documented in cattle), or when hormonal induction of calving or an elected caesarean section takes place before some 90-95% of gestation length has passed. Such calves may suffer from many disorders because of the immature state of their organ development.

Distinctive features of premature calves are: lower body weight and/or smaller body size, round shape head, uncomplete development of teeth and hair, abnormal movements and behaviour.

With respect to their lung function this includes insufficient production of surfactant. While the acid-base balance and blood gas values of these calves usually appear rather normal immediately after birth, respiratory problems gradually develop during the following hour. Tachypnoea exists and the respiration movements, mainly mediated by the diaphragm, are characterized by enforced displacements of the abdominal wall. Grunting can be heard during expiration.

Blood gases and acid-base balance in the blood attain the typical signs of hypoxia, hypercapnea and respiratory acidosis. Hypoxia will damage the pneumocytes that produce surfactant and they cause vasoconstriction in the vascular bed of the lungs. Large parts of the lungs will show atelectasis. Finally a mixed respiratory-metabolic acidosis will become clear because tissue hypoxia and impaired perfusion of peripheral organs will cause the production of lactate. This so-called neonatal asphyxia or late-asphyxia is also known as Respiratory Distress Syndrome (RDS). Although several treatment options have been proposed for such calves (Grunert, 1993; Grove-White, 2000; Bleul, 2009), many of these treatments may appear either to expensive (i.e. surfactant application) or impractical under farm conditions.

When induction of calving before term pregnancy is envisaged or indicated, the use of synthetic corticosteroids might be advantageous in this respect, because these drugs will pass the placental barrier. Apart from their role in the induction of the calving process, they may enhance fetal lung maturation and the production of surfactant production (Zaremba *et al*, 1997).

4.6 Perinatal care

It is important that evaluation of and interference with the calf around birth require a systematic approach, meaning that interferences should take place in the right order. The following protocol is applicable for calves born after full term pregnancy and it has used information from a standard operating procedure for newborn calves at-risk, as proposed by Mee (2008*c*).

The following measures and actions are equally applicable to calves born by the vaginal route or delivered by caesarean section. Immediately after delivery, a further clearing of the airways is the first action to be undertaken and the calf should be placed on its left or right side to allow observation of vital signs. These include head-righting, digital reflexes, onset and regularity of breathing, presence of a heart beat and heart rate, time to reach sternal recumbency spontaneously. Suspending the calf in an upside-down position is usually not necessary, but when applied it should always be for a short period of time.

When onset of breathing is not occurring within 5-10 seconds, rubbing of the chest wall, pouring of cold water over the head and/or pharmacological treatment should be undertaken. Manual support of the first inspirations is preferably done with the calf still lying on its side, by regular lifting of the uppermost chest wall. However, as soon as the calf has started to breath spontaneously, it should be placed in sternal recumbency to facilitate breathing.

At this stage the vitality of the calf should be judged again, taking into account its physical activities, reflexes, the color of the mucous membranes, regularity of breathing and its rectal temperature. In depressed calves with an established respiration, the application of a bicarbonate buffer can be considered. If possible, the severity of the birth asphyxia (and thus the amount of buffer to be used) can be measured animal-side (by portable equipment) after taking a blood sample from either the jugular vein or auricular artery. On farms where omphalitis is a regular phenomenon the use of umbilical antiseptic treatment is advised. Depending on the environmental conditions, drying off the coat and the use of external heating can be considered.

The length of the umbilical cord does not allow it to remain intact after expulsion. The cord usually ruptures spontaneously once the hips of the calf passed the vulva. A preformed weak spot in the walls of the umbilical arteries determine that these vessels rupture within the umbilical ring. Subsequent retraction will cause that the broken ends of the two arteries attain an intra-abdominal location, besides the connection of the intra-abdominal part of the urachus with the apex of the bladder. The umbilical vein ruptures at the site where the two veins fuse into a single vessel, just inside the umbilical ring (Naaktgeboren, 1963). So the piece of the amniotic covering of the cord that remains visible after birth does usually not contain any vessels. Human interference to break the cord manually is not necessary during vaginal deliveries, but can be applied when the calf is lifted out of the uterus during a caesarean section. There is lack of critical evaluation on the use of antiseptic care to prevent navel inflammation (Mee, 2008*c*; Nagy, 2010), although omphalitis may contribute substantially to postnatal calf losses. Mee (2008*c*) recommends that in herds without navel-associated problems, farmers should abstain from treating the umbilicus but, instead, should focus on hygienic conditions within the calving pen and on a timely intake of colostrum to improve immunity of the calf.

A critical physical examination of the newborn calf immediately after delivery is also indicated to reveal the presence of any traumatic injuries. Especially after assisted deliveries such injuries have been found more frequently. A retrospective survey study has been performed on the relationship between clinical factors and autopsy findings in a group of 235 calves that died during the perinatal period (Schuijt, 1990). Excessive traction applied during delivery was found to be the most important cause of rib and vertebral fractures. The author concluded that fetal anterior position at delivery may predispose calves to risky extractions (hiplock) and to the risk of perinatal death secondary to trauma.

4.7 Colostrum intake

Uptake of colostrum by the newborn calf is relevant for both its nutritional and immune status during the (immediate) postnatal period. Calves are born with rather low energy stores and it has been calculated that its endogenous lipids can support summit metabolism for only the first 15 hours, while glycogen reserves are already depleted within 3 hours. The energy content of colostrum (especially in the form of fat, glucose and glucose precursors) will thus enable the calf to sustain gluconeogenesis and support heat production for its thermoregulation. In addition, colostrum contains several vitamins and it is in fact the first source of these nutrient compounds for the calf, because vitamins do not cross the bovine placenta.

Although calves in which the postnatal passive transfer of immunoglobulin has failed sometimes survive, there are numerous studies to indicate that a timely and sufficient uptake of colostrum by the newborn calf is of vital importance for its postnatal health and survival. Colostrum is a rice source of immunoglobulin. Prenatal transfer of antibodies to the fetus is virtually absent during pregnancy because of the cellular structure of the bovine placental barrier. In addition, the relative high levels of corticosteroids in the plasma of calves at birth are immunosuppressive, especially at the level of cellular immunity. So the uptake of immunoglobulin from the mother by means of colostrum has to provide the calf with antibodies before the intestinal closure for these macromolecules occurs. Only in that case the calf can bridge the time until it own immune system is capable to react effectively enough to foreign antigens. Colostrum also contains maternal leucocytes, several types of growth factors and the iron-binding protein lactoferrin. Lactoferrin has both bactericidal and bacteriostatic properties and may play a role in neonatal immunity because it will bind to endotoxin from gram-negative bacteria.

Colostrum contains immunoglobulin of the A, E and M type which are only partly synthesized within the mammary gland. But the predominant type is IGg1 and these immunoglobulin derive from the maternal serum and are in fact concentrated by the mammary gland into the colostrum. They cross the vascular

endothelium and bind to specific receptors on the basement membranes of the secretory epithelium and subsequently reach the lacteal secretory apparatus of these cells by micropinotic endocytosis. Once colostrum is ingested by the calf, immunoglobulin will act locally, within the lumen of the intestines, and systemically once they have passed the intestinal wall and have reached the circulation.

Successful passive transfer of immunoglobulin depends on factors like age and parity of the dam, the quality and volume of colostrum, and the timing and method of colostrum feeding. If a premature calving occurs or has hormonally been induced, it is most likely that the volume and IgG content of colostrum is still not optimal. IgG levels of colostrum are usually highest in third lactation cows. Beef cows also usually have higher IgG levels than dairy cows. There is rapid decrease of the levels of this immunoglobulin during the first 1-2 days after calving. Pooling of colostrum from different dams occurs widespread but is presently under debate, because it might not purposely lead to relative low levels of IgG in the pool. Because gut closure for immunoglobulin rapidly proceeds after 12 hours and mean closure time is at about 24 hours after birth, calves should receive colostrum within the first hours after birth. Hypoxia delays closure time and there are reports indicating that IgG transfer is much lower in newborn calves with a respiratory acidosis and elevated PCO_2.

If less than 2 liters of colostrum from the first milking are given to newborn calves, an inadequate amount of Ig's will be transferred in most cases. Larger volumes of 3-4 liters are usually sufficient. Absorption was similar whether calves were given 4 liters of colostrum with a high IgG content in one or two feedings, but with low IgG levels two feedings (one at birth and one at 6 h after birth) was slightly more advantageous.

The best method for determination of the IgG content of in neonatal blood is radial immunodiffusion, but this (laboratory) method takes to much time and is rather costly. There are several more easy and cheaper tests on the market that can be performed in veterinary practices. On the basis of such measurements it can be decided whether the use of colostrum supplements should be advised.

CHAPTER 5

References

5. References

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CHAPTER 6

Objectives

6. Objectives

It is fundamental to keep in mind that several neonatal looses could and should be prevented by a good management and a very early intervention, diagnosis and treatment. Neonatal care in cattle, however, still remains limited because of the considerable economic impact on the farmer and the low value of calves. For this reason, prognosis, considered both as the chances of survival of the calf and as the long-term outcome as adult, must be accurate as much as possible. Giving the economical impact of neonatal looses, researches on calves are necessary in order to analyze and identify the most important diagnostic and prognostic variables. In order to achieve the most favourable results from birth assistance, the newborn must be recognized early in the course of the disease process. Therefore, predisposing conditions actually present before or immediately after birth should be known.

The aim of this study was therefore to clarify some aspects of the neonatal period in calves (first 14 days after birth), basing on the fact that the process of birth can deeply influence the adaptational process to extrauterine life.

General aims

1. **Verify at what extent birth can influence the adaptational process of the newborn calf**

   - Evaluation of clinical data of calves born spontaneously and by cesarean section *(chapter 7)*

   - Evaluation of the biochemical and metabolic profiles of calves born spontaneously and by cesarean section *(chapter 8)*

   - Evaluation of the hormonal profiles of calves born spontaneously and by cesarean section
     (chapter 9)

2. **Assessment of the $PGF_{2\alpha}$ plasma profile in newborn calves from spontaneous parturition, and comparison with neonates of other animal species** *(chapter 10)*

3. **Evaluation of the hematologic profile of calves born spontaneously and by cesarean section** *(chapter 11)*

4. **Evaluation of Paraoxonase-I as an inflammatory index in newborn calves. Establishment of age-related variations** *(chapter 12)*

CHAPTER 7

Clinical findings

7. Clinical findings

7.1 Materials and methods

The study was conducted between June 2009 and October 2010 in two different European countries, Italy and Belgium. A total number of 42 newborn calves born by two different types of delivery were studied: vaginal delivery without assistance (n=17) and elective caesarean section (n=25).

Calves born by vaginal delivery (VD) were enrolled from a Holstein dairy farm located in northern Italy (45°28' N and 9°41' E). The double-muscled Belgian Blue calves born by caesarean section (CS) belonged to the Institute for Agricultural and Fisheries Research (ILVO), a Scientific Institute property of the Flemish Government's Agriculture and Fisheries Policy Area and situated in the west part of Belgium (51°00'N and 03°44'E).

Calvings were watched over closely in order to check any abnormality or difficulty; parturitions requiring manual or pharmacological assistance were excluded.

According to these criteria, seventeen Holstein-Friesian calves (HF) were enrolled in the VD group. Twenty-five double-muscled Belgian Blue (BB) calves were enrolled in the CS group. The caesarean sections were considered as "elective", since they were performed at the initial stage of parturition (a rectal temperature drop during the last 24 hours and an active phase of cervical dilatation) with no attempt to deliver the calf per vias naturales. The caesarean sections were performed by veterinarians belonging to the Department of Reproduction, Obstetrics and Herd Health of the Veterinary Faculty of Ghent University, as described by Kolkman *et al* (2007).

A thorough history, including the reproductive and clinical history of the cow, details of pregnancy and calving, represents a fundamental part of the calf examination, since prenatal and perinatal events can affect neonatal viability. Many data about the dam can help the veterinarian to promptly identify a calf at risk (Meyer *et al*, 2001; Gundelach *et al*, 2009*)*:

- parity
- gestation length (prematurity)
- BCS at parturition
- duration of the second stage of labour (time between chorionallantois rupture and birth)
- type of delivery

A careful evaluation of the newborn must be done to judge the vitality status and prospects for survival of the calf. Whatever this examination would be, it should be simple, quick, cheap, objective and practicable by everyone; it should be performed for every calf within 10 minutes after birth, in order to promptly identify calves at risk that require some assistance.

Therefore, for all the 42 newborn calves, the following paper was filled in immediately after birth (*figure 8*). Expulsion phase was considered as the time between chorioallantois rupture and birth. Placental retention was defined as no expulsion of the fetal membranes within 24 h of calving.

Immediately after birth, each calf was transferred to a single box with straw until the age of 14 days, and fed with colostrum obtained from the colostrum bank of the herd. From the 3rd day after birth, calves were fed twice a day with an amount of milk equal to 10% of their body weight; the VD calves received pasteurized herd milk, while for the CS calves a milk substitute was reconstituted at 125 g powder/L.

A complete clinical examination was then performed on day 1, 2, 3, 7, 14 of age (table 1).

Fig. 8 - Birth case history

DATE …… **TEMPERATURE** …… ° C

BULL ID …… BREED ……

COW ID …… BREED …… AGE ……PARITY ……..

Reproductive history

……

GESTATION

LENGTH …… gg BCS (7°month) ……

Notes……

PARTURITION

DATE / HOUR …… BCS …… SINGLE
TWINS

SPONTANEOUS followed not followed
ASSISTED *notes*……
CESAREAN SECT. *notes*……

Expulsion phase (length) …… *notes*……

Placental expulsion (length) …… *notes*……

CALF

BREED …… GENDER ……WEIGHT …… kg KIND OF BIRTH……

ALIVE and dead within 24 h
and dead later than 24 h age at death ……..

TEMPERATURE (rectal) …… °C MALFORMATIONS ……

MATURITY……

APGAR

PARAMETERS	0 POINTS	1 POINTS	2 POINTS	SCORE
***A**ppearance*	grey/blue	pale	pink	
***P**ulse* (heart rate,bpm)	absent	<100, irregular	>100, regular	
***G**rimace* Nasal stimulation Ear tickle	 no response no response	 grimace weak ear flick	 sneeze/cough ear flick/head shake	
***A**ttitude*(muscle tone)	limp, lateral	some flexion	sternal, active	
***R**espiratory rate*	absent	<30, irregular	>30, regular, vocalization	
			Total	

TSR …… TSU …… TFS …… colostrum intake …… L

Tab. 1 - Follow-up

CALF CODE ..... Birth date .....

CALF HEALTH EVALUATION

AGE (d)	DATE And h	RECT TEMP (° C)	HEART RATE (bpm)	RESP RATE (apm)	MUCUS MEMB	HYDRAT (0-3)	EYES SCORE		ATTITUDE (0-3)	FEEDING	RESPIRATORY PROBLEMS		FECAL SCORE (0-3)	UMBILICUS SCORE (0-3)
							EYES (0-3)	SCLERAL VES (0-3)			COUGH (0-3)	NASAL DISCHARGE (0-3)		
1														
2														
3														
7														
14														

TREATMENT DAY 1 .....

DAY 2 .....

DAY 3 .....

DAY 7 .....

DAY 14 .....

Blood samples from these calves were collected in order to investigate:

- biochemical and metabolic profile (*chapter 8*)
- hormonal profiles (IGF-I, cortisol, thyroid hormones – *chapter 9*)
- $PGF_{2\alpha}$ (*chapter 10*)
- hematologic profile (*chapter 11*)

7.2 Results

Most of the time no information about length of gestation and conditions of the dam during this period were available, expecially for Belgian Blue breed, because of the use of natural insemination. Thus, it was not possible to establish an influence of maternal factors on conditions of the neonates at birth.

Calvings took place at any time of day and night, and there were no cases of placental retention. The vaginally delivered HF calves were 9 males and 8 females, while the caesarean section BB calves were 13 males and 12 females. All calves were born alive, viable, mature and well developed; no malformations could be detected. Data regarding mean neonatal weight, rectal temperature, time for sternal recumbency (TSR), time to stand up (TSU) and Apgar score in the two groups are reported in table 2.

The clinical follow-up allowed the detection of some transient pathological conditions, mainly consisting of diarrhoea and respiratory problems, but these diseases never required medical treatments, except for one male VD calf that died at 10 days of age and was excluded from the study. Another VD calf (R) showed severe diarrhoea and dehydratation, but no treatment was applied for choice of the farmer; data from this calf were included in the statistical analysis if similar to the mean values and excluded when clearly different. Therefore the final number of calves enrolled was 41 (for clinical features and NEFA) or 40 (for IGF-I and cortisol)

Tab. 2 – Mean (±SD) neonatal weight, rectal temperature, TSR, TSU and Apgar score in the 41 newborn calves

Type of delivery	Weight (kg)	Temperature (°C)	TSR (min)	TSU (min)	Apgar score
VD (N = 16)	34 ± 4.4	38.9 ± 0.2	5.6 ± 3.8	39 ± 17.2	8 ± 1.8
CS (N = 25)	53 ± 7.8	39.1 ± 0.3	2.9 ± 1.5	219 ± 118	8 ± 1.2

VD=vaginal delivery; CS=caesarean section; TSR=time to sternal recumbency; TSU=time to stand up

7.3 Discussion

The normal temperature for a newborn calf should be around 39.5°C, some 0.8°C above the body temperature of the mother; in this study, rectal temperature was around 39°C in both groups of calves, a little lower than expected; anyway it must be mentioned that most of deliveries took place during cold season and in not-heated places.

The Apgar score was almost equal between the two groups of calves; this score was a modified score for calves, and values ≥7 are considered as normal. Scoring was performed within 5 minutes from birth and always by the same person. Type of parturition did not influence the Apgar score and therefore the viability of calves, as evidence also by the time for sternal recumbency.

Mean TSR in fact was similar between groups. All calves had a TRS < 15, except for one calf from spontaneous parturition who presented a TRS of 15 minutes, which is considered as the cut off time-value for non viability. TRS confirmed the viability of calves, being a primary activity that reflects the combined functioning of the circulation and the locomotion and nervous systems.

The TSU in CS was the only parameter not comprised in the range reported for normal calf viability, according to which a calf should stand within 1 hour after birth. The very high mean weight of these subjects (53±7.8 kg) may represent the reason for the prolonged time to stand up. An other hypothesis is that local anaesthesia used in cows for caesarean section could influence the viability of their calves, but TSU was the only parameter affected, making the hypothesis unlikely.

The time for the first suck (TFS), initially included in the birth case-history paper, was in reality standardized, since colostrum was given artificially by the staff of the herds; therefore, the TFS could not represent a measure for viability.

No clinically evident signs of distress were detected at birth, and all the calves were considered as healthy. Clinical conditions during the neonatal period were good for all calves, irrespectively of the type of birth.

7.4 Conclusions

This data confirmed the utility of the Apgar score and TRS as tools for establishing viability in newborn calves. They face the requested features for practice, as they are easy and quick to measure.

According to these parameters, **there are no differences in viability at birth between calves born by spontaneous parturition and calves born by elective caesarean section.** No differences have been found also regarding healthy conditions during the neonatal period.

7.5 References

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CHAPTER 8

Biochemical and Metabolic profile

8. Biochemical and metabolic profile

8.1 Biochemical and metabolic parameters

Aspartate amino transferase (AST) catalyzes the transfer of the alpha amino group of aspartic acid to alpha-ketoglutaric acid, resulting in the formation of oxaloacetic acid and glutamic acid. This aminotransferase requires pyridoxal 5' phosphate (P5P) as an essential cofactor for maximum enzyme activity. P5P is the active metabolite of vitamin B6, therefore reduced vitamin B6 (as occurs rarely in animal patients with liver disease or on certain drugs) can result in decreased aminotransferase activity, unless P5P is included in the assay system for the aminotransferases. AST is useful as an indicator of liver and/or muscle injury in large and small animals.

AST is not organ specific. Skeletal muscle contains the highest concentration, followed by liver and cardiac muscle. Erythrocytes contain enough to raise levels when hemolysis occurs. AST is also found in renal epithelial cells and brain tissue. It is located in the cytoplasm and mitochondria as different isoenzymes. Elevations in the cytoplasmic AST isoenzyme requires only mild hepatocellular injury (and compared to ALT, AST levels may increase less in relatively mild hepatocellular injury), whereas release of the mitochondrial isoenzyme requires (and indicates) more severe cellular injury. The enzyme half life is about 2 or more days in large animals.

Gamma-glutamyl transferase (γGT) is an enzyme that cleaves C-terminal glutamyl groups from amino acids and transfers them to another peptide or to an amino acid. It is important in glutathione metabolism, amino acid absorption and protection against oxidant injury. Although γGT is found in many tissues, the main source is the liver (primarily biliary epithelium), thus γGT is used mainly as a sensitive indicator of cholestasis. It has a half life of 72-96 hours (equine).

Glutamate dehydrogenase (GLDH) is a mitochondrial enzyme that catalyzes the conversion of glutamate to 2-oxoglutarate. Increases in GLDH are used to primarily reflect leakage from damaged or necrotic hepatocytes. Since it is quite a large mitochondrial enzyme, injury needs to be sufficiently severe to damage mitochondria. GLDH is a useful enzyme for hepatocellular injury in large animals and exotic species (birds, amphibians, reptiles). It is now included on large animal panels because it is thought to be a more stable enzyme (with storage) than SDH (which decreases rapidly in stored samples). Low values of GLDH (usually<10 U/L) are seen in health in small animals and horses, whereas healthy cattle and alpacas may have higher values (up to 60 U/L in cattle).

GLDH is found in many tissues in the body, including hepatocytes, kidney, intestine, muscle, and salivary gland. However, most of serum GLDH originates from hepatocytes (in health and disease states). GLDH is located more in the centrilobular areas of the liver, whereas AST is more homogenously distributed. Due to its preferential location in centrilobular areas, liver injury involving these areas (e.g. hypoxia) may result in higher increased in GLDH. The half-life is reported around 14 hours in cattle.

Albumin is a globular protein synthesized in the liver and catabolized by all metabolically active tissues. Albumin makes a large contribution to plasma colloid osmotic pressure due to its small size and abundance (35-50% of total plasma proteins by weight). It also serves as a carrier protein for many insoluble organic substances.

Glucose is derived from digestion of dietary carbohydrates, breakdown of glycogen in the liver (glycogenolysis) and production of glucose from amino acid precursors in the liver (gluconeogenesis). In ruminants, the main source of glucose is gluconeogenesis from volatile fatty acids (propionate) absorbed from rumen by bacterial fermentation. Glucose is the principal source of energy for mammalian cells. Uptake is mediated by a group of membrane transport proteins, called glucose transporters (GLU), some of which are insulin-dependent.

The blood glucose concentration is influenced by hormones which facilitate its entry into or removal from the circulation. The hormones affect glucose concentrations by modifying glucose uptake by cells (for energy production), promoting or inhibiting gluconeogenesis, or affecting glycogenesis (glycogen production) and glycogenolysis.

β-hydroxybutyrate (BHOB), along with acetone and acetoacetate, is considered a ketone. Ketones are produced from the metabolism of non-esterified fatty acids and volatile fatty acids. In ruminants, both NEFAs and volatile fatty acids produced from rumen metabolism can be used to form ketones. Propionate, butyrate and acetate are volatile fatty acids that are produced by rumen fermentation. Of these, mainly butyrate is converted to BHB in the rumen epithelium and the liver. The primary ketone produced by the liver from NEFAs is acetoacetate. This is reduced to BHOB within the mitochondria and spontaneously decarboxylates to acetone. The ketones are excreted into the circulation, taken up by other tissues (e.g. skeletal muscle, mammary gland), where they are oxidized to yield energy or, in the case of the mammary gland, incorporated into milk fat. An increase in ketones in the blood is called ketosis. Since ketones are acids, increased concentrations can result in a primary metabolic acidosis when values are high enough to overcome normal body buffers (primarily bicarbonate, concentrations of which decrease in blood). This is called ketoacidosis but it is not always present in states of ketosis. Ketones are freely filtered by the glomerulus and, since renal absorptive thresholds are low, they are readily found in the urine during ketosis.

Increased BHB concentrations in blood indicate stimulation of lipolysis or excess absorption of butyrate from feeding. Lipolysis is stimulated by any condition leading to negative energy balance (starvation/anorexia, late pregnancy or lactation, insulin lack/inhibition) and exercise.

Non-esterified ("free" or unsaturated) *fatty acids* (NEFA) (see chapter 2.5.2)

Measurement of *creatinine* concentration in serum is included in chemistry profiles mainly to screen for decreased glomerular filtration rate (GFR). Creatinine is produced as the result of normal muscle metabolism. The only source for endogenous creatinine is from the degradation of creatine and phosphocreatine, an energy-storing molecule in muscle. Since muscle mass in health is rather constant during long periods of time and proportional to the cube of body length, the production of creatinine and its plasma level is also very constant. The excretion of creatinine, which is freely filtered at the glomerulus, is almost exclusively via the urine. The constant blood level of creatinine, entirely excreted by the kidneys, was the basis for choosing creatinine as an endogenous marker of GFR. An additional and relatively minor source is creatinine ingested during consumption of animal tissue and absorbed from the intestines. Creatinine is filtered freely through the glomerulus and is not reabsorbed in the tubules. Therefore, creatinine is a more reliable measure of GFR in all species as it is not influenced by diet or protein catabolism.

Measurement of *urea* concentration in serum is used mainly to screen for decreased glomerular filtration rate (GFR). Urea concentration is measured as urea nitrogen. Urea is synthesized by hepatocytes from ammonia generated by catabolism of amino acids derived either from digestion of proteins in the intestines or from endogenous tissue proteins. Urea is excreted by the kidneys, intestine, saliva and sweat. In ruminants, urea is excreted into the gastrointestinal system where it is converted to amino acids and ammonia which are then used for protein production. Concentrations of urea are dependent upon hepatic and renal flow rate. The rate of urea production is dependent on hepatic function and digestion and catabolism of protein, i.e. urea formation is decreased in liver disease and increased with protein catabolism or increased protein digestion in the intestine. Urea is then freely filtered through the glomerulus and passively diffuses out of the tubules at a rate dependent on flow rate through the tubules; the remainder of the filtered urea is excreted in urine. At high flow rates, approximately 40% of filtered urea is reabsorbed. At low flow rates, as happens

in hypovolemic individuals, approximately 60% of filtered urea is reabsorbed and added back to the blood urea concentration.

Serum or plasma concentrations of *sodium* (Na), *potassium* (K), *chloride* (Cl) and *phosphorum* (P) are major electrolytes. In general, electrolyte levels in blood are influenced by changes in free water and by changes in electrolytes themselves, namely rate of intake, excretion/loss, and translocation within the body. Translocation can occur via movement into or out of cells or into specific fluid compartments. As electrolytes are essential to proper functioning of cells, the body maintains electrolyte concentrations within fairly narrow limits.

Sodium concentration is an indication of the amount of Na+ relative to the amount of water in extracellular fluid (free water). Na+ concentration is inextricably linked with extracellular fluid (ECF) concentration, therefore interpretation of sodium levels should always include consideration of the hydration status of the patient (and therefore, changes in free water). Sodium is the major extracellular cation and is a primary determinant of plasma osmolality and ECF volume. The body attempts to maintain a constant ECF volume, as major changes in ECF volume can have profound effects on the cell. The kidney plays a critical role in maintenance of ECF volume, via sodium and water retention. Regulation of body water is accomplished by monitoring of plasma osmolality (determined primarily by sodium concentration) and blood volume through osmoreceptors and baroreceptors.

Potassium is the major intracellular cation (intracellular K+ concentration is approximately 140 mEq/L) and is important for maintaining resting membrane potential of cells. 60-75% of total body potassium is found within muscle cells, with the remainder in bone. Only 5% of potassium is located in circulating blood, therefore potassium concentration in blood is not always a reflection of total body potassium levels. Plasma K+ concentration is tightly regulated; fairly small changes can have marked effects on organ function.

Ingested K+ is absorbed non-selectively in the stomach and small intestine. Regulation of plasma K+ is by renal excretion and movement of K+ from extracellular fluid to intracellular fluid. If these mechanisms are functioning normally, the amount of K+ ingested has little affected on plasma K+. However, if one or more of the regulatory mechanisms is faulty, then the amount of K+ ingested can exacerbate abnormalities in plasma K+. Severe abnormalities of plasma K+ are life-threatening situations.

Chloride is the major extracellular anion, found together with Na+. Chloride is very important for osmolality and acid-base balance. Changes in chloride should always be interpreted with changes in free water, which alters Na+ and Chloride concentrations proportionally. Changes in chloride not related to free water changes are associated with acid-base abnormalities.

Total serum *calcium* (Ca) comprises three major forms: ionized calcium (about 50% of total), protein bound (about 40% of total), and calcium complexed with anions such as bicarbonate, citrate, lactate, and phosphate (about 10% of total). Most of the protein-bound calcium is bound to albumin. The ionized, or free, calcium is the metabolically active form of calcium. Results for Ca are expressed as milligrams per decilitre (mg/dl).

Calcium is absorbed in the intestine with phosphate under the action of vitamin D. Corticosteroids inhibit absorption of calcium. It is stored in the body in bone and excreted through the kidneys.

8.1.1 Samples collection

Blood samples were collected from the jugular vein into plastic tubes with clot activator (Venosafe® - VF-109SP, Terumo) by single-use needles with holders according to the following temporal pattern:

- 30 minutes after birth (always before first colostrum intake)
- 24 and 48 hours after birth (at least 1 hour far away from colostrum intake)

After collection, samples were immediately centrifuged at 1000xg for 20 minutes and the resulting serum was stored at -20°C until analysis.

8.1.2 Laboratory analysis

A basic panel of biochemical tests was performed on all the serum samples using an automated spectrophotometer (ILAB 300 plus, Instrumentation Laboratory, Monza, Italy) at the Clinical Pathology Laboratory of the Large Animal Teaching Hospital of the University of Milan (Lodi, Italy). Specifically, the following parameters were evaluated using reagents provided by manufacturer (Instrumentation Laboratory): γGT (kinetic IFCC method), urea (urease method), glucose (GOD-POD method), total protein (biuret method), albumin (bromochresol green method) creatinine (Jaffè method), AST (kinetic IFCC method), calcium (ortho-cresophthalein method), serum phosphate (molybdate method). Chloride, sodium and potassium were measured using ion selective electrodes (ISE method, included in the ILAB300 plus instrument). D-3 Hydroxybutyrate and GLDH were measured by kinetic enzymatic methods (Randox laboratories ltd, UK).

8.1.3 Statistical analysis

Mean values ±SD of each variable were calculated, and data were analysed using an Excel spread-sheet (Microsoft Corporation, Redmond, WA, USA) with Analyse-it software (Analyse-it® v2.21 Software Ltd, Leeds, UK).

The Mann-Whitney test was used to assess differences between the two groups in each sampling time for each parameter; Kruskall-Wallis test was used to compare the different sampling times within each group of calves for each parameter. Differences were considered as significant if $p \leq 0.05$.

8.1.4 Results

For clinical results, see chapter 7.2.

Mean values ± SD of all parameters in the two groups of calves are reported in figures 9-23. Statistical analysis showed the following differences among different sampling times within each group and between the two groups:

- *Albumin (fig. 9)*
  Differences within the CS group:
  - 30 m vs 24 h = $p \leq 0.0001$
  - 30 m vs 48 h = $p \leq 0.0001$
  - 24 h vs 48 h = $p \leq 0.0001$

  Differences within the VD group:
  - 30 m vs 24 h = $p \leq 0.001$
  - 30 m vs 48 h = $p \leq 0.05$
  - 24 h vs 48 h = $p \leq 0.01$

  Difference between CS and VD groups:
  - at 24 h = $p \leq 0.001$
  - at 48 h = $p \leq 0.01$

- *AST (fig. 10)*
  Differences within the CS group:
  - 30 m vs 24 h = $p \leq 0.0001$
  - 30 m vs 48 h = $p \leq 0.0001$
  - 24 h vs 48 h = $p \leq 0.0001$;

  Differences within the VD group:
  - 30 m vs 24 h = $p \leq 0.001$
  - 30 m vs 48 h = $p \leq 0.0001$
  - 24 h vs 48 h = $p \leq 0.01$

- *Bilirubin (fig. 11)*

  Differences within the CS group:
  - 30 m vs 24 h = p≤0.0001
  - 30 m vs 48 h = p≤0.0001

  Differences within the VD group:
  - 30 m vs 24 h = p≤0.0001
  - 24 h vs 48 h = p≤0.0001

  Difference between CS and VD groups:
  - at 30 m = p≤0.01

- *βHOB (fig. 12)*

  Differences within the CS group:
  - 30 m vs 24 h = p≤0.001
  - 30 m vs 48 h = p≤0.0001

- *Calcium (fig. 13)*

  Differences within the CS group:
  - 30 m vs 24 h = p≤0.001
  - 30 m vs 48 h = p≤0.001

  Differences within the VD group:
  - 30 m vs 24 h = p≤0.01

- *Chlorine (fig. 14)*

  Differences within the CS group:
  - 30 m vs 24 h = p≤0.001
  - 30 m vs 48 h = p≤0.01

  Differences within the VD group:
  - 30 m vs 24 h = p≤0.05
  - 30 m vs 48 h = p≤0.01
  - 24 h vs 48 h = p≤0.05

- *Creatinine (fig. 15)*

  Differences within the CS group:
  - 30 m vs 24 h = p≤0.0001
  - 30 m vs 48 h = p≤0.0001
  - 24 h vs 48 h = p≤0.0001

  Differences within the VD group:
  - 30 m vs 24 h = p≤0.0001

- 30 m vs 48 h = p≤0.001

Difference between CS and VD groups:

- at 24 h = p≤0.0001
- at 48 h = p≤0.01

- *γGT (fig. 16)*

Differences within the CS group:

- 30 m vs 24 h = p≤0.0001
- 30 m vs 48 h = p≤0.0001

Differences within the VD group:

- 30 m vs 24 h = p≤0.001
- 30 m vs 48 h = p≤0.05

Difference between CS and VD groups:

- at 24 h = p≤0.05
- at 48 h = p≤0.05

- *GLDH (fig. 17)*

Differences within the CS group:

- 30 m vs 24 h = p≤0.0001
- 30 m vs 48 h = p≤0.0001

Differences within the VD group:

- 30 m vs 24 h = p≤0.0001
- 24 h vs 48 h = p≤0.0001

Difference between CS and VD groups:

- at 30 m = p≤0.05
- at 24 h = p≤0.05
- at 48 h = p≤0.0001

- *Glucose (fig. 18)*

Differences within the CS group:

- 30 m vs 24 h = p≤0.0001
- 30 m vs 48 h = p≤0.0001

Differences within the VD group:

- 30 m vs 24 h = p≤0.05
- 30 m vs 48 h = p≤0.05

- *Phosphorus (fig. 19)*
  Differences within the CS group:
  - 30 m vs 24 h = p≤0.001
  - 30 m vs 48 h = p≤0.01

- *Potassium (fig. 20)*
  Differences within the CS group:
  - 30 m vs 24 h = p≤0.001
  - 30 m vs 48 h = p≤0.0001

  Difference between CS and VD
  - at 24 h = p≤0.001

- *Sodium (fig. 21)*
  Difference between CS and VD groups:
  - at 48 h = p≤0.001

- *Total proteins (fig. 22)*
  Differences within the CS group:
  - 30 m vs 24 h = p≤0.0001
  - 30 m vs 48 h = p≤0.0001

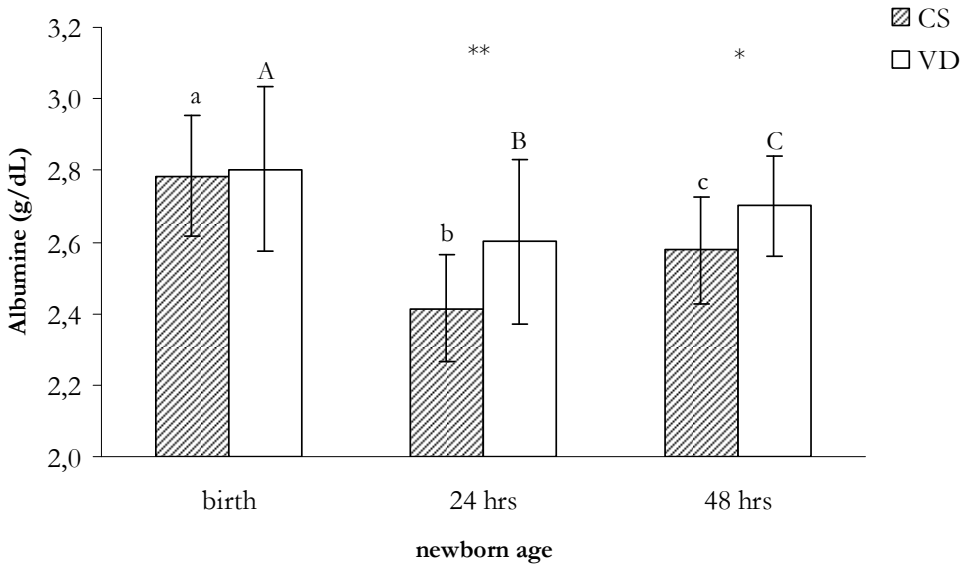
  Differences within the VD group:
  - 30 m vs 24 h = p≤0.01
  - 30 m vs 48 h = p≤0.0001
  - 24 h vs 48 h = p≤0.001

  Difference between CS and VD groups:
  - at 30 m = p≤0.05
  - at 24 h = p≤0.01
  - at 48 h = p≤0.05

- *Urea (fig. 23)*
  Differences within the CS group:
  - 24 h vs 48 h = p≤0.05

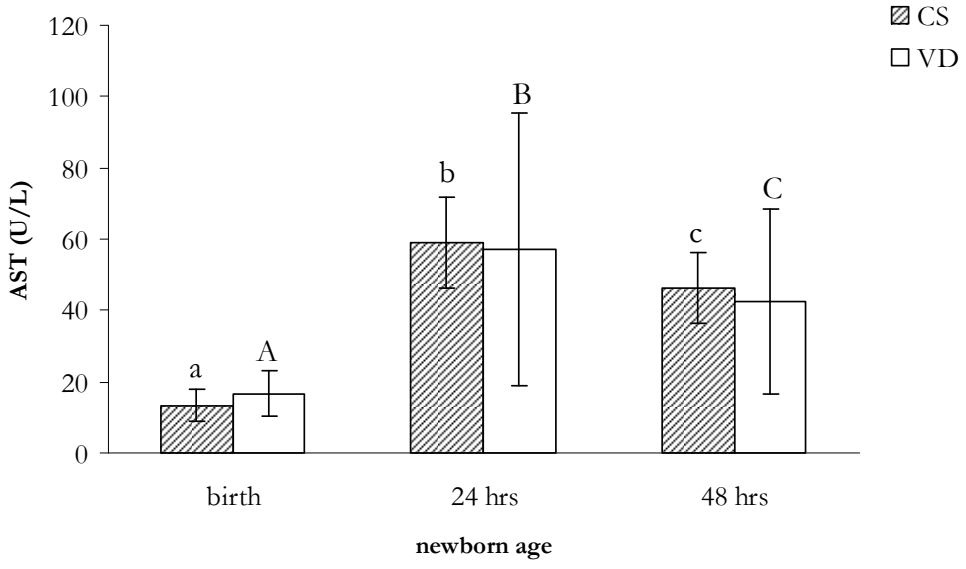
  Difference between CS and VD groups:
  - at 48 h = p≤0.05

Fig. 9 – Mean (±SD) albumin concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



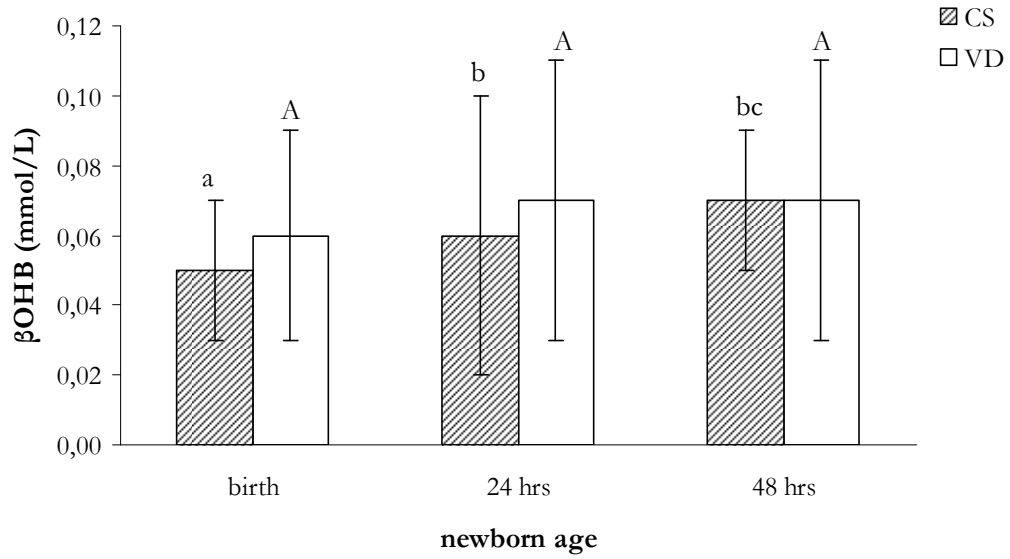
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs c , b vs c=p≤0.0001; A vs B = p≤0.001; A vs C = p≤0.05; B vs C= p≤0.01)
**= difference between CS and VD with p≤0.001; *= difference between CS and VD with p≤0.01

Fig. 10 - Mean (±SD) AST activity in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



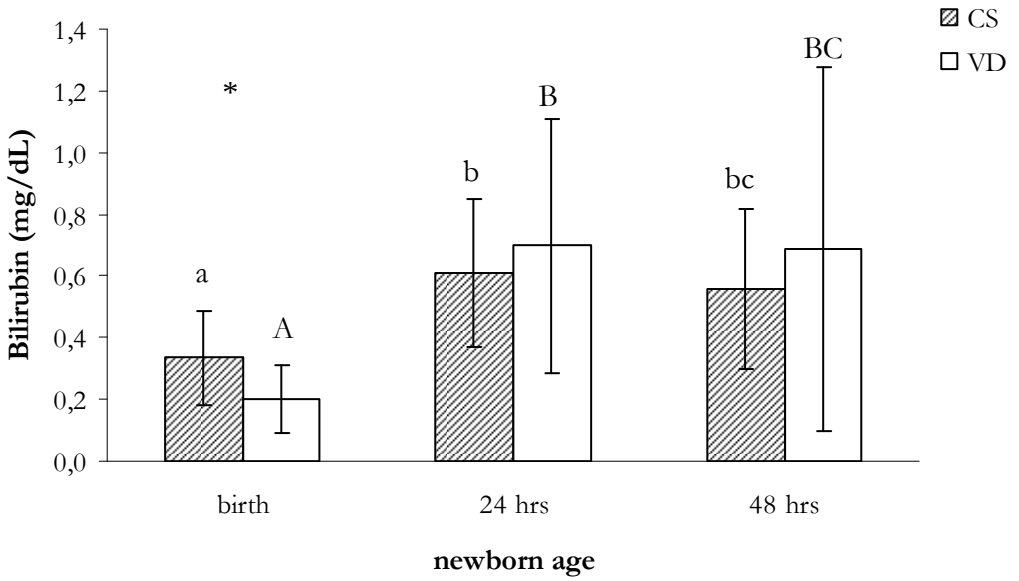
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs c, b vs c =p≤0.0001; A vs B = p≤0.001; A vs C = p≤0.0001; B vs C= p≤0.01)

Fig. 11 - Mean (±SD) βOHB concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



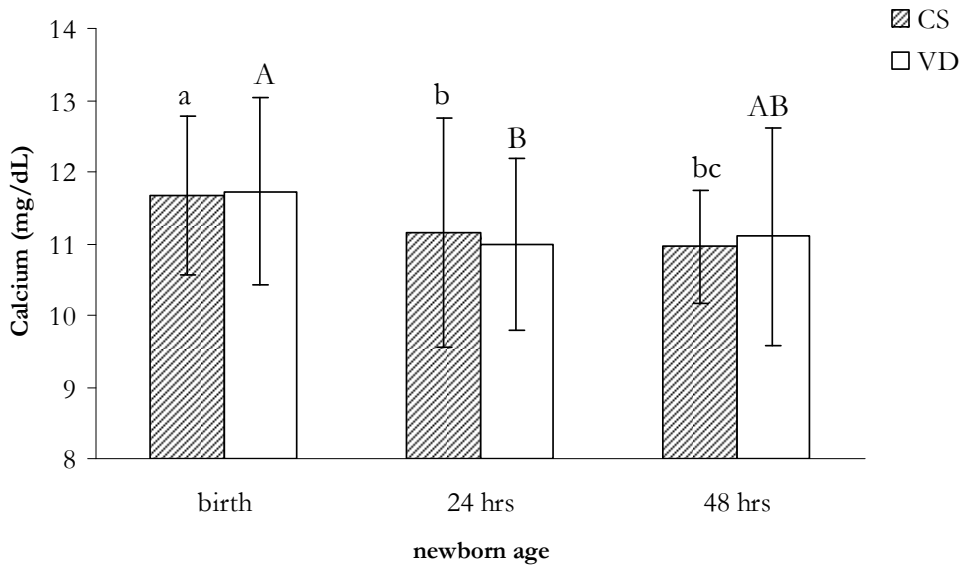
Different letters correspond to significant differences between different sampling time within the same group (a vs b = p≤0.001; a vs bc = p≤0.0001)

Fig. 12 - Mean (±SD) bilirubin concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



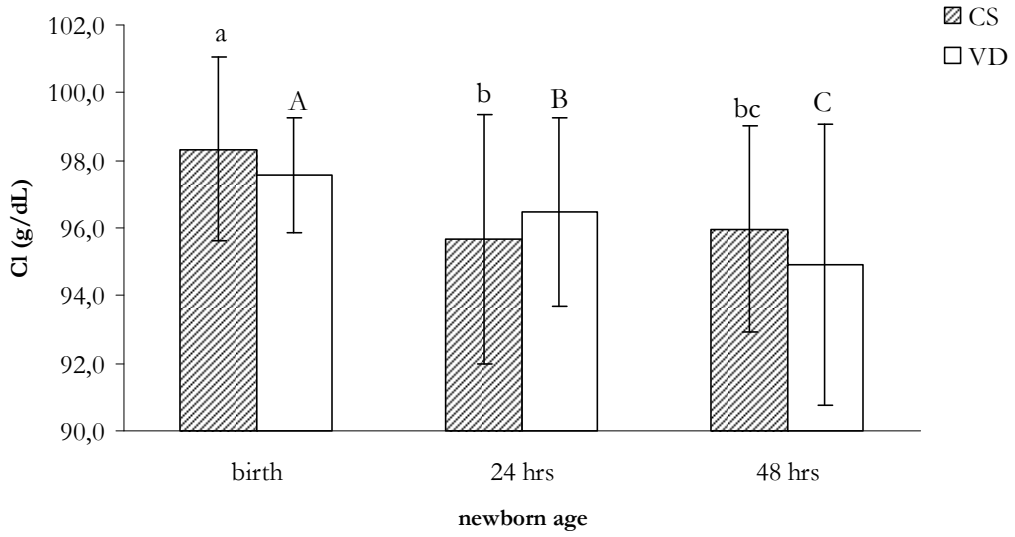
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs bc = p≤0.0001; A vs B, A vs BC = p≤0.0001)
*= difference between CS and VD with p≤0.01

Fig. 13 - Mean (±SD) calcium concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



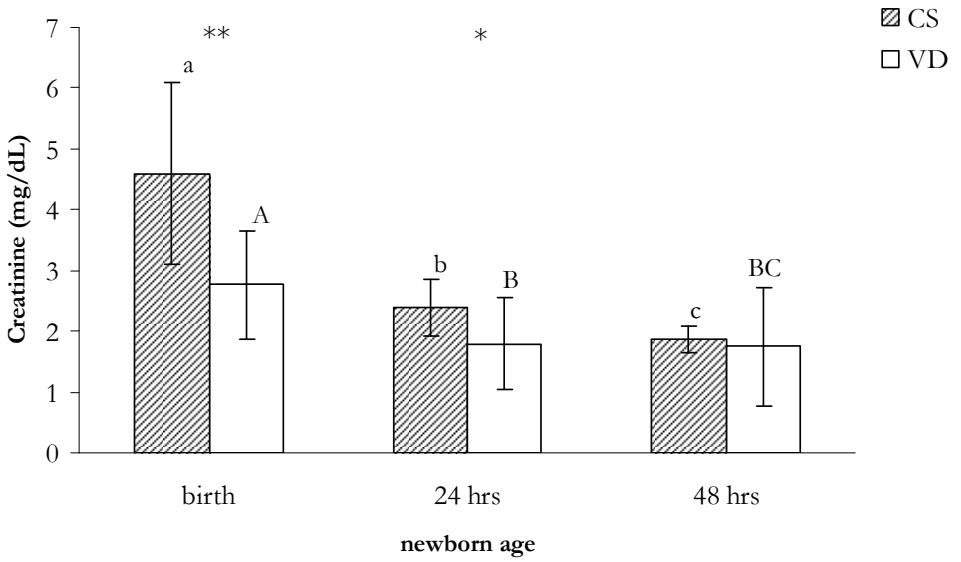
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs bc = p≤0.001; A vs B = p≤0.01)

Fig. 14 - Mean (±SD) chlorine concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



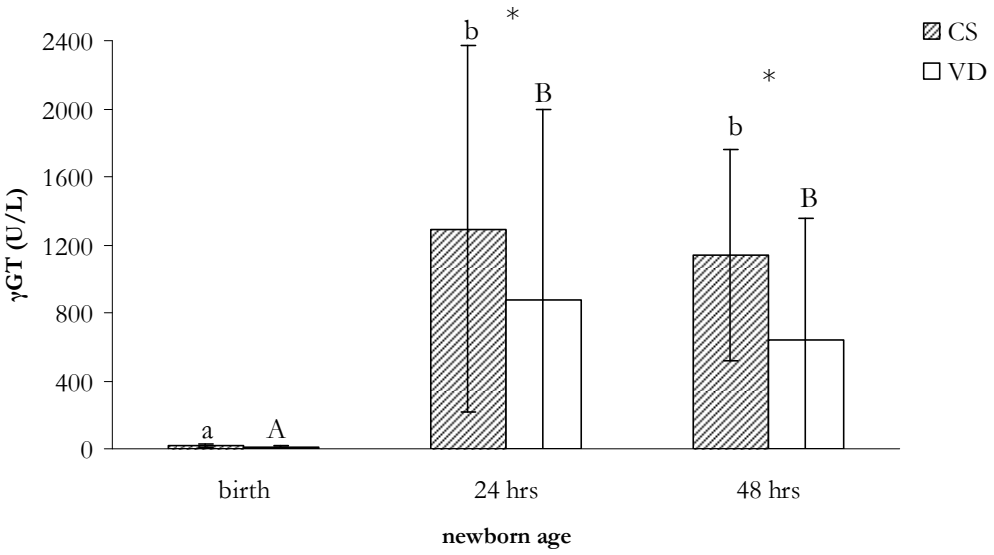
Different letters correspond to significant differences between different sampling time within the same group (a vs b = p≤0.001; a vs bc = p≤0.01; A vs B, B vs C = p≤0.05; A vs C = p≤0.01)

Fig. 15 - Mean (±SD) creatinine concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



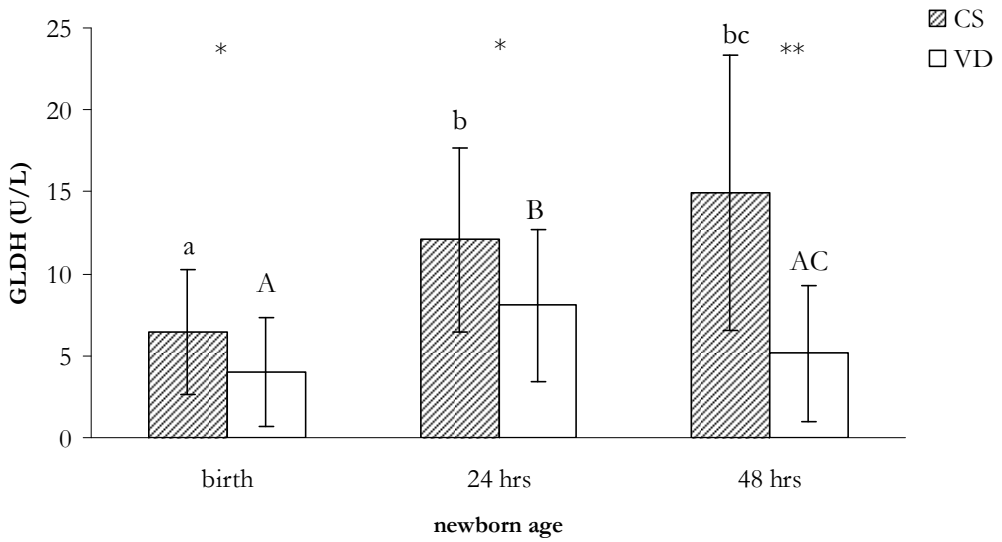
Different letters correspond to significant differences between different sampling time within the same group (a vs b, b vs c, a vs c = p≤0.0001; A vs B = p≤0.0001; A vs C = p≤0.001)
**= difference between CS and VD with p≤0.0001; *= difference between CS and VD with p≤0.01

Fig. 16 - Mean (±SD) γGT activity in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



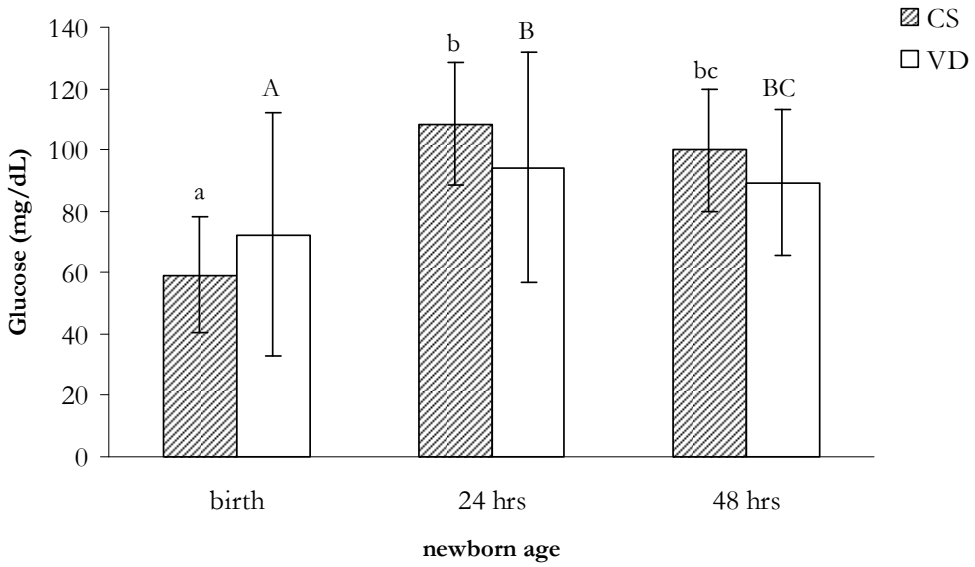
Different letters correspond to significant differences between different sampling time within the same group (p<0.0001)
*= difference between CS and VD with p≤0.05

Fig. 17 - Mean (±SD) GLDH concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



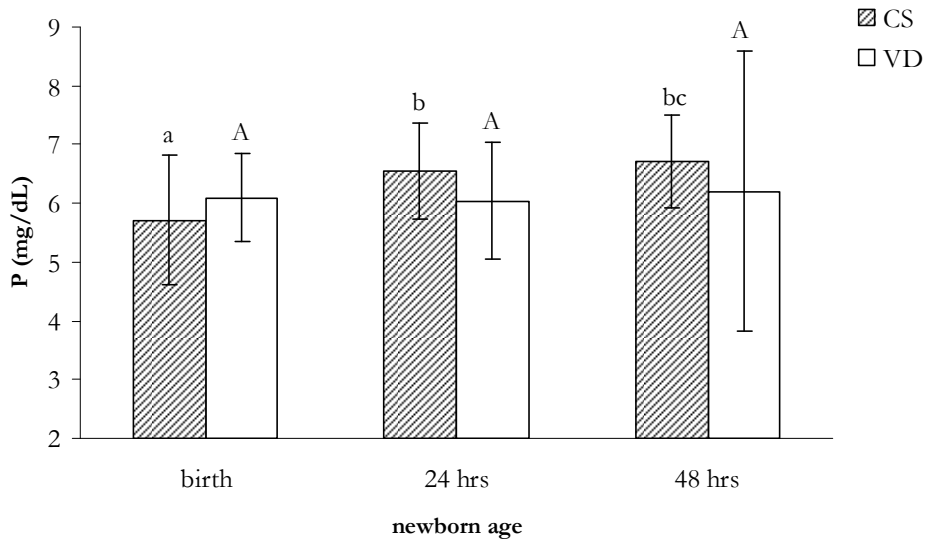
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs bc = p≤0.0001; A vs B, B vs AC = p≤0.0001)
*= difference between CS and VD with p≤0.05; **= differences between CS and VD with p≤0.0001

Fig. 18 - Mean (±SD) glucose concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



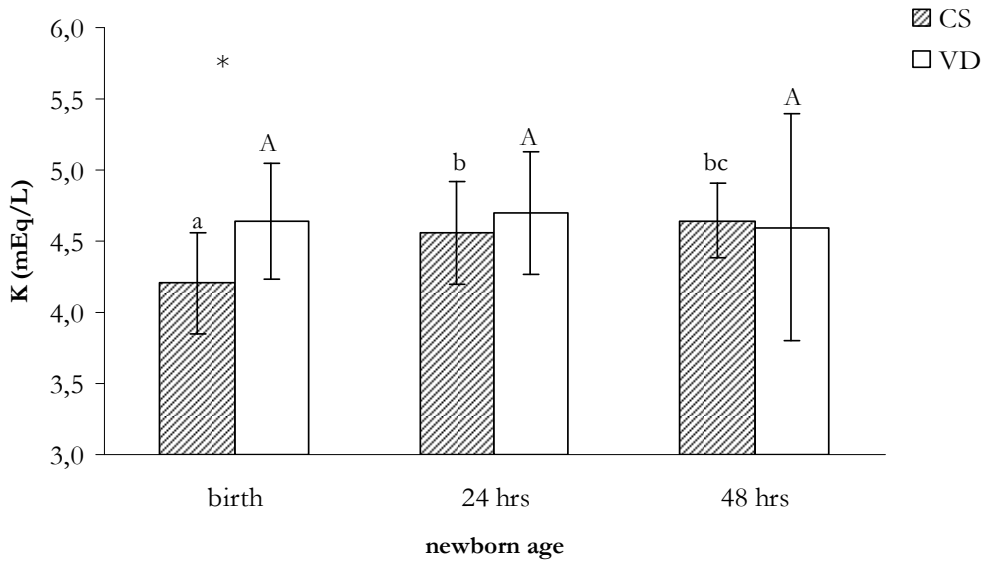
Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs bc = p≤0.0001; A vs B, A vs BC = p≤0.05)

Fig. 19 - Mean (±SD) phosphorus concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



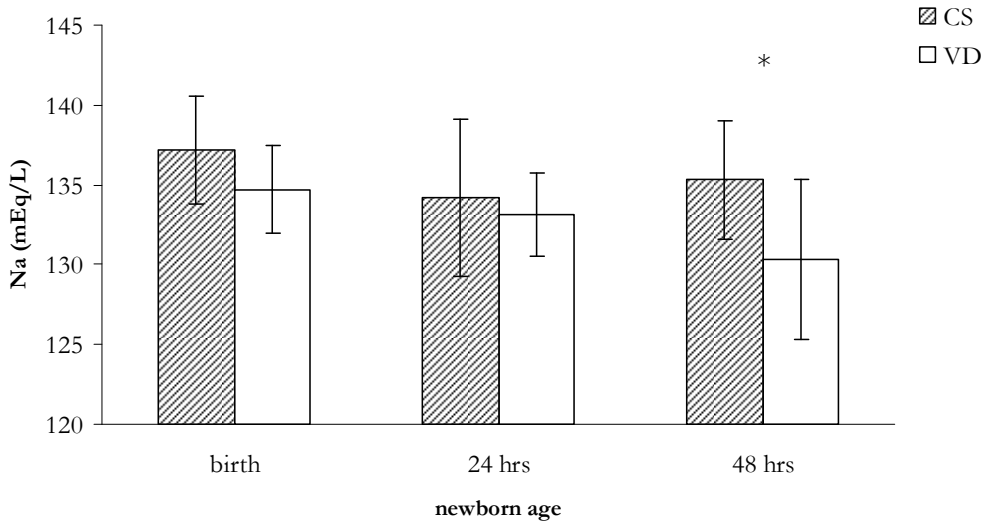
Different letters correspond to significant differences between different sampling time within the same group (a vs b = p≤0.001; a vs bc = p≤0.01)

Fig. 20 - Mean (±SD) potassium concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



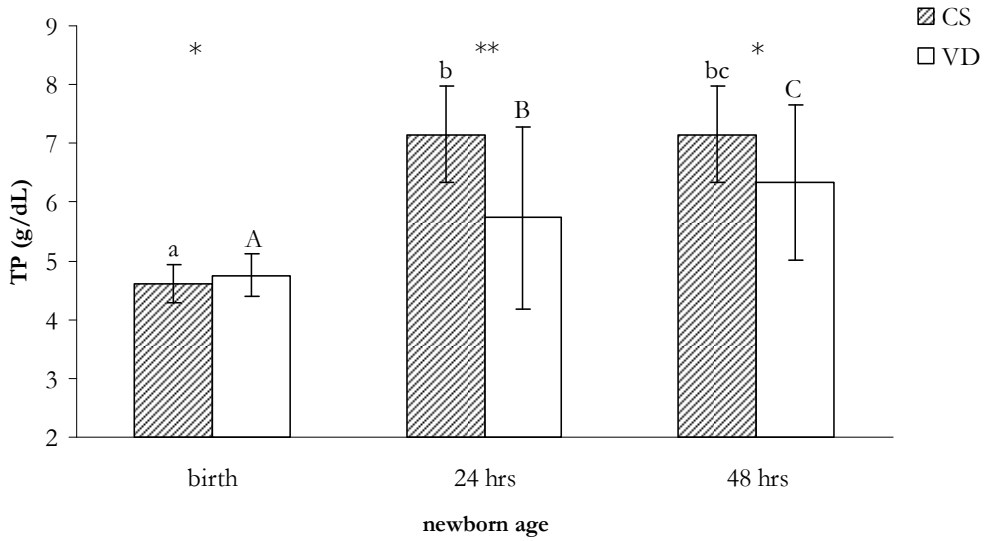
Different letters correspond to significant differences between different sampling time within the same group (a vs b = p≤0.001; a vs bc = p≤0.0001)
*= difference between CS and VD with p≤0.001

Fig. 21 - Mean (±SD) sodium concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



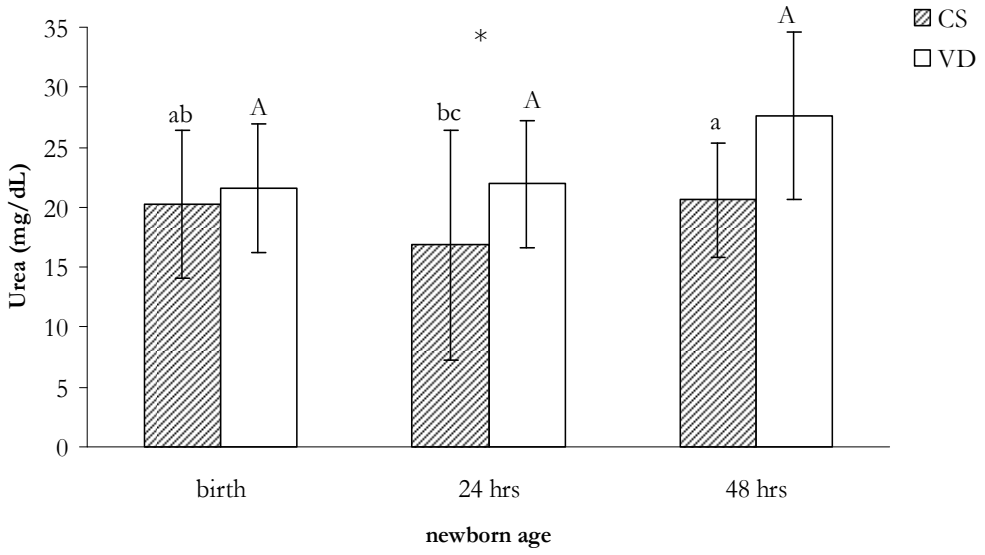
*= difference between CS and VD with p≤0.001

Fig. 22 - Mean (±SD) total protein concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



Different letters correspond to significant differences between different sampling time within the same group (a vs b, a vs bc = p≤0.0001; A vs B = p≤0.01; A vs C = p≤0.0001; B vs C = p≤0.001)
*= difference between CS and VD with p≤0.05
**= difference between CS and VD with p≤0.01

Fig. 23 - Mean (±SD) urea concentrations in serum of newborn calves born by vaginal delivery (VD) and caesarean section (CS)



Different letters correspond to significant differences between different sampling time within the same group (bc vs a = p≤0.05)
*= difference between CS and VD with p≤0.05

8.1.5 Discussion

In neonatal calves there are marked changes of haematological and metabolic traits and of enzymatic and endocrine systems, which partly depend on time of first colostrum feeding as well as on the duration and amount of ingested colostrum. In our study, significant changes of all parameters were seen throughout the time period examined, indicating very fast changes within the first hours after birth.

Similar management systems were adopted for the two groups of calves enrolled; both vaginally delivered calves and caesarean section delivered calves were immediately separated from the dam and fed colostrum within 1 hour from birth and for the first three days of life. At the time of first blood sampling, performed at 30 minutes after birth, no colostrum had been given to calves yet; despite that, significant differences between the two groups of calves were present already at that time.

Albumin concentrations, together with creatinine, calcium and chloride serum levels, decreased from birth to 24 hours and 48 hours after birth; all other parameters, except for sodium, showed an increasing trend from birth to 24 and 48 hours.

Increases in *AST* activity and in *total protein* serum concentrations from birth to later samples can indicate both an increased muscular metabolism and a maturation of proteins synthesis system; since half life of AST in about 2 or more days, the higher concentrations seen at 24 hours can not result from maternal sources but only from endogenous production, and therefore by the calf itself. Lower levels of total protein at birth in the caesarean section delivered calves could also suggest lower physical and muscular efforts of these subjects during the process of birth. During following samples, total protein resulted higher in the CS calves, probably due to the bigger muscular mass of these animals compared to the VD calves.

Increasing concentrations of *bilirubin* and *GLDH* and *γGT* activity from birth to later samples were found in both groups of calves. Again, this probably reflects the maturation of synthesis system in the newborn, especially those located in the liver tissue. Differences between the CS and the VD calves were found in all samples with higher concentrations in the CS calves. Interpretation of these data could be based on different hypothesis. Higher serum concentrations of these enzymes could be due to an increased production, reflecting maturation of hepatic system, or to liver injuries, expecially in centrilobular areas (e.g. hypoxia).

Data regarding *creatinine* concentrations seem to be interesting. Both groups show a decreasing trend from birth to 24 and 48 hours after birth. Many publications in pediatrics and pediatric nephrology mention the fact that plasma creatinine concentration (Pcr) in the newborn period is as high as in the adult. This is unusual because the production of creatinine is dependent on the infant's muscle mass—which is much less than that of the adult— and the healthy

newborn is not in renal failure although the absolute and relative (to adult body size) glomerular filtration rate (GFR) is low. These data are certainly incompatible with the notion that the abnormally high levels of creatinine are attributable only to maternal transfer, as still often cited in textbooks on pediatric nephrology. Some authors (Guignard & Drukker, 1999) hypothesize that the high Pcr of the newborn immediately after birth represent the maternal levels, but shortly thereafter the tubular reabsorption of creatinine seems to be responsible for the continued high plasma creatinine levels of the newborn and in particular the preterm infant. They hypothesize that this latter temporary phenomenon is attributable to a back-flow of creatinine across leaky immature tubular and vascular structures. With time, maturational renal changes will impose a barrier to creatinine. From that point onwards, total body muscle mass, GFR, and tubular secretion will determine the Pcr of the individual (Matos *et al*, 1998).

According to this theory, decreasing serum concentrations of creatinine in newborn calves are due to the gradual maturation of kidney functions, which seems to be somehow slower in the CS group. Acceleration of the process of birth due to the performance of the caesarean section is probably the reason for the higher creatinine levels found in serum of these calves compared to the spontaneous calves. The achievement of comparable levels of creatinine within 48 hours from birth indicates the completion of the adaptational processes of the kidney to extra uterine life.

Also *urea* serum concentrations, which increased with age and were higher in VD calves at 24 hours after birth, could indicate the gradual maturation of newborn kidney and liver; increasing production from hepatic cells or improved filtration and reabsorption from kidney lead to increasing serum levels of urea, and these processes appear faster in the VD calves.

Mean *glucose* concentrations increased from relatively low concentrations after birth within 24 h postnatally and then remained stable. Hypoglycaemia at birth is a quite common situation; newborns are highly dependent on glucose intake, and

their carbohydrate stores are limited and quickly depleted, leading to mobilization of fat depots. For this reason, mean *βhydroxybutyrate* concentrations rapidly increased after birth, but only in the CS group; scarse *βHOB* levels at birth could be due to a scarse energy mobilization during delivery. No significant differences were found between the two groups.

Changes in minerals concentrations with age were found in both groups, with almost no differences between CS and VD calves. Increasing trend was present in *phosphorus* and *potassium* serum concentrations, while *calcium* and *chloridre* levels decreased from birth to later samples. Stable values characterized *sodium* serum levels.

8.1.6 Conclusions

Important changes in biochemical and metabolic parameters are present during the first hours from birth in the newborn calf. Specifically, age seems to affect all parameters, as evidenced by the decreasing or increasing levels or activities of many molecules. **These fast changes are probably due to the maturational processes of liver, kidney and metabolism for adaptation to the extra uterine life.** Basing on these data, the **caesarean section seems to entail a lower physical effort for calves during birth, and a slower or delayed adaptational process.**

8.1.7 References

Guignard JP, Drukker A (1999) Why Do Newborn Infants Have a High Plasma Creatinine? Pediatrics *103* e49

Matos P, Duarte-Silva M, Drukker A, Guignard JP (1998) Creatinine Reabsorption by the Newborn Rabbit Kidney. Pediatric Research *44* 639-641

8.2 Non-Esterified Fatty Acids

8.2.1 Samples collection

Blood samples were taken from the jugular vein into Li-Heparin plastic tubes (Venosafe® VF-109SH, Terumo) by single-use needles with holders according to the following temporal pattern:

- 10 minutes after birth
- 3, 12, 36 hours after birth
- 7, 14 days of age

After collection, samples were immediately centrifuged at 1000xg for 20 minutes and the resulting plasma was stored at -20°C until analysis.

8.2.2 Laboratory analysis

Enzymatic-colorimetric methods were used to determine plasma concentrations of NEFA (NEFA-HR(2); Wako Chemicals, Richmond, VA, USA). This method involves conversion of NEFA to their corresponding enoyl-Coenzyme A (CoA) products with generation of hydrogen peroxide (H_2O_2). The amount of H_2O_2 produced during a reaction can be directly correlated with the total NEFA concentration in a given sample. In the presence of peroxidase (POD), the hydrogen peroxide formed yields a blue purple pigment by quantitative oxidation condensation with 3-Methyl-N-Ethyl-N-(β-Hydroxyethyl)-Aniline (MEHA) and 4-aminoantipyrine (4-AA). Non-esterified fatty acids concentration is obtained by measuring absorbance of the blue purple color. Detection of non-esterified fatty acids was carried out as per manufacturer's protocol. In brief, 2 ml of reagent R1 was added to 50 ml of sample and incubated for 10 min at 37 °C. To this 1 ml of reagent R2 was added and further incubated at 37 °C for 10 min. The absorbance of the solution was read at 550 nm. Intra- and inter-assay coefficients of variation were 2.7% and 5.5%, respectively. Values obtained are expressed as μEq/L.

8.2.3 Statistical analysis

In order to detect changes in NEFA concentrations within each group and between the two groups of calves, a one-way ANOVA was applied, with group and sampling time as fixed factors. ANOVA was followed by a Tukey test for multiple comparisons to identify differences within each group among sampling times. Possible differences between the two groups of calves for each sampling time were investigated with a t-test. Statistical analysis was performed using SAS 9.1 for Windows®, and significativity was set for $p \leq 0.05$.

8.2.4 Results

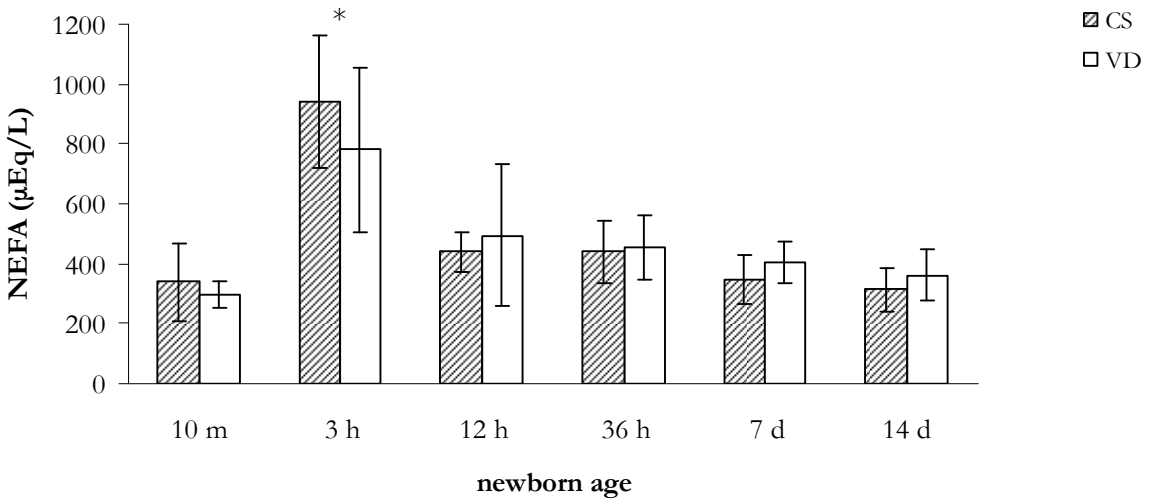
For clinical results, see chapter 7.2.

Mean (±SD) NEFA plasma concentrations (μEq/L) in the 41 newborn calves are reported in table 3 and figures 24-26. Statistical analysis evidenced an influence of time on NEFA concentrations in both groups of calves ($p \leq 0.0001$), while few differences were registered between the two groups of calves ($p \leq 0.05$).

Tab. 3 – Mean (±SD) NEFA plasma concentrations in the Caesarean section delivered (CS) newborn calves and in the vaginal delivered (VD) newborn calves

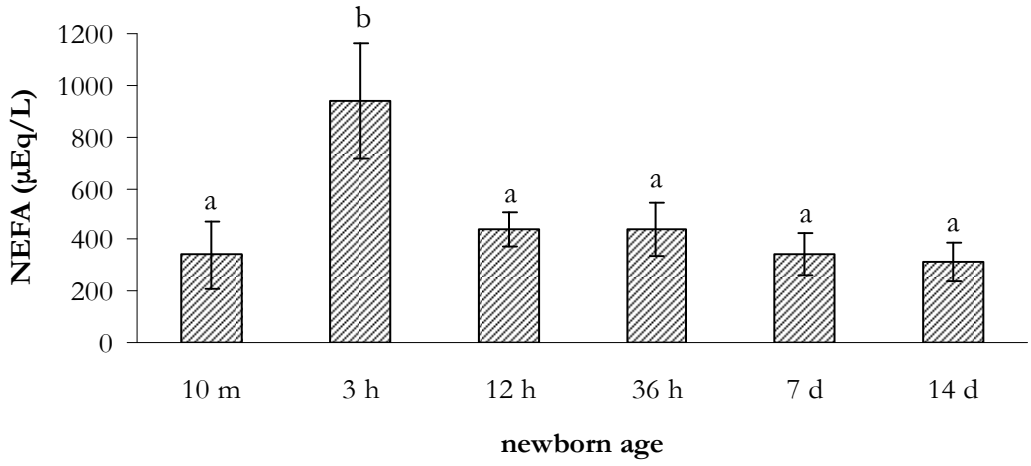
Newborn age	CS	VD
10 min	340.4 ± 129.9	294.6 ± 44.0
3 hrs	940.0 ± 222.6	780.5 ± 272.1
12 hrs	441.7 ± 66.7	494.4 ± 234.4
36 hrs	439.7 ± 106.0	457.3 ± 106.9
7 d	344.6 ± 82.0	405.2 ± 70.1
14 d	313.3 ± 72.6	362.8 ± 85.2

Fig. 24 – Mean (±SD) NEFA plasma concentrations in the Caesarean section delivered (CS) newborn calves and in the vaginally delivered (VD) newborn calves



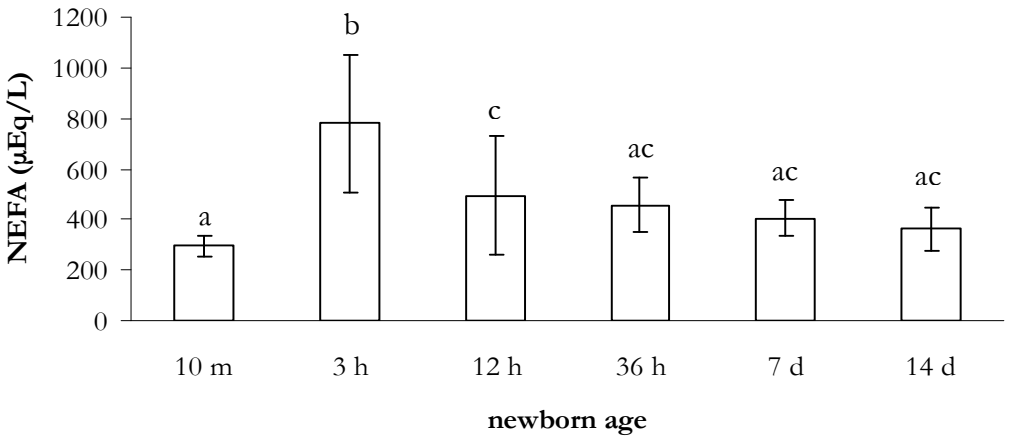
*= difference between CS and VD ($p \leq 0.05$)

Fig. 25 – Mean (±SD) NEFA plasma concentrations in the Caesarean section delivered newborn calves



Different letters correspond to significant differences among sampling times ($p \leq 0.01$)

Fig. 26 – Mean (±SD) NEFA plasma concentrations in the vaginally delivered newborn calves



Different letters correspond to significant differences among sampling times ($p \leq 0.01$)

8.2.5 Discussion

NEFA plasma levels showed a rise during the first 3 hours after life compared to birth, followed by a declining trend reaching stable values already at 12 hours after birth; these results were similar to previous studies on calves (Ronge & Blum 1988; Vermorel *et al*, 1989; Hadorn *et al*, 1997; Hammon & Blum 1998).

The rather low levels of NEFA registered at birth underline the special conditions of newborn energy state at birth; energy demand is satisfied thanks to maternal sources, while as soon as this link between mother and fetus is interrupted, the newborn has to provide for energy uptake itself. The maintenance of normoglycaemia depends also on the maturation of the gluconeogenic pathway and appropriate concentrations of the hepatic enzymes required for gluconeogenesis. Oxidation of NEFA generates not only energy to drive gluconeogenesis, but also acetyl coenzyme A, which activates pyruvate carboxykinase, the first rate-limiting enzyme in the gluconeogenetic pathway.

These conditions, together with the uptake of first colostrum, can explain the fast and huge increase in NEFA levels at 3 hours after life.

Vermorel *et al* (1983) found a reduced mobilization of body lipids in dystocial calves in comparison with eutocial calves, reflected by lower levels of plasma fatty acids; therefore a difficult birth may have an influence also on energy metabolism. According to the results of this study, the types of parturition considered do not influence NEFA levels in the calf; very similar NEFA pattern were in fact registered in CS and VD calves. The only difference between groups was detected at 3 hours after life, with higher NEFA plasma levels in the CS group. As explanation, it can be hypothesized that this difference is due to the intake of different colostrum quality; colostrum administration was made after the first blood sample but within 1 hour after birth, so it is consistent with the rise in NEFA levels at 3 hours.

8.2.6 Conclusions

NEFA plasma levels in newborn calves were deeply influenced by age and probably by the type of colostrum administered, **but not by the mode of delivery.**

8.2.7 References

Hadorn U, Hammon H, Bruckmaier M, Blum JW (1997) Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J Nutr *127* 2011-2023.

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CHAPTER 9

Hormonal profiles

9. Hormonal profiles

9.1 Insulin-like Growth Factor-I

9.1.1 Samples collection

Blood samples were taken from the jugular vein into Li-Heparin plastic tubes (Venosafe® - VF-109SH, Terumo) by single-use needles with holders according to the following temporal pattern:

- 10, 20, 30 minutes after birth
- 3, 6, 12, 24, 36 hours after birth
- 7, 14 days of age

After collection, samples were immediately centrifuged at 1000xg for 20 minutes and the resulting plasma was stored at -20°C until analysis.

9.1.2 Laboratory Analysis

IGF-I plasma levels were evaluated using a modified RIA technique (Renaville *et al*, 1993). In this method, a cryoprecipitation step was used to eliminate aggregated IGF binding proteins in plasma extracts. Briefly, after acid-ethanol extraction (87.5% ethanol and 12.5% HCl 2M, v:v), an aliquot of the supernatant was neutralized with 0.855 M Tris base at a ratio of 5:2. The samples were then stored at -20 °C for 1 hour and immediately centrifuged at 3000 x g for 30 min at 4°C. After centrifugation, the supernatant fluid was diluted in assay buffer ($NaH2PO_4$, 0.6 mol/liter; EDTA, 3.72 g/liter; protamin sulfate, 200 mg/liter; Tween 20, 500 µl/liter; NaN3, 200 mg/liter; pH 7.5) (final dilution of plasma: 1/28). The diluted extracts were measured by RIA method using ^{125}I-labeled human IGF-I (9,000 cpm; specific activity: 50 µCi/µg) and a rabbit anti-serum (1/80,000 final dilution) raised against recombinant human IGF-I. (Novozymes Biopharma; Thebarton, SA 5031, Australia). Recombinant human

IGF-I (rhIGF-I, Roche S.p.A, Italy) was used for iodination as tracer and as standard in the radioimmunoassay. The tracer was prepared with Na ^{125}I by the iodogen method. Following incubation for 24 h, antibody-bound hormone was precipitated with goat anti-rabbit gamma globulin. The sensitivity of the assay, calculated as the interpolated dose of the response to a concentration of zero minus the statistical error, was 1.8 ng/ml. Intra- and inter-assay coefficients of variation were 8.5 and 12.7%, respectively.

9.1.3 Statistical analysis

In order to detect changes in IGF-I concentrations within each group and between the two groups of calves, a one-way ANOVA was applied, with group and sampling time as fixed factors. ANOVA was followed by a Tukey test for multiple comparisons to identify differences within each group among sampling times. Possible differences between the two groups of calves for each sampling time were investigated with a t-test. Statistical analysis was performed using SAS 9.1 for Windows®, and significativity was set for $p \leq 0.05$.

9.1.4 Results

For clinical results see chapter 7.2.

Mean (±SD) IGF-I plasma concentrations (ng/ml) in the 40 newborn calves are reported in table 4 and figures 27-29; comparison between mean values in the VD calves and the values in calf R are reported in figure 30. Statistical analysis evidenced an influence of time on IGF-I concentrations of both groups of calves ($p<0.0001$), while no differences were registered between the two groups of calves ($p=0.09$).

Tab. 4 – Mean (±SD) IGF-I plasma concentrations in the vaginal delivered (VD) newborn calves and in the Caesarean section delivered (CS) newborn calves

Newborn age	CS	VD
10 min	57.3 ± 39.3	62.4 ± 29.0
20 min	59.0 ± 40.2	70.2 ± 31.6
30 min	58.4 ± 41.1	71.0 ± 30.9
3 hrs	49.5 ± 36.7	57.2 ± 32.5
6 hrs	45.4 ± 33.2	54.5 ± 34.9
12 hrs	40.4 ± 33.7	46.6 ± 26.7
24 hrs	39.3 ± 30.3	44.0 ± 29.3
36 hrs	27.8 ± 19.4	29.6 ± 17.0
7 d	9.9 ± 5.8	7.0 ± 2.9
14 d	7.1 ± 4.5	7.1 ± 3.7

Fig. 27 – Mean (±SD) IGF-I plasma concentrations in the Caesarean section delivered (CS) newborn calves and in the vaginally delivered (VD) newborn calves

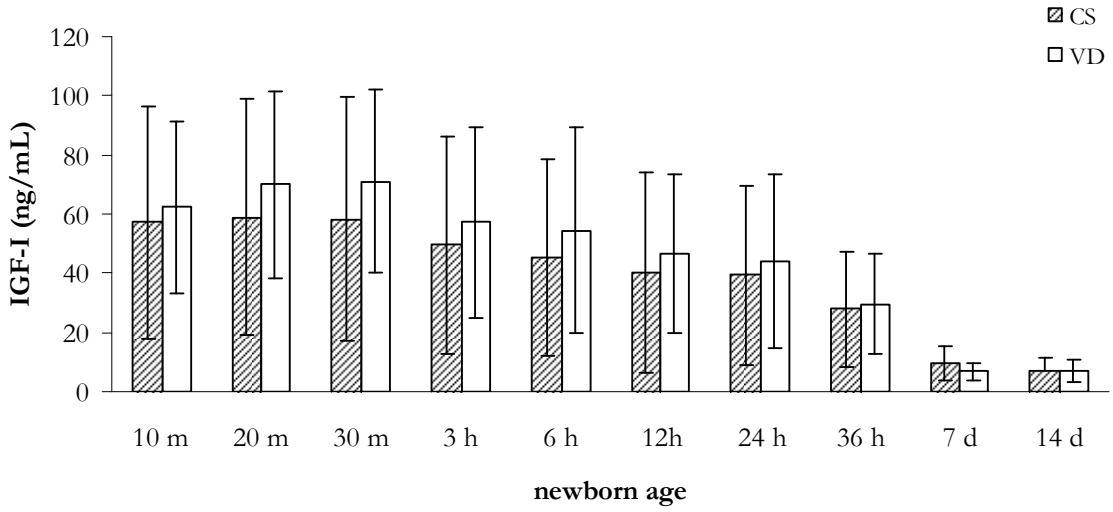
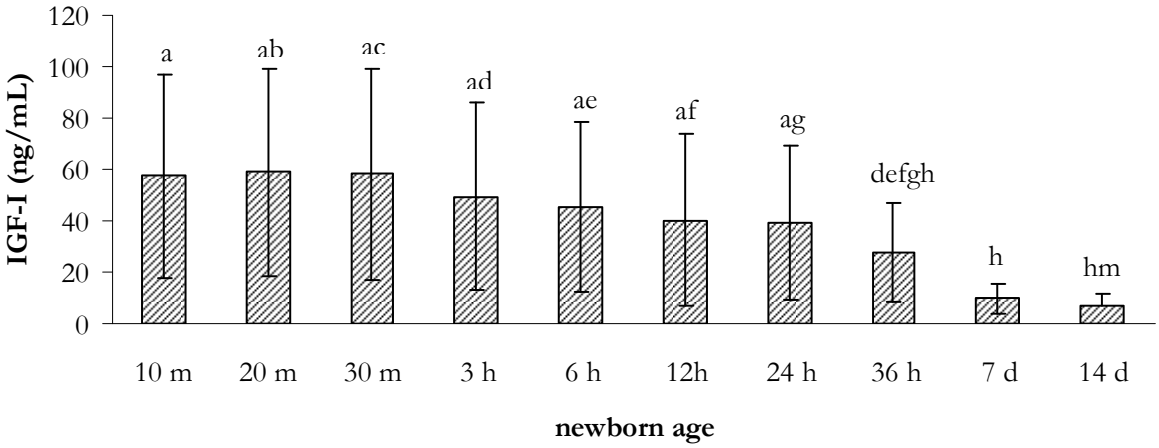
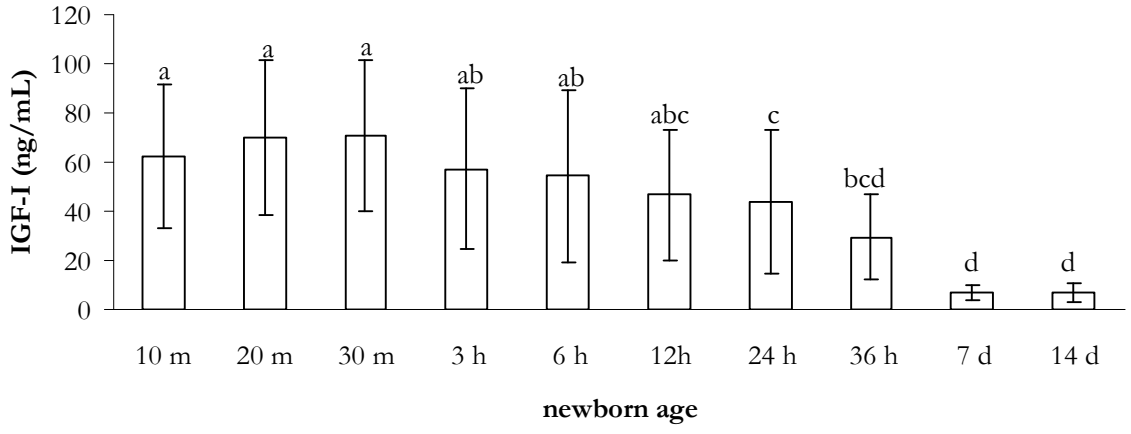


Fig. 28 – Mean (±SD) IGF-I plasma concentrations in the Caesarean section delivered newborn calves



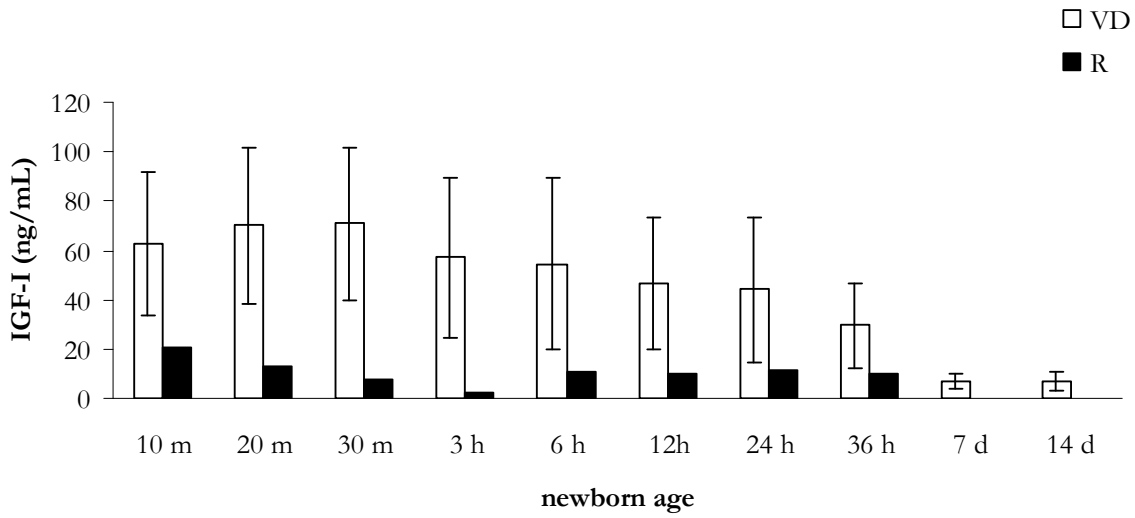
Different letters correspond to significant differences among sampling times (p≤0.01)

Fig. 29 – Mean (±SD) IGF-I plasma concentrations in the vaginally delivered newborn calves



Different letters correspond to significant differences among sampling times (p≤0.01)

Fig. 30 – Comparison between mean (±SD) IGF-I plasma concentrations in the vaginally delivered (VD) newborn calves and in the calf dead at 7 days of age (R)



9.1.5 Discussion

Systemic changes of metabolic and endocrine profiles in neonates generally depend on composition, amounts, time and duration of feeding colostrum. Oral administration of IGF-I in calves had no effect and feeding of colostrum whey extracts had only minor effects on metabolic and endocrine traits; thus, mammary secretions deeply influence regulatory functions of neonatal tissues.

In this study, a decrease of IGF-I plasma concentrations was observed in both the considered groups of calves, and important statistical differences between different sampling times were observed. Our results appear in contrast with previous reports on IGF-I in the neonates of other species, but do agree with some data on calves.

Levels of circulating IGF-I in humans are low at birth and rise progressively during childhood (Hammerman, 1987); in the newborn lamb, plasma IGF-I levels increase rapidly after birth, primarily as a result of the onset of growth hormone stimulated IGF-I production by the liver (Gluckman & Butler, 1983). During postnatal life in fact, most of circulating IGF-I derives from the liver, and the increasing levels in human neonates have been also correlated with body weight gain. Anyway, because calves are born relatively mature compared with other species such as rats, mice and humans, differences with other species with respect to IGF circulating levels receptor numbers and thus of GIT responses to ingested food components can be expected and justified.

Indeed, we found a gradual decrease of IGF-I plasma levels in both groups of calves from 2 to 14 days of age, as previously reported by Hadorn *et al* (1997). Specifically, in the CS group (*figure 28*) statistic evidenced similar levels of IGF-I for the first 24 hours from birth, and significantly different IGF-I concentrations at 7 and 14 days after birth. Sample at 36 hours from birth represented a midway step between higher and lower IGF-I levels. A similar situation was detected in the VD group (*figure 29*).

Previous findings can explain our results. Both IGF-I and -II have been characterized in ruminant blood relatively to the stage of lactation. Ronge & Blum (1988) showed that IGF-I concentration in blood was inversely related with milk production: a drastic drop after parturition was followed by a gradual increase as lactation persisted. IGF binding proteins (IGFBPs) are present at high concentration in the mammary gland secretions, equivalent or higher in concentration than in blood during the prepartum period. They drop precipitously after the colostral phase, with the onset of copious milk secretion: therefore, the decreasing trend of plasma IGF-I found in these newborn calves could be partially explained by the decline of this hormone in colostrum and milk of cows, as described also by Hadorn *et al* (1997).

Colostrum and milk contents of IGF-I were not measured in this study, but we can hypothesize that some differences could exist, due to the different breed, attitude and herd management of the mothers. It must be remembered that even nutrition strategy of calves in this study was different between the two groups; after 3 days of colostrum, VD calves were fed with pasteurized herd milk, while the CS received a milk substitute. Although all these factors mean a different nutritional intake, no significant differences could be detected comparing circulating levels of IGF-I in two groups of calves (*figure 27*).

This leads to the hypothesis that beyond nutritional factors, other factor can affect IGF-I plasma levels in the newborn; according to some authors in fact, only small amounts of IGF-I are absorbed by newborn calves (Vacher *et al*, 1995; Hammon & Blum 1997).

IGF-I should be absorbable by the gut within the first 24 h of life whether it is bound by IGFBP or not. A study from Sparks *et al* (2003) showed a wide variation in serum IGF-I concentrations in calves at birth; moreover, calves born with higher serum IGF-I had a significant decrease in serum IGF-I concentration between birth and 48 hours, whereas calves born with low serum IGF-I had no significant change in serum IGF-I during the same interval. Although absorption of IGF-I from the colostrum into the systemic circulation is a possibility, those results indicate that the concentration of serum IGF-I at birth is important in determining serum IGF-I concentrations at 48 hours. Hammon *et al* (2000) reported that plasma levels of IGF-I decrease between 0 and 48 hours after birth and that these IGF-I levels during the first 24 hours of life are significantly influenced by how soon after birth the first colostrum is fed. In the study from Sparks *et al* (2003), birth weight was not significantly correlated with serum IGF-I at either 0 or 48 hours, and it was therefore unlikely that birth weight accounts for differences in serum IGF-I levels among bovine neonates.

This agree with our data, where similar IGF-I plasma levels were found among calves of different breed and attitude and with very different mean birth weight; therefore, our results do not agree with data from many species, where

the concentration of IGFs have been found to be positively correlated to birth weight (Daughaday *et al*, 1982; Gluckman & Butler, 1983; Lee *et al*, 1993).

Neither type of delivery seems to influence IGF-I plasma levels in the newborn calves; differences could be expected in relation with the maturity degree of calves, but in our study all calves were judged at term and mature.

Regarding data from the vaginally delivered calf R, who died at 7 days of age, a clear difference with mean IGF-I values of VD group was notable. Clinical data of this calf at birth were not dissimilar from others, with TSR, TSU and Apgar score included in the normal ranges. Starting from 24 hours after birth, the calf began to show a depressed attitude, deydratation, pale mucus membranes and loosy faeces; clinical diagnosis consisted of neonatal diarrhoea. Although clinical examination at birth could not evidence signs of illness, IGF-I plasma concentrations were already clearly different from mean values, indicating the presence of some kind of alteration.

More data are necessary to establish a real relation, but a prompt investigation at birth may help veterinarians identifying calves at risk for development of neonatal pathologies.

9.1.6 Conclusions

In conclusion, **IGF-I plasma levels in newborn calves were influenced by age**, being significantly lower at 7 days compare to birth; critical point for this decrease appeared to be around the second day of life. **Differences in breed, attitude, neonatal weight and type of birth do not seem to affect plasma concentrations of IGF-I** in calves during the first two weeks of life.

9.1.7 References

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9.2 Cortisol

9.2.1 Samples collection

Blood samples were taken from the jugular vein into Li-Heparin plastic tubes (Venosafe® VF-109SH, Terumo) by single-use needles with holders according to the following temporal pattern:

- 10, 20, 30 minutes after birth
- 6, 24 hours after birth
- 7, 14 days of age

After collection, samples were immediately centrifuged at 1000xg for 20 minutes and the resulting plasma was stored at -20°C until analysis.

9.2.2 Laboratory analysis

Plasma cortisol was measured using a solid-phase microtitre RIA after diethyl ether extraction. Briefly, a 96-well microtitre plate (Optiplate, Perkin-Elmer Life Science, Boston, MA, USA) was coated with anti-rabbit γ-globulin serum raised in a goat diluted 1:1000 in 0.15 mM sodium acetate buffer, pH 9 and incubating overnight at 4°C. The plate was then washed twice with PBS 0.1% BSA, pH 7.4 and incubated overnight at 4°C with 200 μl of the anti-cortisol serum diluted 1:12.000. The antiserum (USBiological, Massachusetts) was raised in the rabbit against cortisol-3 carboxymethyloxime–BSA and showed the following cross reactions: cortisol 100%, prednisolone 36%, 11-deoxycortisol 5.7%, corticosterone 3.3%, cortisone <0.7%. Afterwards, the anti-serum solution was decanted, and the plate was washed with RIA buffer. Finally, standards (5-200 pg/well), quality control, unknown extracts and tracer (1,2,6,7–3H-cortisol, Perkin-Elmer Life Sciences, 30 pg/well) were added (final volume: 200 μl), and plate were incubated overnight at 4 ◦C. The separation of bound from free hormone was performed by decanting the reaction mixture and washing the

plate with RIA buffer. Bound radioactivity was counted on the β-counted after the addition of 200 µl scintillation cocktail (Microscint 20, Perkin- Elmer Life Sciences). The intra- and inter-coefficients of variation for C were 4.8% and 8.7% respectively. The sensitivity of the assay was defined as the dose of hormone at 90% binding (B/B0) and was 2.94 pg/well.

9.2.3 Statistical analysis

In order to detect changes in cortisol concentrations within each group and between the two groups of calves, a one-way ANOVA was applied, with group and sampling time as fixed factors. ANOVA was followed by a Tukey test for multiple comparisons to identify differences within each group among sampling times. Possible differences between the two groups of calves for each sampling time were investigated with a t-test.

Statistical analysis was performed using SAS 9.1 for Windows®, and significativity was set for $p \leq 0.05$.

9.2.4 Results

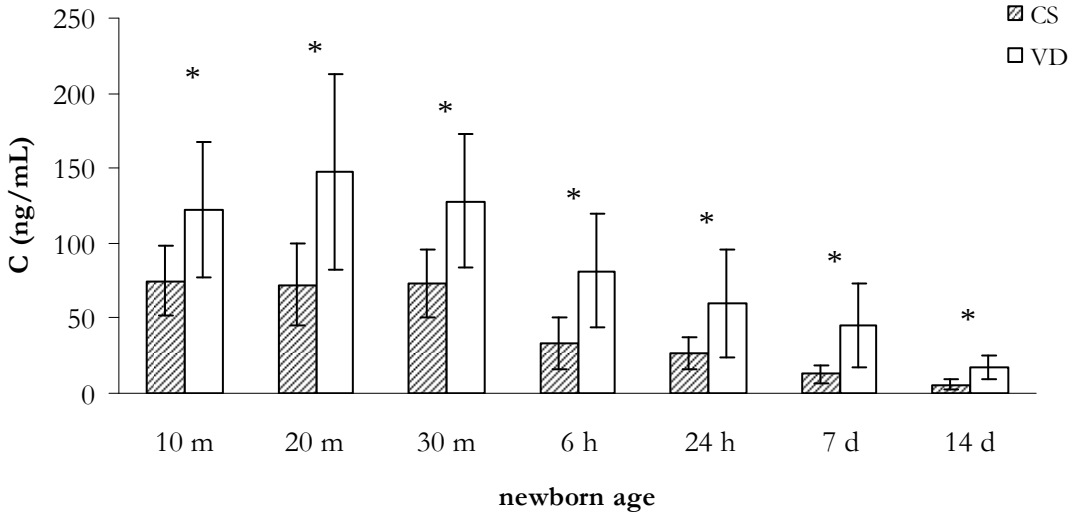
For clinical results, see chapter 7.2.

Mean (±SD) cortisol plasma concentrations (ng/mL) in the 40 newborn calves are reported in table 5 and figures 31-34. Statistical analysis evidenced an influence of both time ($p \leq 0.05$) and group ($p \leq 0.0001$) on cortisol concentrations of newborn calves.

Tab. 5 – Mean (±SD) cortisol plasma concentrations in the Caesarean section delivered (CS) newborn calves and in the vaginally delivered (VD) newborn calves

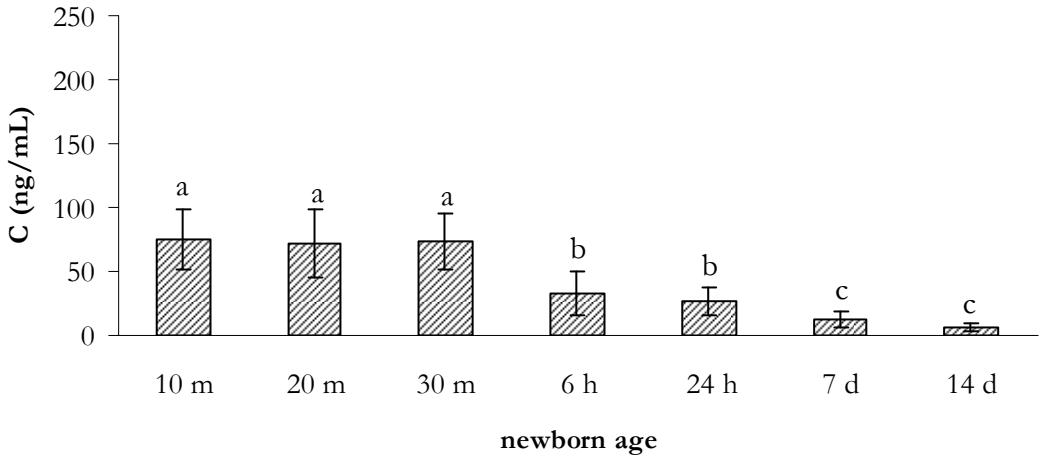
Newborn age	CS	VD
10 m	75.07 ± 23.75	122.64 ± 44.93
20 m	72.00 ± 27.20	147.48 ± 65.06
30 m	73.18 ± 22.02	128.30 ± 45.15
6 h	32.98 ± 17.18	81.62 ± 38.11
24 h	26.50 ± 10.64	59.66 ± 35.82
7 d	12.66 ± 6.43	45.14 ± 28.20
14 d	5.96 ± 3.40	17.44 ± 7.77

Fig. 31 – Mean (±SD) cortisol plasma concentrations in the Caesarean section delivered (CS) newborn calves and in the vaginally delivered (VD) newborn calves



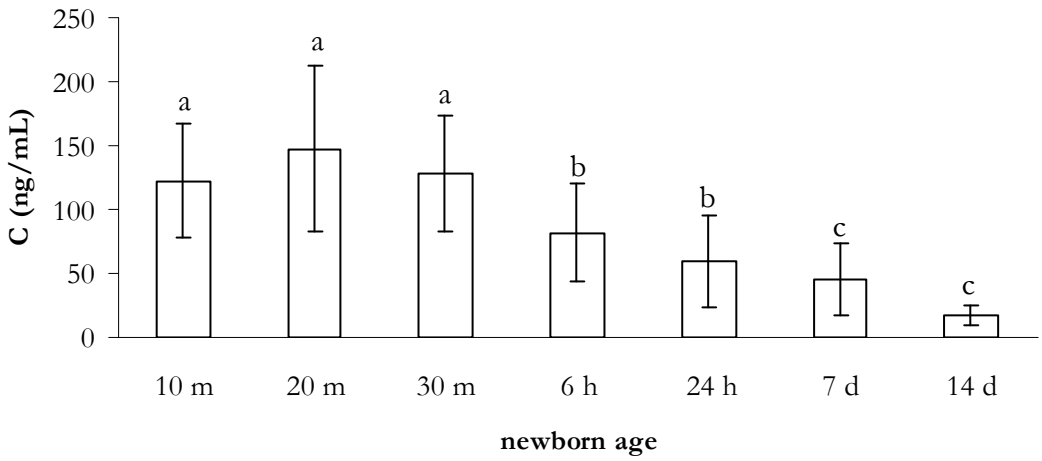
*=significant difference between CS and VD ($p<0.0001$)

Fig. 32 – Mean (±SD) cortisol plasma concentrations in the Caesarean section delivered newborn calves



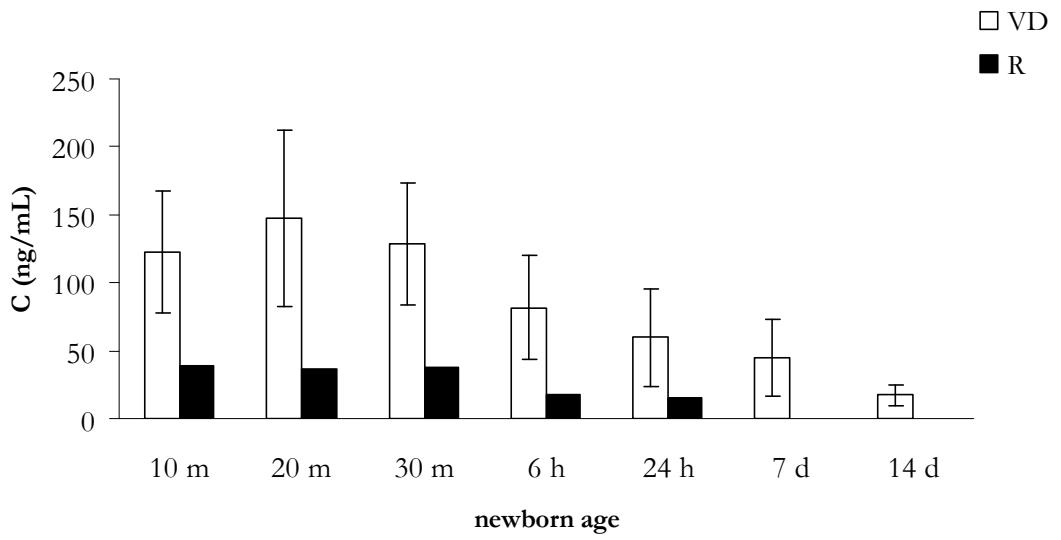
Different letters correspond to significant differences between sampling times ($p<0.05$)

Fig. 33– Mean (±SD) cortisol plasma concentrations in the vaginally delivered newborn calves



Different letters correspond to significant differences between sampling times ($p<0.05$)

Fig. 34 – Comparison between mean (±SD) cortisol plasma concentrations in the vaginally delivered (VD) newborn calves and in the calf dead at 7 days of age (R)



9.2.5 Discussion

Cortisol plasma levels showed a very similar trend in both groups of calves; high concentrations at birth were followed by a reduction at 6 hours and a further decrease on day 7 after birth.

These data are in agreement with previous studies on calves, which found that cortisol concentrations appear high at birth, and tend to decrease during the first week of life (Lee *et al*, 1995; Hadorn *et al*, 1997; Hammon & Blum, 1998). The high concentrations at birth are partially due to the important role of cortisol in triggering the onset of calving (Comline *et al*, 1974; Hunter *et al*, 1977), to achieve an efficient synchronization between the control of parturition and fetal lung maturation and surfactant production. This is the reason for calves that are born before some 90% of the normal gestation length usually suffer from respiratory problems after birth; this is most likely associated with inadequate synthesis of surfactant by their lungs (Bleul, 2009).

In animal and human studies, decreased secretion and deposition of surfactant in the alveolar space, and decreased levels of catecholamines and stress hormones have been found to occur in individuals with RDS.

When comparing the endocrine response between newborn babies born by elective caesarean section (ECS) and babies born by vaginal delivery (Zanardo *et al*, 2006), blood levels of cortisol were found to be significantly lower in the ECS group.

The same results were found in this study. Comparison between calves from caesarean section and calves from vaginal delivery evidenced significantly different cortisol plasma concentrations, with almost double cortisol levels in the vaginally delivered calves; differences were present from birth until the last sample**.**

Similar results were found by Hoyer *et al* (1990) where calves subjected to severe pulling had the higher glucocorticoid concentrations at birth (110.4 ng/ml) followed by calves requiring no assistance (88.3 ng/ml), calves requiring only slight assistance (83.8 ng/ml), and calves that had been delivered by caesarean section (82.9 ng/ml). On the contrary, Massip (1980) examined blood samples taken from 24 calves, six born normally, 11 by caesarean section and seven after difficult delivery, and mean values for plasma cortisol concentrations did not differ much with the three types of delivery.

It should be remembered that neonatal calves born by caesarean section seem to be more predisposed to develop a respiratory distress syndrome, and therefore a respiratory acidosis, during the first hours of their life (Cambier *et al*, 2000). These findings lend support to the suggestion that some clinical respiratory consequences of ECS, in example the higher risk for developing a RDS, might be initiated and enhanced by the reduced production at birth of stress hormones and cortisol (Gerten *et al*, 2005).

For this and other reasons, there is much evidence that labour and vaginal delivery have beneficial short- and long-term effects on neonatal respiratory adaptation to extra uterine life. Stress caused by vaginal deliver, mainly consisting of physical and muscular efforts, is beneficial for lung maturation and expansion and for surfactant production.

Data regarding cortisol concentrations in the calf dead at 7 days of life seems to confirm the importance of a good immune-neuroendocrine response at birth to develop an appropriate postnatal adaptation; this can maybe be an efficient tool for facing possible diseases during and after the neonatal period.

9.2.6 Conclusions

Cortisol pattern in the newborn calves are characterized by high plasma levels at birth, coming partially from the endocrine cascade that start the mechanism of parturition and partially from the stressful conditions for the newborn during birth.

Elective caesarean section seems to entail a lower level of stress with consequent lower levels of plasma cortisol in the newborn calf; higher risk for development of respiratory distress could be a direct consequence of this condition.

9.2.7 References

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9.3 Thyroid hormones

9.3.1 Samples collection

Blood samples were taken from the jugular vein into Li-Heparin plastic tubes (Venosafe® VF-109SH, Terumo) by single-use needles with holders according to the following temporal pattern:

- 10, 20, 30 minutes after birth
- 6, 24 hours after birth
- 7, 14 days of age

After collection, samples were immediately centrifuged at 1000xg for 20 minutes and the resulting plasma was stored at -20°C until analysis.

9.3.2 Laboratory analysis

Samples analysis for detection of T_3 and T_4 plasma levels are currently underway.

CHAPTER 10

Comparative study on 15-ketodihydro-$PGF_{2\alpha}$ plasma concentrations in newborn horses, donkeys and calves

Published in:
Reproduction in Domestic Animals
doi:10.1111/j.1439-0531.2011.01804.x

Reprod Dom Anim doi: 10.1111/j.1439-0531.2011.01804.x
ISSN 0936-6768

Comparative Study on 15-Ketodihydro-$PGF_{2\alpha}$ Plasma Concentrations in Newborn Horses, Donkeys and Calves

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Contents

The aim of this study was to compare the plasma profiles of 15-ketodihydro-$PGF_{2\alpha}$ (PGM) in healthy neonates of three different species from birth until the third week of life. Twentyfour horse foals, 12 donkey foals, and 9 calves were studied. Blood samples were collected at 10, 20 and 30 min after birth, at 3, 24 and 72 h after birth, and at 7, 10, 17 and 21 days of life. All mothers experienced normal gestation lengths and normal, spontaneous deliveries. All newborns were judged mature and viable. Hormone concentrations were higher ($p < 0.05$) in horse foals 20 and 30 min after birth compared to later samples, and at 10 min in donkey foals compared to later samples ($p < 0.05$). In calves, higher ($p < 0.05$) concentrations of PGM were observed 10, 20, 30 min and 3 hours from parturition compared to later samples. These findings may be related to increased fetal placental unit production during parturition, while the relatively high PGF2α levels in the days after parturition may be connected with their role in completing organ maturation. Despite the existing differences between these species, the statistical analysis did not discover significant differences in PGM profiles during the first 3 weeks of life in donkey, horse and cattle newborns. The low levels observed 10 days after birth are possibly due to a fast completion of maturational development in these species.

Introduction

The transition from fetal to neonatal state involves three phases: late gestation, parturition, and the establishment of independent homeostatic regulation following placental separation. The first hours of life are characterized by changes in respiration, circulation, glucose homeostasis and by the onset of independent oral feeding and thermoregulation. These stages are regulated by a series of fetal and placental endocrine events involving several hormones and factors (Gluckman et al. 1999). Among these, the prostaglandins (PGs) play a major role during parturition and in the final multi-system maturational processes of the newborn, but their exact pattern is poorly understood (Skinner and Challis 1985; Challis et al. 1997; Ousey 2004, 2006; Panzani et al. 2009).

Prostanoids act upon the fetus, influencing fetal wellbeing and adaptation after birth. They contribute to the development of the fetal hypothalamic-pituitary-adrenal axis (Kitterman 1987), and are involved in the maturation and function of various systems (Pace-Asciak 1978). For example, PGs operate at the cardiovascular and respiratory systems levels, regulating the tone of the ductus arteriosus and of the tracheobronchial tree (Friedman and Demers 1978; Mitchell et al. 1978; Reznichenko et al. 1993). They also contribute to kidney development, regulating perfusion and glomerular filtration rate and controlling water and electrolyte balance (Antonucci et al. 2007). PGs also play important control on hemodynamic, fluid and electrolyte homeostasis (Friedman and Demers 1980; Benzoni et al. 1981).

Both PGE and PGF significantly increased in umbilical plasma after the onset of labour because of the fetal placental unit production (Saeed et al. 2003). Human neonates born at term display high PG concentrations, with a rapid decrease after birth in the circulating levels of prostaglandin E but not of prostaglandin F (PGF) and 15-ketodihydro-PGF2α (PGM) (Mitchell et al. 1978). These high levels at birth could be related to fetal placental unit production, leading to an increase in the umbilical PG concentration compared to maternal plasma concentration (Saeed et al. 2003). In newborn foals, plasma PGM concentrations increased from 10 min to 20 and 30 min after parturition, then

consistently declined to levels <100 pmol/l at 10 days after birth, remaining low until 21 days of life (Panzani et al. 2009). The higher concentrations of $PGF_{2\alpha}$ in the days after parturition compared to adults could be explained by their role in completing organ maturation (Panzani et al. 2009).

Donkeys, horses and cows are monotocous characterized by important differences in placentation and in peripartal plasmatic changes of PGs. In mares, the birth of live horse fetuses is associated with an increase in PG concentrations in both fetal and maternal plasma during the last two hours before parturition (Barnes et al. 1978). PGs begin to increase up to 50 nmol/l during the first stage of labour, and continue to rise up to 250 nmol / l during the second stage (Barnes et al. 1978; Ousey 2004, 2006). To our knowledge, only one study has been carried out on endocrine changes during peripartum in the jenny (Veronesi et al. 2007); PGs were high during the last 10 days of pregnancy (11.7– 18.6 nmol / l) and declined on the day of parturition (1.5 nmol / l) (Veronesi et al. 2011). Finally, cows' PGM levels markedly increased at the end of pregnancy and around parturition (13.7–21.2 nmol/l), then declined, but the release continued for several postpartum days (Edqvist et al. 1978; Konigsson et al. 2001).

To date, many investigations are performed in the horse, while few studies have been conducted on the physiology of reproduction in the donkey. It should be stressed that the donkey is not just a smaller horse, but has special and unique qualities (Burnham 2002; Pugh 2002; Carluccio et al. 2008b) which justify a separate investigation. As for the donkey foals, also some aspects of the calf physiology remain to be clarified, as to our knowledge only one study has investigated PGM concentrations in early postnatal calves (Kornmatitsuk et al. 2004). Moreover, these are species with considerable gestational lengths, a healthy pregnancy ending with the birth of a live, viable newborn is an essential prerequisite for reproductive success; for this reason, it is of some interest to analyse the PGM plasma profiles in the healthy newborns of these three species.

Therefore, the aim of the current study was to evaluate plasma PGM levels in horses, donkeys and cattles during the first three weeks after birth, and to compare the PGM neonatal profile of these species.

Materials and Methods

Animals

Twenty-four Standardbred mares, belonging to a private stable in the north of Italy, were evaluated. In addition, 12 Martina Franca jennies housed in the Chiareto country estate, belonging to the Faculty of Veterinary Medicine of Teramo, Italy, were enrolled in the present study. Clinical data were recorded for each mare and jenny: age, gestational length, and foal and placenta expulsion times (Table 1). Foal expulsion time was defined as the interval between the rupture of the allantoic sac and the complete expulsion of the foal. Placental retention was defined as no expulsion of the fetal membranes within 3 h of foaling (Jeffcott 1972; Campitelli et al. 1982/83).

To assess viability of each horse and donkey newborn, the following characteristics were evaluated: the Apgar index within 10 min of birth, the presence of suck and righting reflexes, and the times to stand up (TSU) and to the first suck (TFS) (Table 2). According to the modified Apgar score index used, a score of 2, 1, or 0 was assigned regarding the heart rate and rhythm (>60 bpm and regular rhythm, irregular rhythm or <60 bpm, or absent rhythm), the respiratory rhythm (regular, irregular, or absent), the body tone (sternal / active, hypotonic, or atonic), the color of eyes and mouth's mucous membranes (pink, pale, or hyperaemic / cyanotic), and the response to nasal and ear stimulation (avoidance of stimulation, grimace / weak response, or absent response). An Apgar index ≥7 was considered normal. Physical and behavioural characteristics were also used to assess foal maturity, including birth weight, body size, hair coat, head shape, ear position, and absence of periarticular laxity (Rossdale et al. 1984; Koterba 1990; Lester 2005).

Nine Holstein-Friesian cows belonging to a farm located in northern Italy were also enrolled in this study. For each cow the following data were recorded: age, parity, gestation length, calf expulsion time (from the appearance of the allantoic sac and the complete expulsion of calf), and placental expulsion time. Placental retention was defined as no expulsion of the fetal membranes within

24 h of calving (Muller and Owens 1974). The interval between birth and sternal recumbency (TSR) (Schuijt and Taverne 1994) and TFS (Schulz et al. 1997) were used to evaluate calf viability. Birth weight, body temperature, and maturity were also recorded. A modified Apgar score was measured within 10 min of birth; all the parameters and scores were the same used for horse and donkey foals, except for the heart rate and rhythm (≥100 bpm and regular rhythm, irregular rhythm or <100 bpm, or absent rhythm). An Apgar index ≥7 was considered normal.

Blood samples

Blood samples were collected from the jugular vein into heparanized tubes from each foal and calf according to the following sampling schedule: 10, 20, and 30 min after parturition; 3, 24, and 72 h after birth; and 7, 10, 17, and 21 days after birth. After collection, samples were centrifuged at 1000 g for 20 min and the resulting plasma was stored at -20 °C until analysis.

15-Keto-dihydro-PGF_{2a} assay

The profile of $PGF_{2\alpha}$ was monitored by measuring its main initial plasma metabolite, 15-ketodihydro-$PGF_{2\alpha}$ (PGM) by RIA (Kindahl et al. 1982). This metabolite is the compound of choice for monitoring PGs, both for its stability and for its reflection of increased PG production during biological processes. The assessment of PGM is also advantageous because no special precautions are needed for sample collection. Duplicate 0.2 ml samples of unextracted plasma were assayed. Prior to addition of antibody and radioactive tracer, 0.3 ml 0.25% bovine gamma globulin in buffer were added and, for horse and donkey samples, the tubes were heat-treated for 30 min at 45 °C. If a high PGM concentration (>800 pmol / l) was measured, the analysis was repeated with 50 µl unextracted plasma and 0.45 ml 0.25% bovine gamma globulin in buffer. The antibody cross

reacted with 15-keto-$PGF_{2\alpha}$ (16%), 13,14-dihydro-$PGF_{2\alpha}$ (4%), and 15-keto dihydro-PGE_2 (1.7%). Other tested PGs cross-reacted at low levels (<0.1%). The detection limit of the assay using 0.2 ml plasma was 75 pmol / l. The intra- and inter-assay coefficients of variation were 8.5% and 14%, respectively.

Statistical analysis

For the statistical analysis within each group, a one-way ANOVA test followed by a Tukey test for multiple comparisons was used. When differences between groups were analysed, a two-way ANOVA test was performed. Differences were considered significant when $p < 0.05$.

Results

Data concerning maternal age, pregnancy length, and fetus and placental expulsion times for mares, jennies, and cows are reported in Table 1. All deliveries were spontaneous and eutocic. All newborns were mature and viable; the clinical characteristics of the horse foals, donkey foals, and calves are reported in Table 2.

Tab.1 – Clinical characteristics of mares, jennies and cows enrolled in this study. Data are presented as mean ± SD

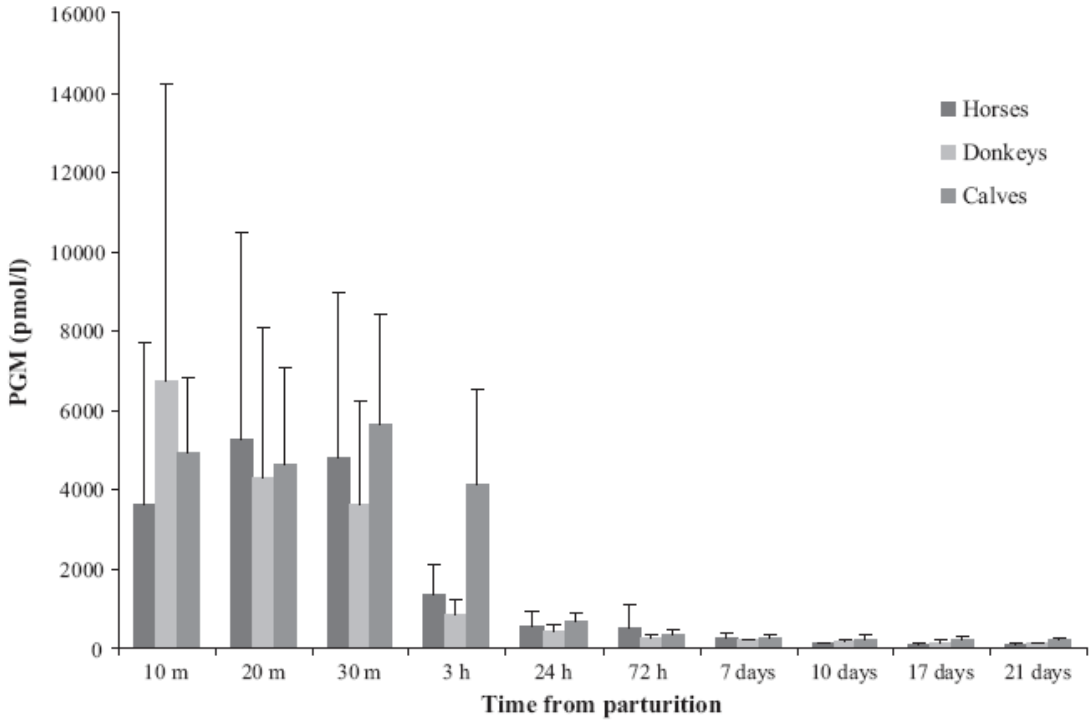
	Age (years)	Gestation length (d)	Fetus expulsion time (min)	Placenta expulsion time (min)
Mares (n = 24)	11.7 ± 4.79	337.6 ± 9.58	20.3 ± 11.23	47.7 ± 28.3
Jennies (n = 12)	9.8 ± 3.92	371 ± 7.10	16.1 ± 4.70	21.7 ± 7.97
Cows (n = 9)	4.1 ± 1.9	269.8 ± 3.3	28.1 ± 18.9	840 ± 455

Tab. 2 – Clinical characteristics of newborn horses, donkeys and calves. Data are presented as mean ± SD

	Birth weight (kg)	Apgar score	TSR (min)	TSU (min)	TFS (min)
Horses (n = 24)	45.9 ± 7.72	9.4 ± 1.05	–	62.4 ± 42.63	94.7 ± 53.01
Donkeys (n = 12)	29.4 ± 3.59	9.3 ± 0.67	–	65.1 ± 29.08	99 ± 34.37
Calves (n = 9)	37.1 ± 5.5	9 ± 1	4.3 ± 4.2	–	70 ± 15

TSU, time to stand up; TSR, time to sternal recumbence; TFS, time to first suck.

Fig. 1 – PGM profile (mean±SD) during the first 3 weeks of life in newborn horses, donkeys ans calves



The mean±SD values of PGM concentrations in the three groups at various sampling times were recorded (Table 3).

Table 3. PGM concentrations (pmol/l) during the first 3 weeks of life in newborn horses, donkeys and calves. Data are presented as mean ± SD. Different letters correspond to difference ($p < 0.05$) within each group

	10 min	20 min	30 min	3 h	24 h	72 h	7 days	10 days	17 days	21 days
Horses	$3628^{abc} \pm 4058.9$	$5258^{a} \pm 5205$	$4783^{ab} \pm 4190.8$	$1330^{cd} \pm 784.3$	$529^{cd} \pm 390.3$	$509^{d} \pm 567$	$243^{d} \pm 124.8$	$111^{cd} \pm 28.2$	$96^{cd} \pm 26.7$	$91^{cd} \pm 17.9$
Donkeys	$6743^{a} \pm 7489.5$	$4293^{ab} \pm 3800.6$	$3624^{ab} \pm 2606$	$843^{b} \pm 377.8$	$423^{b} \pm 150.7$	$258^{b} \pm 99.8$	$196^{b} \pm 10.2$	$167^{b} \pm 62.9$	$147^{b} \pm 42.3$	$115^{b} \pm 25.7$
Calves	$4918^{a} \pm 1901.6$	$4630^{a} \pm 2430.1$	$5634^{a} \pm 2771.6$	$4135^{a} \pm 2391.8$	$660^{b} \pm 230.9$	$351^{b} \pm 128.4$	$246^{b} \pm 82.6$	$220^{b} \pm 113.3$	$193^{b} \pm 94$	$193^{b} \pm 66.6$

Differences ($p<0.05$) in horse foal PGM concentrations were observed at 20 (5258±5205.0 pmol / l) and 30 (4783±4190.8 pmol / l) min compared to samples taken from later time points. Plasma levels at 10 min (3628±4058.9 pmol / l) were higher ($p<0.05$) than the samples from 72 h and 7 days. At three (1330±784.3 pmol / l) and 24 (529±390.3 pmol / l) h, PGM plasma concentrations were lower ($p<0.05$) compared to samples from 20 and 30 min. In the donkey group, higher values ($p<0.05$) were observed at 10 (6743±7489.5 pmol / l) min after birth compared to later samples. Finally, calves showed higher values ($p<0.05$) at 10 (4918±1901.6 pmol / l), 20 (4630±2430.1 pmol / l), and 30 (5634±2771.6 pmol / l) min, and 3 h (4135±2391.8 pmol / l) compared to all later time points.

No significant differences were measured at any sampling time among horse foals, donkey foals and calves; the profiles for the three species are plotted together in a common figure (Fig. 1).

Discussion

All mares, jennies and cows experienced normal pregnancies, with spontaneous, eutocic deliveries. All data regarding parturition for the mares and jennies fell within normal reported ranges (Jeffcott 1972; Campitelli et al. 1982 / 83; Carluccio et al. 2008a; Panzani et al. 2009). Similarly, data regarding calf expulsion time was in agreement with previous reports, but our measured mean placenta expulsion time was slightly longer (Table 1) (Roberts 1986).

All foals and calves were born mature, well developed and viable, as confirmed by recorded clinical data (Table 2) and by observations during the entire study period. The Apgar index

within 10 min of birth, the presence of suck and righting reflexes, TSU, TFS, and TSR, together with the other physical and behavioural characteristics considered, were all within normal reported ranges (Rossdale et al. 1984; Koterba 1990; Schuijt and Taverne 1994; Schulz et al. 1997). For this reason, all newborns were included in this study and evaluated for PGM plasma profiles.

In all groups, high PGM plasma concentrations were observed immediately after birth (Table 3), as previously described in newborn babies and horse foals (Mitchell et al. 1978; Panzani et al. 2009). These high concentrations could partially originate from the maternal blood during late gestation and parturition. Our measurements agree with previous studies concerning increased fetal placental unit production during parturition (Saeed et al. 2003). PGM levels registered in the foals are in agreement with previous data from a similar number of subjects (Panzani et al. 2009); donkey foals showed an everdecreasing trend, but no other studies are available for comparison.

PGM in calves were very high during the first 3 h of life, then significantly declined at later time points. In another study, significantly higher PGM levels were found immediately after birth compared to 1 h of life; the values obtained in this previous work overlap with our measurement at 3 h (Kornmatitsuk et al. 2004). The apparently higher PGM concentrations for a longer period in calves may be related to the different placenta or suggest the possibility of a different PG involvement in the first hour of life.

When PGM concentrations between the groups were compared, no significant differences were found, even though foals seem to exhibit a different trend than calves (Fig. 1). Perhaps the high individual variability, especially in horse and donkey foals, and the low number of animals enrolled, underlies this data. Nevertheless, considering the very higher PGM concentrations found in mares at parturition in comparison to jennies and cows (Konigsson et al. 2001; Ousey 2004; Veronesi et al. 2007) the authors would expect a similar difference also in the neonates. The finding of similar PGM values among horse foals, donkey foals and calves, suggests that PGM in the neonate only partially derives from placental transfer, reflecting for the majority the release from the neonate

itself. The relatively high PGM levels in the first days after parturition can reflect their role in completing organ maturation. However, when compared to human infants, horse, donkey, and cattle newborns already exhibit low levels 10 days after birth, possibly due to different time for completion of maturational development (Panzani et al. 2009). It should be kept in mind that these species are considered as preys, and the birth of a viable and independent neonate is an essential requirement for ensuring survival. It could be argued that both the generally lower PGM levels of these species compared to the human, and the increase of PGM values in foals between 10 and 20 min after birth may be due to this feature. Our hypothesis is that this increase results from a neonatal PGM production, and it is probably essential for the very fast maturational processes.

In conclusion, the results of this study suggest a role of high PG concentrations immediately after birth in completing organ maturation in horse and donkey foals and in calves.

Moreover, despite several studies highlighting the existing differences between these species, this study did not discover significant differences in PGM profiles during the first 3 weeks of life in donkey, horse and cattle newborns.

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All the authors contributed equally to the preparation of the manuscript.

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CHAPTER 11

Mode of Delivery is Associated with Different Haematological Profiles in the Newborn Calf

Published in:
Theriogenology
dx.doi.org/10.1016/j.theriogenology.2011.09.010

Available online at www.sciencedirect.com
SciVerse ScienceDirect
Theriogenology xx (2011) xxx
Theriogenology
www.theriojournal.com

Mode of delivery is associated with different hematological Profiles in the newborn calf

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Abstract

Several studies on babies have shown that the type of delivery can influence the hematological and immune status of the newborn. In bovine medicine, some authors reported the hematological pattern of the newborn calf, but never related it with the calving process or other perinatal factors. The purpose of the present study was to evaluate the hematological profile in newborn calves in relation to the type of delivery. A total of 41 healthy calves were enrolled; 16 Friesian calves which were born by vaginal delivery without assistance (VD), and 25 Belgian Blue calves that were born by elective Caesarean section (CS). As soon as the calves were born, a complete clinical examination was performed to verify viability and maturity. At 10 min after birth, 2 mL venous blood was collected to perform the blood gas and acid-base evaluation. Blood samples were

subsequently collected from the jugular vein within 30 min after birth, and at 1, 2, 3, 7, and 14 days of age. An automatic analyzer was used to determine hemoglobin concentration (Hb), hematocrit (Ht), and red and white blood cell counts, while differential leukocyte count was performed microscopically. Statistical analysis was applied to assess differences between the groups and within the group for all parameters between each sampling time ($P \leq 0.05$). All the calves were born alive, viable, and mature. There were no acidotic calves, but statistical analysis revealed many differences, as higher pH, base excess (BE) ($P \leq 0.05$), PO_2 ($P \leq 0.001$), and sO_2 ($P \leq 0.0001$) in the VD group. Levels of hemoglobin concentration, hematocrit, and red blood cell number were constantly higher in CS calves ($P \leq 0.001$). In comparison with the VD calves, white blood cell and neutrophil absolute number were higher at birth and at 14 days of age in the CS group ($P \leq 0.001$ and $P \leq 0.05$). The mode of delivery, therefore, seems to have an influence on the oxygenation levels and on the hematological and nonspecific immunity profile of the newborn calf.

Keywords: Calf; Delivery; Hematology; Newborn

Introduction

The birth process can have profound effects on the immune system of the newborn, who is rapidly challenged by environmental microflora and is depending on innate immunity onset. Studies on newborn babies[1–4] demonstrated the early capacity of the neonate to mount a powerful inflammatory response already during the process of labor. This increase and redistribution of immune cells at birth could be an adaptive response designed to enhance the vigilance at the major defense barriers of the body [5]. There are many data suggesting that the method of delivery is able to modify the immunologic balance in the newborn infant [6]. Labor process in fact normally leads to leukocytosis, that is selective for neutrophils, monocytes, and natural

killer cells [1,2,7,8], but differences were found according to the type of delivery. Several studies [5,9,10] compared hematological values among three groups of newborn babies: born vaginally, by elective Cesarean section, or by Cesarean section after labor. The results indicated that the mode of delivery influences not only the white blood cell count, but also hemoglobin (Hb) and hematocrit (Ht) levels, red blood cell distribution width (RDW), platelet count, and the number of nucleated red blood cells [10]. Babies who were born by Cesarean section after labor had the highest Ht and Hb levels [10], while babies born by elective Cesarean section had the lowest Ht and Hb levels and the lowest leukocyte and neutrophil counts [6,10]. Oka et al. [11] demonstrated that a transient increase of blood Hb concentration in the human newborn can be induced also by hypoxic conditions, as a balancing reaction through spleen contraction. Ghosh et al. [12] found higher Hb level and red blood cell count in newborn babies with evidence of perinatal asphyxia compared with healthy babies. Because stress and temporary hypoxia in babies are more frequent and prolonged in vaginal delivery compared with Cesarean section, and even more in Cesarean section after labor, it was speculated that different stress and oxygenation levels could be the reason for the differences in the hematological pattern [7]. Elevated white blood cell counts in neonates at term have also been associated with both metabolic and respiratory acidosis [10]. Contrary to what happens in the human neonates, neonatal calves born by Cesarean section seem to be more predisposed to develop a respiratory distress syndrome, and therefore a respiratory acidosis, during the first hours of their life [13]. Moreover, some authors [13,14] found that double- muscled calves, which are usually born by Cesarean section, present a lower blood oxygen equilibrium curve (OEC) compared with dairy calves, and this probably contributes to the higher sensitivity of doublemuscled calves to hypoxia. Because this difference between breeds disappears after the first month of life [14], it could be due to the different type of birth. Some studies investigated the hematological profile of healthy newborn calves [15–17], but never related it with the birth process or other perinatal factors. Our hypothesis was that the type of delivery, associated with different oxygenation conditions at birth, could have an impact on the hematological profile and nonspecific

immune response of the calf. Specific hematological reference ranges according both to the age of the calf and the type of delivery, could promote the ability of clinicians for more accurate interpretations of disorders.

Therefore, the aim of the present study was to investigate the hematological profile of newborn dairy calves born by vaginal delivery without assistance and of newborn double-muscled calves born by Cesarean section during the first 14 days of age, and to verify any relation with oxygenation conditions.

Materials and methods

Farms

The study was conducted between June 2009 and October 2010 in two different European countries, Italy and Belgium. A total number of 42 newborn calves were studied after two different types of delivery: vaginal delivery without assistance (N=17) and elective Cesarean section (N=25). Calves born by vaginal delivery (VD) were enrolled from a Holstein dairy farm located in northern Italy (45°28=N and 9°41=E). The double-muscled Belgian Blue calves born by Cesarean section (CS) belonged to the Institute for Agricultural and Fisheries Research (ILVO), a Scientific Institute property of the Flemish Government's Agriculture and Fisheries Policy Area and situated in the west part of Belgium (51°00=N and 03°44=E).

The experiment was approved by two ethical committees belonging to the University of Milan and to the Institute for Agricultural and Fisheries Research institute.

Animals

Calvings were watched over closely in order to check any abnormality or difficulty; parturitions occurring preterm (before 210 days of gestations) [18] or

requiring manual or pharmacologic assistance were excluded. According to these criteria, 17 Holstein-Friesian calves (HF) were enrolled in the VD group.
Twenty-five double-muscled Belgian Blue (BB) calves were enrolled in the CS group. The Cesarean sections were considered as "elective", because they were performed at the initial stage of parturition (a rectal temperature drop during the last 24 h and an active phase of cervical dilatation) with no attempt to deliver the calf per vias naturales. The Cesarean sections were performed by veterinarians belonging to the Department of Reproduction, Obstetrics and Herd Health of the Veterinary Faculty of Ghent University, as described by Kolkman et al. [19].

For both groups of parturition, placental expulsion time was recorded, and placental retention was defined as no expulsion of the fetal membranes within 24 h of calving [20].

For all 42 newborn calves, the following data were recorded immediately after birth: sex, weight, rectal temperature, maturity (body size and weight, head shape, coat characteristics), and evidence of malformations. The interval between birth and sternal recumbency (TSR) [21] and the interval between birth and stand up (TSU) [22] were used to evaluate calf viability. For the same reason, a modified Apgar score [23] was measured within 10 min of birth; scores of 2, 1, or 0 were assigned in relation to the heart rate and rhythm (≥100 beats per min and regular rhythm, irregular rhythm, or <100 beats per min, or absent), the respiratory rate and rhythm (≥30 breaths per min and regular rhythm, irregular rhythm, or <30 breaths per min, or absent), the body tone (sternal/active, hypotonic, or atonic), the color of the mucous membranes of eyes and mouth (pink, pale, or hyperemic/cyanotic), and to the response to nasal and ear stimulation (avoidance of stimulation, grimace/weak response, or absent response). An Apgar index ≥7 was considered as normal.

Immediately after birth, each calf was transferred to a single box with straw until the age of 14 days, and fed with colostrum obtained from the colostrum bank of the herd. From the third day after birth, calves were fed twice a day with an amount of milk equal to 10% of their body weight; the VD calves received pasteurized herd milk, while for the CS calves a milk substitute was reconstituted at 125 g powder/L.

Blood samples

At 10 min after birth, 2 mL of blood was collected from the jugular vein by single-use needles with holders and used to perform the blood gas and acid-base balance evaluation. An advanced, portable clinical analyzer (i-STAT System; Abbott Laboratories, North Chicago, IL, USA) together with specific cartridges (CG8; Abbott Laboratories) was used to immediately determine the following parameters: glucose, sodium, potassium, ionized calcium, pH, TCO_2, PCO_2, PO_2, sO_2, HCO_3, and base excess (BE).

Additional blood samples were then taken from the jugular vein into K3-EDTA glass tubes (VT-100STK, Venoject, Terumo, Italia srl, Rome, Italy) by single-use needles with holders. Sampling was performed approximately 20 to 30 min from birth (Day 0), and at 1, 2, 3, 7, and 14 days of age. Before each sampling, all the calves were subjected to a complete clinical examination, in order to identify any abnormality that could influence the hematologic profile. All samples were immediately placed at 4 °C and transferred to the Clinical Pathology Laboratory of the Large Animal Teaching Hospital of the University of Milan (Lodi, Italy) or to the Laboratorium Mediwaf (Ghent, Belgium). Anticoagulated blood was used for a complete blood cell count (CBC) using an automated veterinary hematology analyzer (ADVIA 120 with multispecies software, Siemens Healthcare Diagnostics, Deerfield, IL, USA) in both laboratories. Hematologic analyses included: hemoglobin concentration (Hb), hematocrit (Ht), and red blood cell (RBC) and white blood cell (WBC) counts. Stained blood smears were examined microscopically to perform a differential leukocyte count, by counting 100 nucleated cells.

The first blood sample (approximately 20 to 30 min after birth) was collected before colostrum uptake; because calves were immediately separated from their dams, administration of colostrum was performed artificially through bottles. All the subsequent blood samples were collected at different day times, but at least 1 h apart from milk administration.

Statistical analysis

Mean values ± SD of each variable were calculated, and data were analyzed by an Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA) using Analyze- it software (Analyze-it v2.21 Software, Ltd., Leeds, UK). The Mann-Whitney test was used to assess differences between the two groups in each sampling time for each parameter; Friedman test was used to compare the sample at birth with the following samples within each group of calves for each parameter. Differences were considered as significant if $P \leq 0.05$.

Results

All pregnancies were uncomplicated, and all deliveries were at term; thus all calves could be included in the study. Calvings took place at any time of day and night, and there were no cases of placental retention. The vaginally-delivered Holstein-Friesian calves were nine males and eight females, while the Cesarean section BB calves were 13 males and 12 females. All calves were born alive, viable, mature, and well developed; data regarding mean neonatal weight, rectal temperature, TSR, TSU, and Apgar score in the two groups are reported in Table 1. The clinical follow-up allowed the detection of some transient pathologic conditions, mainly consisting of diarrhea and respiratory problems, but these diseases never required medical treatments, except for one male VD calf that died at 10 days of age and was excluded from the study. Therefore, the final number of calves enrolled in the VD group was 16 instead of 17. Data regarding the acid-base evaluation in the two groups are reported in Table 2. The comparison between the acid-base profile of the two groups showed some significant differences for Na, PCO_2, and sO_2 ($P \leq 0.0001$), for PO_2 ($P \leq 0.001$), and for K, pH, and base excess ($P \leq 0.05$) (Table 2).

Mean Hb concentrations, mean Ht values, mean RBC and WBC counts, mean neutrophil (PMN) and lymphocyte (LYMPH) counts in the two groups at the different sampling times are reported in Figures 1–6.

Hemoglobin decreased from birth to Day 1, Day 2, and Day 3 in both groups ($P \leq 0.001$ in VD, $P \leq 0.0001$ in CS), and from birth to Day 7 and 14 ($P \leq 0.001$) only in the CS group. The hematocrit was constantly lower in all samples compared with the sample at birth in both groups with different significances. Specifically, in the VD group the sample at birth showed higher Ht than the sample at Day 1 ($P \leq 0.001$), Day 2 ($P \leq 0.05$), Day 3 ($P \leq 0.001$), Day 7 ($P \leq 0.05$), and Day 14 ($P \leq 0.0001$); in the CS group the sample at birth showed always higher Ht than the other samples with $P \leq 0.0001$. A significant decrease was found in the RBC count of the VD group between birth and Day 1 ($P \leq 0.001$), Day 2 and Day 3 ($P \leq 0.01$); a decrease was found also in the CS groupbetween the sample at birth and Day 1, Day 2, and Day 3 ($P \leq 0.001$). Changes in WBC counts were registered only in the CS calves; an increase was evident between birth and Day 1 ($P \leq 0.05$), while WBC counts on Day 2, Day 3, Day 7, and Day 14 were lower compared with the sample at birth ($P \leq 0.001$). Neutrophil counts in the VD calves showed a decrease only on Day 14 compared with birth ($P \leq 0.01$). The CS group presented an increase in PMN between birth and Day 1 ($P \leq 0.001$), and a decrease between birth and Day 2, Day 3, Day 7, and Day 14 ($P \leq 0.0001$). Lymphocyte counts in the VD group increased only between birth and Day 14 ($P \leq 0.01$), while in the CS group there was an increase from birth to Day 1 ($P \leq 0.01$), Day 3 ($P \leq 0.01$), and Day 7 and Day 14 ($P \leq 0.0001$).

The comparison between the two groups evidenced constantly higher Hb and Ht levels in the CS, with different significances (Figs. 1 and 2). Also RBC count was higher in the CS calves, with different significances at birth and at Day 1, 2, and 3 ($P \leq 0.001$) and at Day 7 and 14 ($P \leq 0.01$) (Fig. 3). Differences in the WBC count were found at birth ($P \leq 0.001$) and at 14 days of age ($P \leq 0.01$), with higher levels in the CS neonates (Fig. 4). These differences were due to higher PMN counts at birth and at Day 14 ($P \leq 0.001$ and $P \leq 0.01$, respectively; Fig. 5), while no significant differences were registered in the lymphocyte count between the two groups (Fig. 6).

Tab. 1 – Mean clinical data of the vaginally delivered newborn calves (VD) and the Caesarean section newborn calves (CS)

Type of delivery	Weight (kg)	Temperature (°C)
VD (N = 16)	34 ± 4.4	38.9 ± 0.2
CS (N = 25)	53 ± 7.8	39.1 ± 0.3

Type of delivery	TSR (min)	TSU (min)	Apgar score
VD (N = 16)	5.6 ± 3.8	39 ± 17.2	8 ± 1.8
CS (N = 25)	2.9 ± 1.5	219 ± 118	8 ± 1.2

TSR, time for sternal recumbency; TSU, interval between birth and stand up.

Tab. 1 – Mean venous blood gas and acid-base parameters in the newborn calves of the two groups

	VD (N = 16)	CS (N = 25)
Glu (mg/dL)	55 ± 18.3	41 ± 10.7
Na (mEq/L)	137 ± 1.1*	141 ± 1.7
K (mEq/L)	4.8 ± 0.3†	4.3 ± 0.4
iCa (mmol/L)	1.4 ± 0.1	1.4 ± 0.1
pH	7.40 ± 0.06†	7.32 ± 0.05
TCO_2 (mmol/L)	30 ± 2.1	30 ± 2.1
PCO_2 (mm Hg)	46 ± 4.2*	56 ± 5.2
PO_2 (mm Hg)	104 ± 40.2‡	29 ± 4.1
sO_2 (%)	93 ± 14.2*	48 ± 9.9
HCO_{3-} (mmol/L)	28.1 ± 2.2	28.5 ± 2.2
BE (mmol/L)	4 ± 1.1†	1 ± 2.6

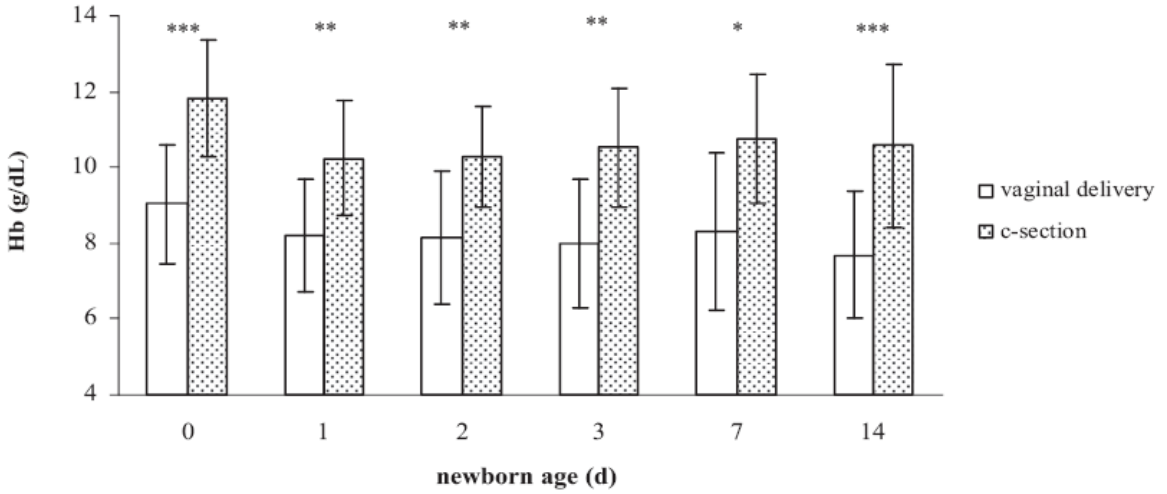
BE, base excess; CS, Cesarean section newborn calves; VD, vaginally delivered newborn calves.

* $P < 0.0001$.

† $P \leq 0.05$.

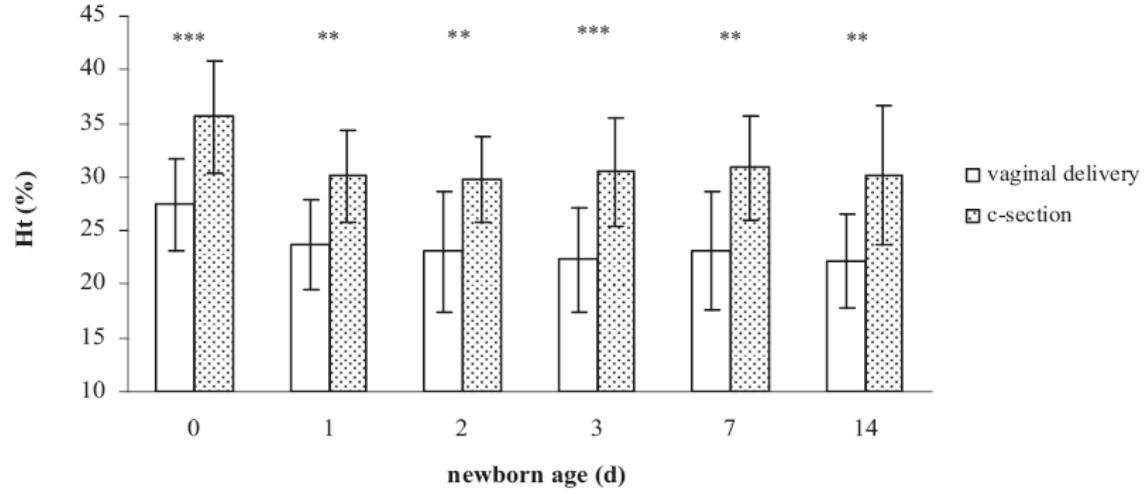
‡ $P < 0.001$.

Fig. 1 – Mean (±SD) levels of hemoglobin concentration (Hb) in the newborn calves during the first 14 days of age



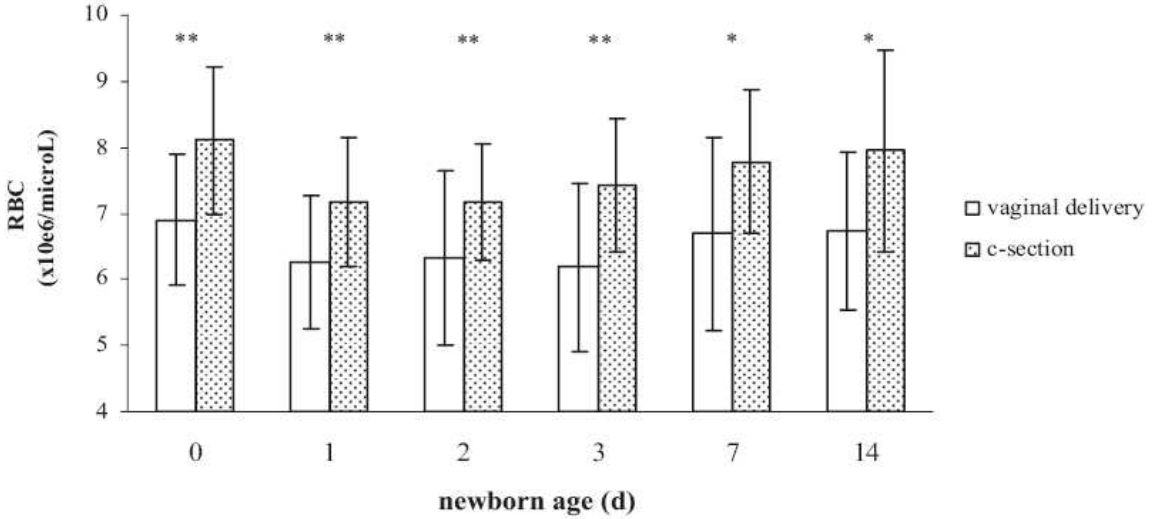
*= p≤0.05; **=p≤0.001; ***=p≤0.0001

Fig. 2 – Mean (±SD) levels of hematocrit (Ht) in the newborn calves during the first 14 days of age



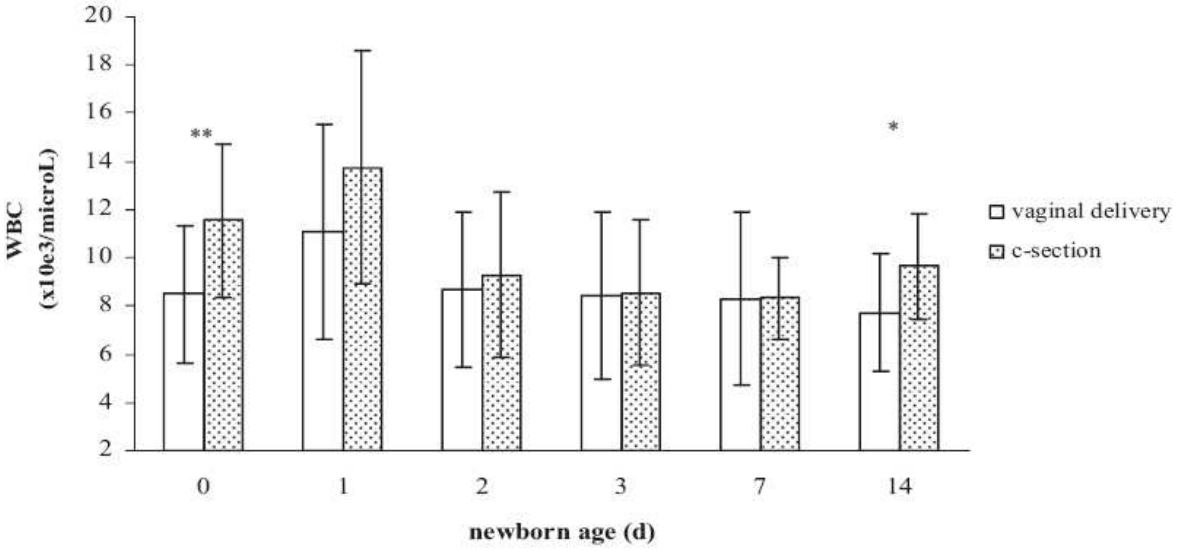
**= p≤0.001; ***=p≤0.0001

Fig. 3 – Mean (±SD) red blood cells (RBC) in the newborn calves during the first 14 days of age



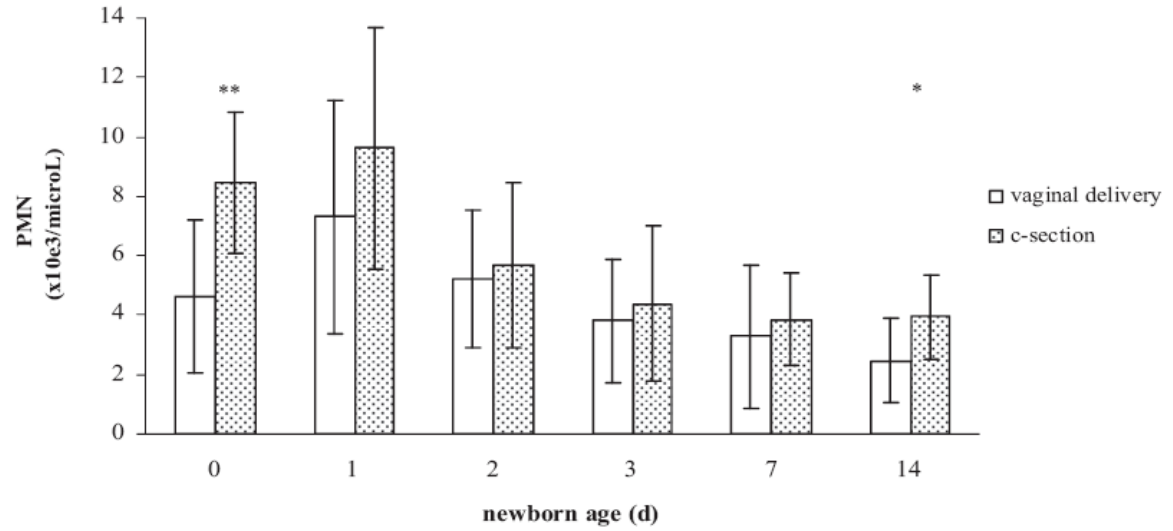
*= p≤0.05; ***=p≤0.001

Fig. 4 – Mean (±SD) white blood cells (WBC) in the newborn calves during the first 14 days of age



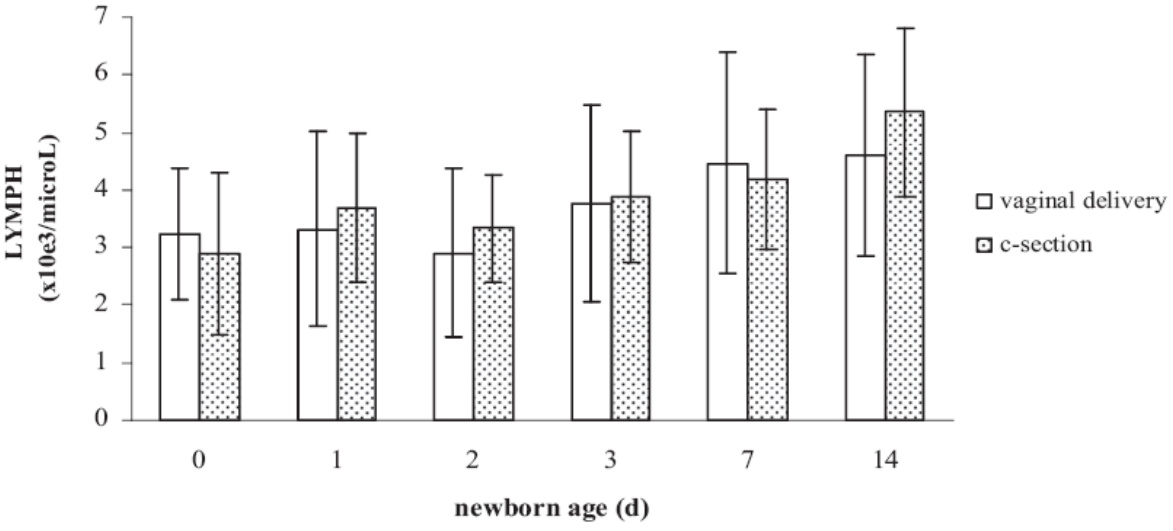
*= p≤0.05; **= p≤0.001

Fig. 5 – Mean (±SD) neutrophil (PMN) count in the newborn calves during the first 14 days of age



*= p≤0.05; **= p≤0.001

Fig. 6 – Mean (±SD) lymphocytes (LYMPH) count in the newborn calves during the first 14 days of age



Discussion

The most relevant finding of the present study is the detection of many significant differences in the hematological patterns between newborn calves from vaginal delivery and newborn calves from Cesarean section.

Because data from literature speculated that different oxygenation levels could be the reason for hematological differences in newborn babies [7], this study considered not only the different type of delivery but also blood gas and acid-base evaluation, in order to identify any case of respiratory distress.

Neonatal asphyxia, characterized by hypercapnia and hypoxemia, is a life-threatening condition in newborn animals, and usually leads to acidosis, due to an inadequate oxygen uptake by the lungs and thus inadequate oxygenation of body tissue cells [24]. The degree of hypoxia and hypercapnia, as well as the resulting respiratory and metabolic acidosis, depends not only on the length of the period between the complete separation from maternal circulation and the beginning of independent respiration, but also on the disturbance of gas exchange during parturition [25]. Therefore, the type of birth has a great influence on blood gas and acid-base status of the newborn [25].

In the present study, blood gas evaluation evidenced a very high PO_2 and sO_2 in the VD, which is not in agreement with previous data [26], while mean PCO_2, PO_2 and sO_2 of the CS do agree with previous studies [24,25,27,28]. Blood sampling and gas analysis were always performed in double by the same operator through the same portable analyzer, making procedural mistakes unlikely. These data suggest different oxygenation conditions between the two groups of calves, possibly due to the difference in breed or in the type of delivery. Kornmatitsuk et al. [28] also found a mean sO_2 at 10 min after birth of approximately 95% in healthy calves and approximately 70% in weak calves from difficult parturition, which could suggest a strong influence of parturition on newborn oxygenation. No systematic studies have been performed so far to compare these parameters in neonatal calves from different breeds, but some characteristics were identified in newborn BB calves. Two Belgian studies [29,30] investigated the composition of pulmonary surfactant and its physical characteristics in BB calves, and

detected some differences with Holstein-Friesian calves. The difference between breeds could be responsible for these results; anyway, it must be mentioned that, also in those studies, all the BB calves were born by Cesarean section. Moreover, other differences recorded between the two breeds by other authors progressively disappear after the first month of life [14]; therefore, the breed factor could be less decisive than the type of delivery. Anyway, no firm conclusions are allowed about this point, and further investigations are required.

According to our results, the Cesarean section can be correlated with greater chances of respiratory distress in calves. A blood pH of 7.2 is usually considered the cutoff point to differentiate between acidotic and normal calves [25,31]; in our study, there were no calves with acidosis and the acid-base profiles of the two groups were in agreement with other previous studies on newborn calves [24,25,31–33]. Although slight hypoxia and hypercapnia were detected, calves of the CS group did not develop a respiratory or metabolic acidosis.

All the hematological parameters examined fell within the reference intervals reported by other authors [15–17] but some differences between groups could be highlighted; presumably, the different blood oxygenation level represents an explanation of this fact. The higher WBC and PMN counts at birth in the CS calves could be explained by the hypercapnia and hypoxia conditions, confirming data from newborn babies [6,7,10]. Because the stress of labor is higher in presence of neonatal disorders (i.e., respiratory distress syndrome) in newborn babies, and because it has been shown to depress neutrophil functions [6], the higher neutrophil count could represent a counteracting response of the immune system to make the inflammatory reaction more efficient. Stress, in fact, stimulates hypothalamic-pituitary-adrenal (HPA)-axis activation, inducing a cortisol release [5]. Cortisol and adrenal steroids in general are able to increase the demargination of neutrophils, and to prolong their half-life in circulation [5]. Therefore, the redistribution of neutrophils may be the result of an interaction between the immune and the neuroendocrine system, and the mode of delivery can deeply modify this interaction.

The hypoxic status of the CS calves could be due to the absence of a physical compression of the fetal thorax and of a temporary suspension by the hind legs:

both these events normally occur during spontaneous delivery thanks to the passage through the vagina, having a positive effect on development and expansion of neonatal lungs, inducing gas exchanges in a short period of time, and contributing to the elimination of fetal fluid from the upper airways [33]. The lower oxygenation of CS calves may also explain the higher RBC count found at all the sampling times when compared with the VD. Hypoxia in fact, leads to an increased sympathetic activity, causing spleen contraction and resulting in increased blood Hb, Ht, and RBC count [11,12]. It can be speculated that the lower delivery of oxygen from Hb in the double-muscled calves is balanced by a higher Hb concentration, and higher Ht and RBC count, which result from a sympathetic reaction to hypoxia [14].

It must be kept in mind that all the 41 calves were viable at birth, because mean TSR and Apgar score were in agreement with the normal ranges reported for newborn calves [22]. Therefore, no clinically evident signs of distress have been detected, and all the calves were considered as healthy. The TSU in CS was the only parameter not comprised in the range reported for calf viability according to which a calf should stand within 1 h after birth [22]. The very high mean weight of these subjects (53±7.8 kg) may represent the reason for the prolonged time to stand. The detection of hematological differences in absence of clinical signs of disease or distress is relevant, because it allows the clinician to more carefully consider some tricky situations. A practical consequence of the hematological differences evidenced by the present study is that perinatal care of newborn calves from Cesarean section must be more intense, because they can face more stress and respiratory difficulties, even if not clinically evident. A positive implication of this stressful condition is the natural enhancing of the immune system, with higher levels of WBC and, specifically, of neutrophils; possible infections could be faced by these calves thanks to specific balancing and adaptive mechanisms already present at birth. This is particularly important because the impact of delivery is much longer than expected [6], and it can have negative consequences on subsequent growth and health.

In conclusion, this study evidenced many differences in the hematological profile of newborn calves from different breeds and different types of delivery, probably related to different degrees of respiratory distress. Even though all the parameters were included in the previously reported ranges for newborn calves, differences in hematological parameters should be kept in mind for a more effective application of neonatal care procedures.

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CHAPTER 12

Preliminary data about paraoxonase activity (pon1) in serum from newborn calves: age related variation and comparison between healthy and sick animals

Preliminary Data About Paraoxonase Activity (PON1) in Serum From Newborn Calves: Age Related Variation And Comparison Between Healthy And Sick Animals

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Introduction

Paraoxonase (PON1) belongs to the paraoxonases family together with PON2 and PON3. PON1 is the most investigated member and the better understood. It is an ester hydrolase expressed in the liver and excreted in blood associated with high density lipoproteins (HDL) (James & Deakin, 2004). It is characterized by several different properties. The ability to hydrolyze the substrate of the organophosphate insecticides, the paraoxon, is responsible for the enzyme name and it is also used as a marker for the enzyme activity (van Himbergen *et al*, 2006). PON1 also shows esterase activity against both aromatic and cyclic esters (Sorenson *et al*, 1995, Khersonsky & Tawfik, 2005). In human medicine the biological properties of PON1 have been investigated. Specifically, the ability of the enzyme to protect low- and high-density lipoproteins (LDL and HDL) from oxidant agents has been recognized (Jaouad *et al*, 2003). The binding of PON1 with lipoproteins and its role in protecting LDL and HDL from oxidation have been demonstrated to prevent atherogenesis (Shih et al., 2000; Tward et al., 2002). During the acute phase response, besides the quantitative and structural changes of the acute phase proteins, also alterations of lipoproteins have been described (Khovidhunkit *et al*, 2000; Cabana *et al*, 2003). Specifically, HDL seems to play a crucial role in the innate immune system. During infection and inflammation a reduction in level of plasma proteins (cholesterol acyltransferase, cholesterol ester transfer protein, phospholipids

transfer protein) involved in the HDL-mediated reverse cholesterol transport and in inhibition of plasma lipid oxidation is recorded (Navab *et al*, 2011). In particular the decrease of the hepatic lipase apolipoprotein A-I and of the paraoxonase is relevant because of their antioxidant role during the atherogenetic process (Van Lenten *et al*, 2006). Moreover, the lipid composition of the acute phase HDL is altered during inflammation. Specifically, the huge increase in serum amyloid A is responsible for the displacement of the apolipoprotein A-I particle, which contains paraoxonase, from HDL, thus reducing the HDL anti-inflammatory properties (Hyka et al., 2001).

In human medicine, during the last decade, paraoxonase was mainly investigated from one hand due to its involvement during cardiovascular diseases, specifically concerning the atherogenesis as a model of chronic inflammation (Mackness *et al*, 2006; Camps *et al*, 2009). From the other hand it was described a PON1 decreased activity during sepsis due to a lower expression in liver and to its inhibition by the nitric oxide metabolites produced during inflammation, thus high lightening the role of PON1 as a fast negative APP (Feingold *et al*, 1998; Novak *et al*, 2010).

In veterinary medicine, by contrast, there are very few reports about PON1, mainly focused on its changes in the transition dairy cows, where paraoxonase activity was found to be negatively and positively correlated with the other positive and negative acute phase proteins, respectively (Trevisi *et al*, 2009). Because of its hepatic expression it was also used as a marker of liver function (Bionaz *et al*, 2007). Validation studies assessing the reliability of PON1 measurement in bovine were never performed and no information is currently available concerning the possible role of PON1 activity as a marker of inflammation in newborn calves. The aims of the present study were to investigate the activity of PON1 in newborn calves. We also investigated the possible age-related variation of paraoxonase activity because, as previously reported, most of the APP are influenced by the age (Kanakoudi *et al*, 1995; Brunn *et al*, 1998). Last, we investigated the possible usefulness of paraoxonase by comparing results obtained from healthy and sick newborn calves. A preliminary method validation step was also performed.

Materials and methods

Serum paraoxonase activity

Serum PON1 activity was spectrophotometrically measured using an automated analyzer (Cobas Mira Roche, Basel, Switzerland), by adapting to the instrument an enzymatic method already reported in bovine and in other species (Eckerson *et al*, 1983; Ferrè *et al*, 2002; Bionaz *et al*, 2007). The analytical principle of the method is the following: the paraoxon substrate (Paraoxon-ethyl, 90% Sigma–Aldrich) was used as well as the other required buffers. Specifically, the reaction buffer Reagent was prepared using glycine buffer (0.05 mM, pH 10.5) containing 1 mM of paraoxon-methyl and 1 mM of CaCl2. The enzymatic reaction incubate at 37°C 8 μL of samples were incubated at 37°C, with 89 μL of distilled water, and 100 μl of reaction buffer. The rate of hydrolysis of paraoxon to p-nitrophenol was measured by monitoring the increase in absorbance at 504 nm using molar extinction coefficient of 18.050 L• mol-1 • cm-1 as suggested by Feingold and others (1998). The unit of PON activity expressed as U/mL is defined as 1 nmol of p-nitrophenol formed per minute under the assay conditions.

Using the same instrument, serum total protein concentration was also measured in each sample by the biuret method (Roche).

Method validation

The method validation protocol included the assessment of the precision and accuracy. In particular the following tests were performed:

Intra-assay precision - The evaluation of the intra-assay imprecision was performed using three different pooled sera with low, medium and high paraoxonase activity (as assessed on a preliminary run of test on sera randomly collected from adult cows), which were measured 20 fold in the same run.

Inter-assay precision - The inter assay precision was assessed by 1 measurement every day for 20 consecutive working days of each pooled sera described above aliquoted and frozen at -20°C.

Accuracy - in the absence of a calibrator or of the purified molecule, the accuracy of the method was evaluated by two indirect recovery assays:

1) *Accuracy by linearity under dilution (LUD)* - The pooled serum with high PON1 activity was diluted with different proportions of demineralized water to obtain samples containing 100% to 10% of the analyte. Each sample was analyzed in triplicate and the correspondence between expected and obtained values was assessed with least square linear regression. Demineralized water alone (without any bovine serum) was also analyzed in triplicate to determine the lower limit of detection (LLOD)

2) *Accuracy by spiking–recovery test (SRT)*- the pooled serum with low PON1 activity was added with increasing volumes of the pooled serum with high PON 1 activity. Samples obtained after mixing the pooled sera are described above were then analyzed in triplicate and the correspondence between expected and obtained values was assessed with least square linear regression.

Age related variation of PON1 activity

All the analyses were performed on sera collected from clinically healthy Holstein adult and young cows (see below for details) and from Holstein calves. Specifically, samplings were performed as follows:

- Nine calves were sampled several folds during the first day of life (10, 20 and 30 minutes after birth – 3, 6, 12 and 24 hours after birth) because most of complications of calving occur in day 1 (Mee, 2008). Then, samplings in this

group were repeated daily during the first week of life and every 3 or 4 days until day 21.

- Seven calves aged 28 to 120 days were also sampled once to define the distribution of values in young weaned calves.
- 45 adult female cows (age >2 years): not lactating, not pregnant were also sampled to assess the possible difference between newborn-young and adult calves.

PON1 activity in sick calves

Forty-five clinically healthy calves and 17 calves of different ages affected by inflammatory conditions were included in order to compare results obtained in sick animals to those of the age-matched controls.

Specifically the following groups were compared to each others:

- Calves aged less than 7 days: PON1 activity recorded in 5 calves affected by neonatal diarrhoea was compared with results obtained in 20 clinically healthy age-matched calves
- Calves aged 10 days: PON1 activity recorded in 4 calves affected by diseases (diarrohea/respiratory disease) was compared with results obtained in 10 clinically healthy age-matched calves
- Calves aged 1 to 3 months: PON1 activity recorded in 8 calves mainly affected by respiratory diseases was compared with results obtained in 15 clinically healthy age-matched calves.

Statistical analyses

For each of the pooled sera employed in precision and accuracy testing, mean value and standard deviation (SD) regarding PON1 activity were calculated using an Excel spreadsheet (Microsoft Corp, Redmond, WA) with Analyse-it software (Analyse-it Software Ltd, Leeds, UK). The same software was used to perform all the statistical analyses as follows:

- the coefficient of variation recorded in precision testing was calculated using the formula (CV = SD/mean x 100)
- to evaluate the accuracy of measurement in both LUD and SR tests, the correspondence between expected and obtained values was assessed with least square linear regression;
- the PON1 activity recorded in the different time-samplings of newborn calves were used to calculate mean, SD, median and min-max ranges. Results obtained at the different time-samplings were compared to each other using a non parametric t-test for paired data (Wilcoxon test);
- the PON1 activity recorded in newborn, in young and in adult bovine were compared to each other using a non parametric ANOVA test for independent values (Kruskall Wallis) followed by a Bonferroni test;
- For each age range, the PON1 activity recorded in healthy and in sick calves were compared to each other using a non parametric t-test for independent values (U Mann Whitney).

Results

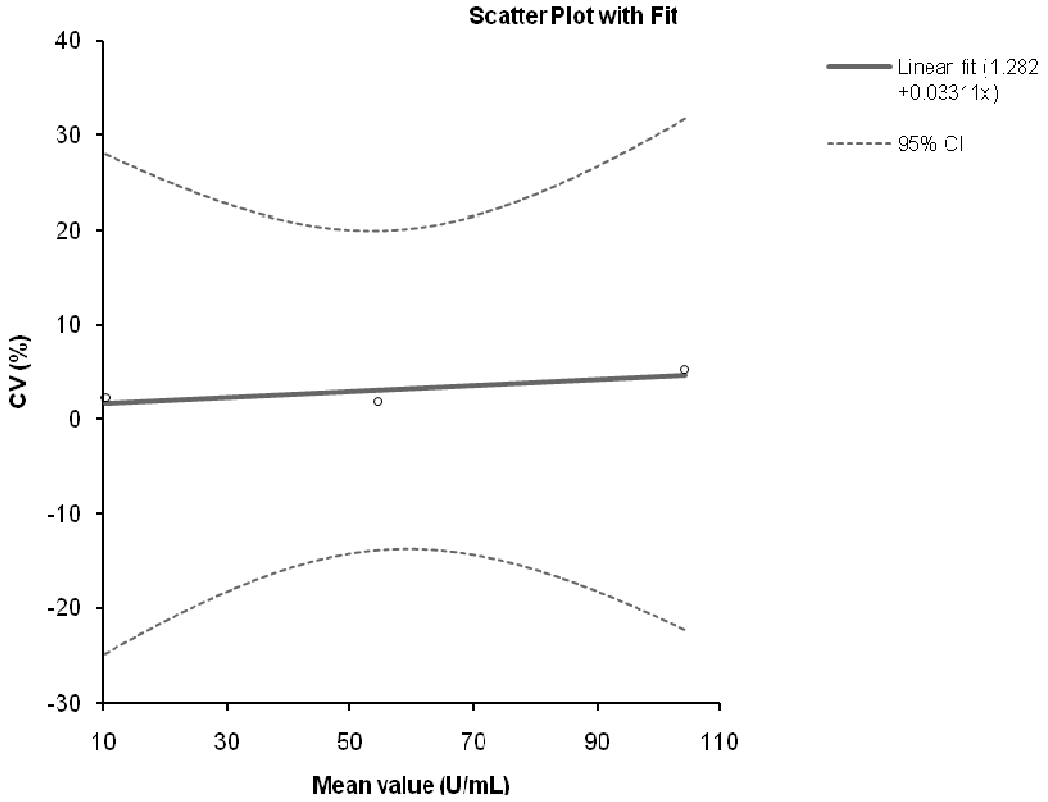
Method validation

The intra-assay precision results showed a very low coefficient of variation (CV) (*table 1, figure 1*) irrespective of the level of paraoxonase activity, thus reflecting a good repeatability of the method. Similar results were obtained also during the assessment of the inter-assay precision: very low CVs without any significant differences among different PON1 activity levels were recorded (*table 2, figure 2*), thus reflecting a good reproducibility of the method.

Tab. 1 – Intra-assay precision

	Low	Mid	High
Mean value (U/mL)	10.4	54.6	104.2
SD (U/dL)	0.2	1.0	5.5
CV (%)	2.3	1.9	5.3

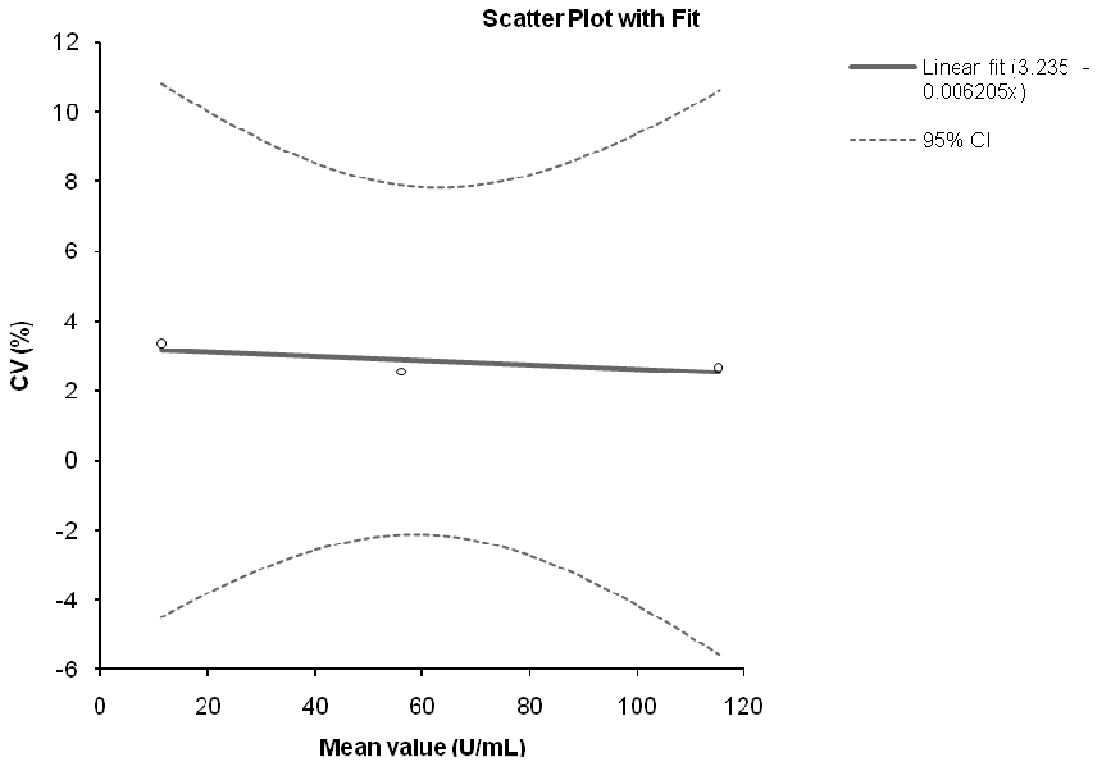
Fig. 1 – Intra-assay precision



Tab. 2 – Inter-assay precision

	Low	Mid	High
Mean value (U/mL)	11.4	55.9	115.2
SD (U/dL)	0.4	1.4	3.1
CV (%)	3.4	2.5	3.7

Fig. 2 – Inter-assay precision



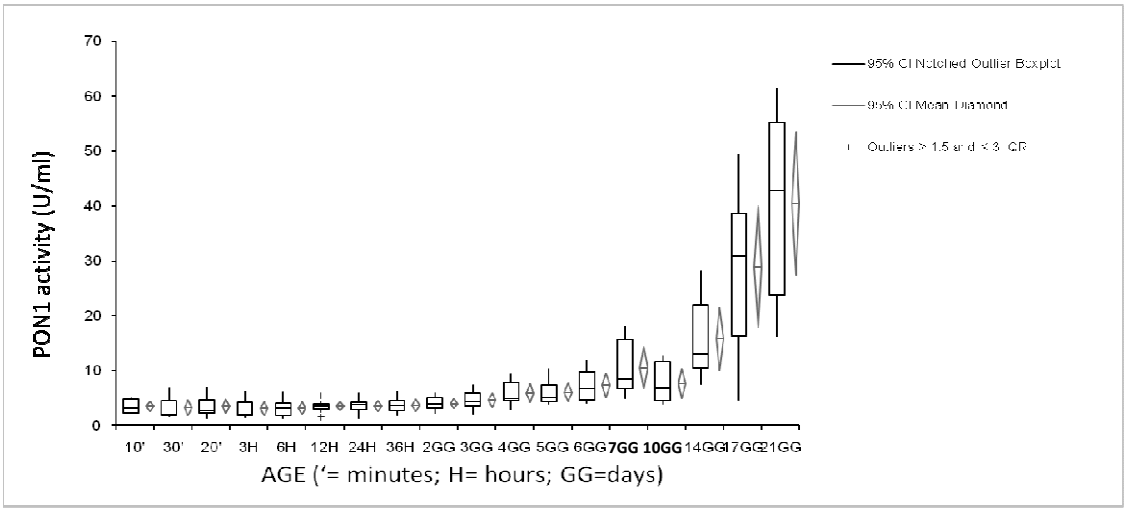
Age-related variation

Concerning the influence of age on paraoxonase activity, results showed that in newborn calves, from birth to day 21 there was an increase proportional to the age of the calves (*figure 3*). This trend was evident even if not every sample was significantly higher than the previous one. Specifically, significant differences were recorded between samples from 3 day old calves compared to the first sample ($p \leq 0.001$) Four day old calves showed higher PON1 activity than 3 day old calves and so on for the showed samplings ($p \leq 0.001$) (*table 3*). Moreover calves aged 28 to 120 days did not show different PON1 activity compared to those of 21 days of age. In contrast, adult cows had the highest PON1 activity compared to all the other groups ($p \leq 0.001$) (*table 4, figure 4*).

Tab. 3 – Age-related variations

	10min	**3 d**	**4 d**	**5 d**	**6 d**	**7 d**	**10 d**	**14 d**	**17 d**	**21 d**
Mean ± SD	3.5 ±1.2	4.6 ±1.7	5.9 ±2.6	6.0 ±2.2	7.3 ±2.9	7.6 ±3.5	10.5 ±5.0	15.7 ±7.5	28.9 ±14.2	40.4 ±17.0
Min-Max	2.3-5.1	2.1-7.4	2.8-9.4	3.9-10.4	4.1-11.8	4.0-12.6	4.9-17.9	7.5-28.2	4.6-49.4	16.2-61.4

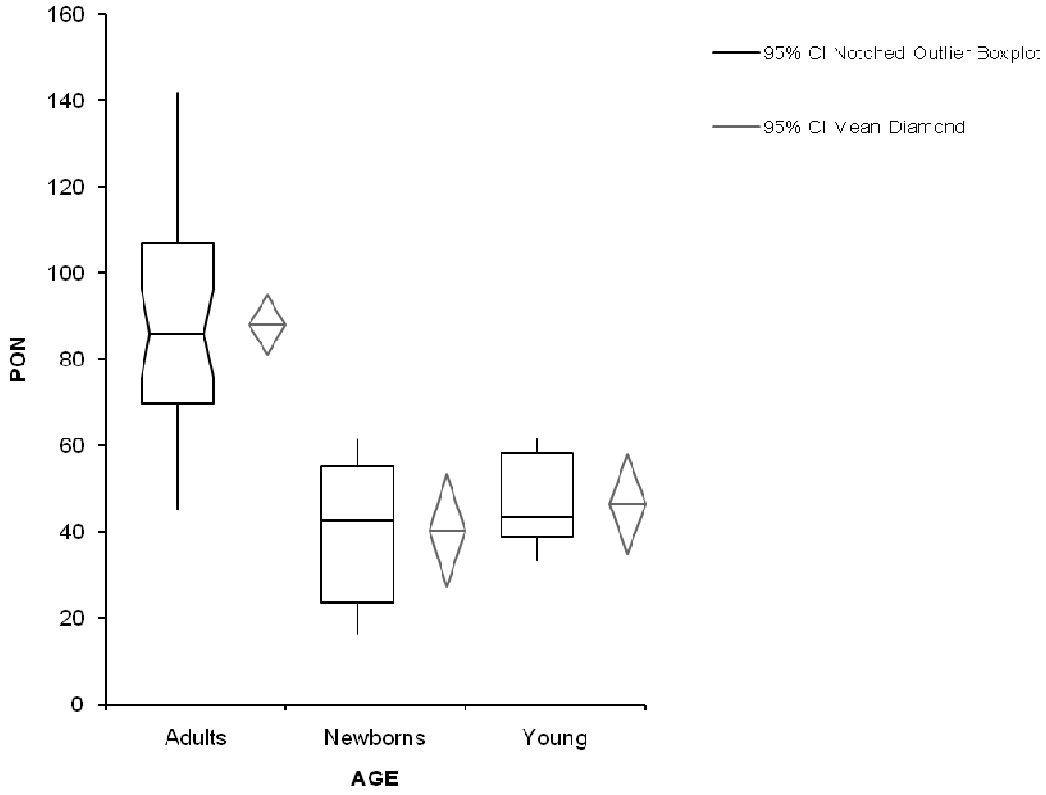
Fig. 3 – Age-related variations



Tab. 4 – Age-related variations

PON1 by age	**n**	**Mean**	**SD**
Adults	45	88.03	22.7
Newborns (21 d)	9	40.40	16.9
Young	6	46.50	11.1

Fig. 4 – Age-related variations



Comparison between sick and healthy calves

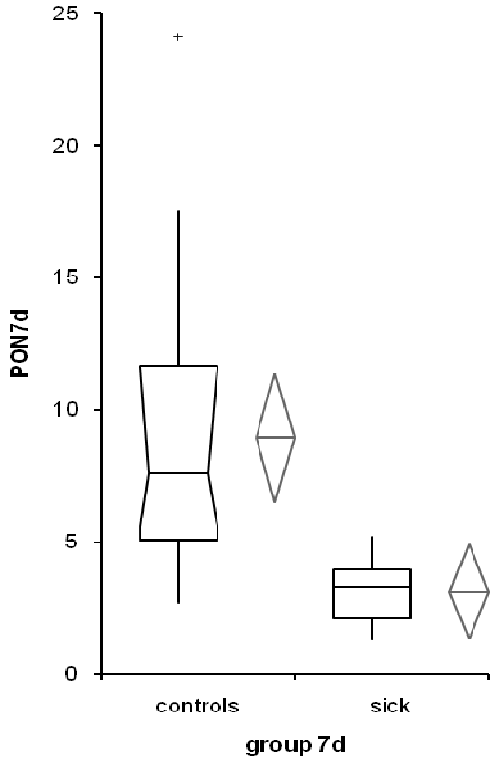
We were able to compare sick animals and the age-matched controls only for three different ages.

Specifically, 7 day old sick calves had a significantly lower PON1 activity than controls ($p \leq 0.01$) (*table 5, figure 5*). Similar results were obtained in calves of about 1 to 3 months of age ($p \leq 0.01$) (*table 6, figure 6*). In the 10 days group, non significant differences were present between sick and controls (*table 7, figure 7*).

Tab. 5 – Comparison between 7 days-old sick animals and age-matched controls

PON1 group 7 days	**n**	**Mean**	**SD**
Controls	20	8.9	5.16
Sick	5	3.1	1.42

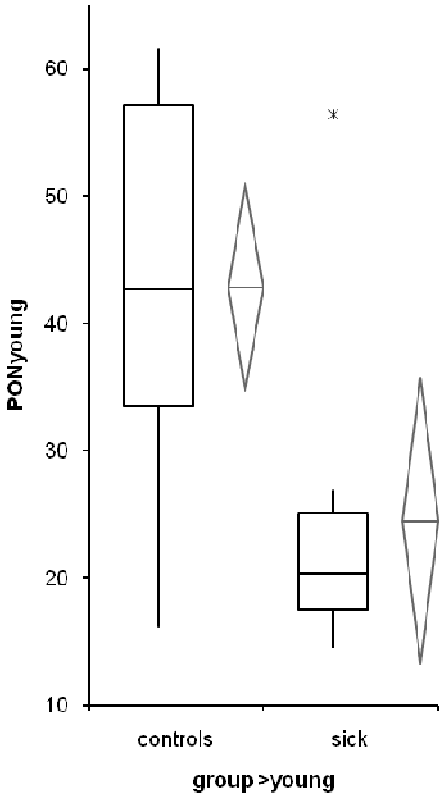
Fig. 5 – Comparison between 7 days-old sick animals and age-matched controls



Tab. 6 – Comparison between 1 to 3 months-old sick animals and age-matched controls

PON1 group young	n	Mean	SD
Controls	15	42.8	14.75
Sick	8	24.5	13.44

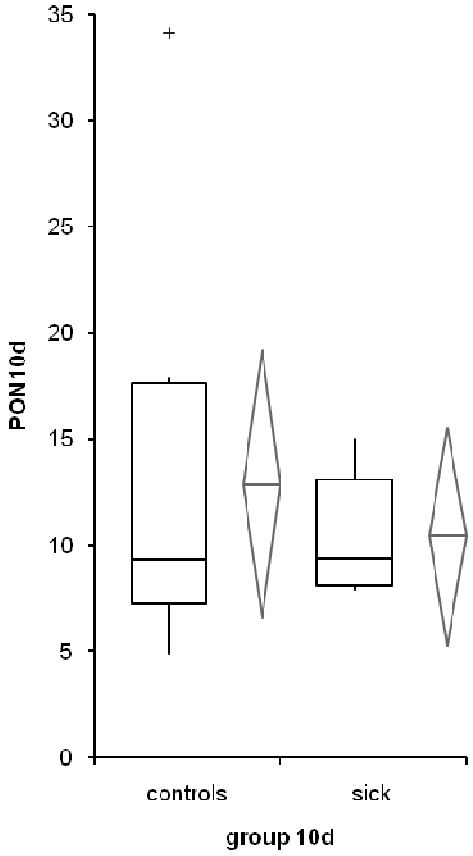
Fig. 6 – Comparison between 1 to 3 months-old sick animals and age-matched controls



Tab. 7 – Comparison between 10 days-old sick animals and age-matched controls

PON1 group 10d	n	Mean	SD
Controls	10	12.9	8.82
Sick	4	10.4	3.24

Fig. 7 – Comparison between 10 days-old sick animals and age-matched controls



Discussion and conclusions

The analytical performances of the method used to measure PON1 activity were satisfactory. The activity of paraoxonase seems to be influenced by the age of the animals, so **it would be advisable to use age-related reference intervals when newborn calves have to be examined**.

Paraoxonase was lower in animals affected by inflammatory conditions, so due to its faster decrease during inflammation compared to albumin together to the fact that the method is cheap and rapid to perform, **the usefulness of PON1 as negative acute phase protein should be considered in newborn calves**.

Increased number of patients should be examined in order to give more power to the present results. Moreover PON activity should be correlated to other robust markers of sepsis and inflammation (other APP, leukogram).

Due to its biological properties also the lipidic and oxidative status of the animals should be taken into account during the evaluation of this enzyme, before to extend its use as marker in the field.

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CHAPTER 13

General discussion

5. General discussion

During recent years, an increase in perinatal mortality has been reported in dairy calves, quite typically not associated with an increase in the rate of dystocia (Mee, 2006; Mee, 2008*b*). These perinatal losses not only represent a tremendous economic loss but also mirror an important welfare problem on cattle farms.

In order to reduce the losses occurring during the perinatal and neonatal period, management of the parturient cow and newborn calf have to be addressed critically. As for all newborns, also calf's survival and subsequent health conditions require a perfect transition from fetal to extra-uterine life. Critical observation and/or investigation of the calf during and immediately after calving is important, considering that several problems encountered during the first days after delivery can originate from the calving period itself.

In a crude analysis for fetal and neonatal outcomes related to the type of delivery in the human specie (Villar *et al*, 2007) the highest rates of neonatal morbidity and mortality were seen in the elective caesarean group. Elective caesarean delivery could increase neonatal morbidity and mortality because lack of labour affects the physiological process for initiation of respiration. Caesarean delivery is known to be associated with respiratory distress syndrome and transient tachypnoea possibly mediated by the lower release of catecholamine and prostaglandins, as well as the lack of the mechanical compression of the lungs during labour needed to facilitate postnatal lung adaptation.

Moreover, several studies on babies (Gasparoni *et al*, 1992; Gronlund *et al*, 1992; Herson *et al*, 1992; Hasan *et al*, 1993; Usmani *et al*, 1993; Steinborn *et al*, 1999; Redźko *et al*, 2005; Yektaei-Karin *et al*, 2007) demonstrated that the type of delivery can deeply influence the adaptational process in the newborn, modifying the immune response, the oxygenation status and the developing endocrine axis; the influence of delivery is not only immediate but extended in time, possibly affecting also the future health status of the subject.

The main focus of this experimental thesis was on the effects of two different type of delivery on the conditions of the calf at birth and during the neonatal period; differences in clinical features and biochemical, metabolic, hormonal, hematological and inflammatory profiles, have been described and discussed in details.

In the **first** study (*chapter 7-8-9*), clinical data and biochemical, metabolic and hormonal profiles from newborn calves born by spontaneous parturition or by caesarean section were compared. For each newborn many clinical data (Apgar score, rectal temperature, extimated weight) were collected within 5 minutes from birth. Time for sternal recumbency and time to stand up were registered as soon as evident. Biochemical and metabolic profiles during the first 2 days of life (birth, 24 h, 48 h) were determined. Plasma concentrations of IGF-I, cortisol and thyroid hormones were investigated for the first 14 days of life.

No significant differences between calves from spontaneous parturition and from caesarean section were found concerning clinical features at birth and during neonatal period (*chapter 7*). Many differences between the two groups were found regarding some biochemical, metabolic and hormonal parameters. These fast changes in biochemical and metabolic parameters in the newborn calf are probably due to the maturational processes of liver, kidney and metabolism for adaptation to the extra uterine life; basing on our data, the caesarean section seems to entail a lower physical effort for calves during birth, and a slower or delayed adaptational process**.**

In the **second** study (*chapter 10*) the plasmatic profile of prostaglandin $F_{2\alpha}$ was determined in 10 newborn calves from spontaneous parturition, and compared with those from neonates of other animal species. Plasmatic levels of prostaglandin metabolite in calves remained high for the first 3 hours of life, unlike newborn horse and donkey foals, in which the metabolite already decreased at 30 minutes after birth.

Differences in placentation type or in gestation length could be the reason for the difference between these species.

In the **third** study (*chapter 11*) the hematological profile of the newborn calves during the first 14 days of life was investigated in relation to the type of delivery. Data from 16 Holstein Friesian calves from spontaneous parturition and from 25 Belgian White and Blue calves from caesarean section were compared. Differences were found regarding many parameters; red blood cells count was always higher in the CS group, as hemoglobin concentrations and hematocrit levels. White blood cells count was higher in the CS group at birth and at 14 days after birth, and it was due to an higher neutrophil count.

Different type of delivery can lead to different degree of stress and oxygenation in the newborn, which can represent the reason for variation in hematological pattern; difference in breed could anyway also play a role in this.

In the **fourth** study *(chapter 12)* a new acute phase protein (PON1) was investigated in newborn calves from spontaneous parturition, and in calves up to 120 days old. Samples from some calves with inflammation were examined and compared with those of age-matched controls. The profile of PON1 activity in the newborn calves was characterized by a gradual increase starting from day 3 of life, probably due to maturation of hepatic system; this result evidences the importance to establish age-related reference intervals. In sick calves, PON1 was significantly lower than in age-matched controls, confirming the role of PON1 as a negative acute phase protein also in calves.

General conclusions

From this thesis, the following general conclusion can be drawn:

- during the first minutes, hours and days after delivery, a wide number of physiological changes take place in the newborn calf;
- circulating levels of many hormones, which are responsible for maturation and adaptation of the neonate, rapidly change with age; most of these

hormones reach stable values after the first 24 hours of life, so that the first day after birth has a key role in the adaptational process of the newborn to the extra uterine life;

- prostaglandins $F_{2\alpha}$ metabolite plasma concentrations in the newborn calf remain high for the first 3 hours of life, unlike newborn horse and donkey foals, in which the metabolite already decreased at 30 minutes after birth. Differences in placentation type or in gestation length could be the reason for the difference between these species;
- PON1 activity in the newborn calves is characterized by a gradual increase starting from day 3 of life, probably due to maturation of hepatic system; this result evidences the importance to establish age-related reference intervals;
- in sick calves, PON1 is significantly lower than in age-matched controls, confirming the role of PON1 as a negative acute phase protein also in calves;
- the type of delivery does not seem to influence the immediate clinical conditions of the newborn calf, above all in terms of viability;
- the type of delivery seems to influence the biochemical, metabolic, haematological and hormonal profiles of the newborn calf: these differences are probably due to a different stress stimulation, which is normally lower during elective caesarean section.

Future perspectives

The main limitation of this experimental trial lays on the enrollment of two different cattle breeds. The difference in breed (and attitude) represents an important confounding factor that can deeply influence results. Future researches in this field should take into consideration different type of delivery within the same breed. Comparison of prostaglandin $F_{2\alpha}$ plasma levels and of PON1 activity in calves from different type of delivery should be made.

Examination of possible long-term influences of the type of delivery on the health status of the calf should be performed.

Acknowledgements

Acknowledgments

At the end of these three years at university, I would like to thank firstly my promoters, Cristina and Geert.

Cristina, you've been an example of how passion can become a work, and how work can be a passion. Your great and true interest for the unknown is contagious; thanks for teaching me how to be a good student, a good researcher, and a better person. You've been a Friend, besides and more than a teacher.

Thanks *Geert* for your helpfulness, and for your criticisms that really improved my project. Thanks for hosting me in Gent, and for making my research feasible. Thank you also for trying to cook italian food… you did your best!

Thanks to *dr. Leo Fiems*; without your help and support this research would not have been possible.

I would like to thank also my third (unofficial) promoter, *professor Cairoli*.
For sharing my passion for bovine's world, and encouraging me in learning more and more. For teaching me that honesty and friendship are the best "results" we can achieve in our life. I will really miss our talks about work, politics and life, that taught me more than thousands books.

Thanks to my "colleagues" Sara and Alessandro.
To *Sara*, for being like a sister: we shared all the emotions of our private lives during these last years. You were always present in bad and good moments, as friends should do. I really wish we could go on this way..
To *Alessandro*, for his will of learning and working. Sacrifices you're doing will be repaid.. but take a break sometimes! Thanks for being a quiet but true friend.

Thanks to *dr. Battocchio*, for pushing me towards the undiscovered land of scientific curiosity.

Many thanks to colleagues from pathology, *Saverio, Alessia, Gabriele* and *Luca,* and to *Pieranna Martino, Massimo Faustini* and *Antonella Comin* for the precious help in processing and analysing my data.

Thanks to all friends from Gent University.
To *prof. deKruif*, for authorizing my research; to *Marcel*, for the wonderful day in Gent; to *Jeff*, for lending me his office-room during the weekends; to *Lars*, for helping me with lab analysis; to *Els*, *Ria*, *Steven* and *Sandra* for technical (and moral) support; to *Marnik* for the beautiful room-with-no-windows; to *Petra*, for helping me with samples' shipments; to *Jan*, *Maarten*, *Catharina*, *Katrien* and *Kim*, for teaching me something about horses; to my dear office-mates *Ruben* and *Benedicte* (and the French guy); to *Miel*, for growing up our chicks, and for being my personal driver for one day; to *Alfonso*, *Sebastiaan*, *Stefaan*, *Wendy*, *Cyriel*, *Hilde*, *Emily*, *Sofie*, *Karlien*, *Mohmmad, Davy...* and to everybody from who I received a smile, a help, or a word.

Special thanks for special people who made my Belgian life unforgettable. *Vanessa*, thanks for simply teaching me what means to have a true friend; you've been mine. *Iris*, thank you for sharing with me your house, your travels, your favourite music (that's also mine!), your thoughts and above all your passion for life.

Thanks to *dr. Egidio Calcini*, *professor Marcel Taverne* and *professor Hans Kindahl*, for being excellent teachers.

Many thanks to *Manzoni family* and to the entire Mirandola farm's staff: thanks for helping me in collecting data, and for offering me a shelter and a warm tea during the cold winter nights. Thanks to *Gisella* for help and support with samples' collection; I'll never call you again in the middle of the night!

At the end, my "personal" acknowledgments.

To my *father* and *mother*: Thanks for the unconditioned love, for the precious help as grandparents, and for the interest in my job. I wish you could always be proud of me, and happy for the life we're living.

Thanks to my sister *Claudia* and my brother *Luca*: life would not be the same without you! Forever together, as always.

Thanks to my beloved husband *Daniele*, for sharing all my choices, rejoicing with me for victories and encouraging me in defeats. With you, "me" has become "we", and there's nothing better than having you at my side.

Thanks to my little son *Davide*; you were inside me when I started to write this thesis, and you're here with me now that it is finished. There's really something magic when suddenly one plus one is 3; this is the magic we call *Love*.

This thesis is dedicate to you two, and to the wonderful future that is waiting for us

Curriculum Vitae

Curriculum Vitae

Monica Probo was born in 1983 in Lodi, Italy.
Secondary school education started in 1997 at the Liceo G.Gandini in Lodi, and high school graduation was obtained with honours in 2002.
From September 2002 until June 2007 she studied veterinary medicine at the Veterinary Faculty of Milan (Italy), focussing on reproduction in large animals. During the last year, she attended a course on herd health management. She graduated in 2008 with honours.
In June 2008 she obtained a grant for young researchers, developing researches on donkey stallion reproduction.
In 2009, she started the PhD course at the Graduate school of Veterinary Sciences for Animal Health and Food Safety, following a Doctoral Program in Veterinary Clinical Sciences with subspecialty in ruminants' reproduction. During this course, she moved to the Faculty of Veterinary Medicine of Gent (Belgium) for few months, collecting data for her experimental research and attending clinical activity at the Department of Reproduction, Obstetrics and Herd Health of the faculty.
She attended many national and international congresses on reproduction, and she published some articles on peer-reviewed journals (see list of publications). From 2009 she is a resident of the European College of Animal Reproduction with subspecialty in Ruminant Reproduction and Herd Health.

List of publications

Book text

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- XIV SIVE/FEEVA Congess, 25-27 january 2008, Venezia, Italy
- VI SIRA National Congress, 12-13 june 2008, Lodi, Italy
- VI AAAA Biannual meeting, 12-13 july 2008, Budapest, Hungary
- LXII SISVet National Congress, 24-26 september 2008, S. Benedetto del Tronto, Italy
- VII SIRA National Congress, 2-3 july 2009, Messina, Italy
- XIII ESDAR Annual Conference, 10-12 september 2009, Gent, Belgium
- LXIII SISVet National Congress, 16-18 september 2009, Udine, Italy
- VIII SIRA National Congress, 17-18 june 2010, Bologna, Italy
- XIV ICPD Congress, 20-24 june 2010, Gent, Belgium
- LXIV SISVet National Congress, 7-10 september 2010, Asti, Italy
- XIV ESDAR Annual Conference, 15-19 september 2010, Eger, Hungary