pDepartment of Obstetrics and Gynaecology

Department of Clinical Science and Education

Söder Hospital

Karolinska Institutet, Stockholm, Sweden

LACTATE DETERMINATION INANTE- AND INTRAPARTUM SURVEILLANCE

Eva Wiberg-Itzel

Stockholm 2007

Cover page `mitchondria producing lactate´ (From `Energetic Transference Occurring in the Biosphere Part III: Lactate Clearance and Anaerobic Training Adaptations´ published with permission, from the author).

Published by Elanders, Vällingby 2007

ISBN 978-91-7357-213-2

ABSTRACT

Lactate concentration is reported to be high in amniotic fluid (AF). Prelabour rupture of membranes (PROM) occur in about 20% of all pregnancies. The condition is associated with fetal and maternal complications, and might be a marker of imminent delivery Therefore among women with suspected prelabour rupture of the membranes (PROM); it is of great importance to accurately confirm the diagnosis.

In our studies we wanted to assess whether lactate determination in vaginal/amniotic fluid could be used as a diagnostic test for prelabour rupture of membranes, and could predict onset of labour in women with suspected PROM.

In our PROM studies we selected women with a history of suspect PROM after 34 weeks gestation for determination of lactate concentrations in vaginal fluid.

A lactate concentration $\geq$ 4.5 mmol/l was found to be the best cut-off value for a positive 'Lac-test' and showed a sensitivity of 86% and specificity 92%.The median time interval between examination and spontaneous onset of labour among the women with "high" lactate ($\geq$ 4.5 mmol/l) were 8.4 hours and for those with low" lactate concentration (< 4.5 mmol/l) 54 hours. Among women with "high" lactate concentration 88% started in labour within 24 hours, as compared with 21% for those with "low" lactate concentration.

Labour dystocia is clinically defined as slow or arrest of progress during labour and is a common obstetrical problem worldwide. In our study we looked for an association between high lactate concentration in amniotic fluid and labour dystocia. We selected women in active labour attending labour ward, and performed at least two consecutive measurements of lactate concentration in amniotic fluid during labour. Among women with spontaneous vaginal deliveries (n=23) the mean lactate concentration in AF during labour was 8.9 mmol/l and among women with labour dystocia (n=31) the corresponding value was 10.9 mmol/l ($p < 0.001$). Of 29 women with a high lactate concentration ($\geq$10.1 mmol/l) in at least two consecutive measures, 86% were delivered instrumentally/operatively due to dystocia. Using this definition of a positive test gives a sensitivity of 81% a specificity of 82%, a positive predictive value of 86%, and a negative predictive value of 76%.

Fetal surveillance during labor is often based on fetal heart rate monitoring using the cardiotocograph (CTG). A normal CTG is reassuring for a well oxygenated fetus. However, a non-reassuring trace occurs in up to 50% of all recordings, but only a small proportion of these fetuses are at risk of hypoxia. In a multicentre trial we wanted to compare pH vs. lactate analysis, regarding prevention of acidemia at birth. 2992 women in labour were randomised to pH (n=1,496) or lactate analysis (n=1,496). Protocol violations were significantly less frequent in women randomised to lactate compared with women randomised to pH analysis, 11.0% vs. 1.5%. There were no significant differences between the groups in the rate of metabolic acidemia (RR 0.96) or pH <7.00 (RR 0.88) in cord artery blood at birth.

We have with this thesis shown the usefulness of determination of lactate in AF and fetal blood sampling. Lactate in AF can be used in the diagnosis of suspected PROM, in the prediction of spontaneous onset of labour for women with suspected PROM, and also in the diagnosis of labour dystocia. We have shown lactate analysis of fetal scalp blood to be at least as good as pH analysis in the management of intrapartum fetal distress

Key words: lactate, amniotic fluid, PROM, prediction of onset of labour, diagnosis of dystocia, fetal distress, hypoxia.

Stockholm 2007

LIST OF PUBLICATIONS

This thesis is based on studies reported in following papers, referred to in the text by their roman numerals.

I. Eva Wiberg-Itzel, Sven Cnattingius, Lennart Nordstrom. Lactate determination in vaginal fluids: a new method in the diagnosis of prelabour rupture of the membranes. BJOG. 2005 Jun; 112(6):754-8.

II. Eva Wiberg-Itzel, Hans Pettersson, Sven Cnattingius, Lennart Nordstrom. Association between lactate in vaginal fluid and time to spontaneous onset of labour for women with suspected prelabour rupture of the membranes. BJOG. 2006 Dec; 113(12):1426-30.

III. Eva Wiberg-Itzel, Hans Pettersson, Sven Cnattingius, Lennart Nordstrom. Association between lactate concentration in amniotic fluid and dysfunctional labour. *(Submitted*).

IV. Wiberg-Itzel E, Lipponer, Norman M, Herbst A, Prebensen D, Hansson A, Bryngelsson A-L, Christoffersson M, Sennström M, Wennerholm U-B, Nordstrom L. Fetal scalp blood pH or lactate analysis in the management of intrapartum fetal distress. A randomised controlled trial. (Submitted).

CONTENTS

LIST OF ABBREVIATIONS

AFP	Alpha feto protein
AF	Amniotic fluid
ATP	Adenosine triphosphate
CI	Confidence intervals
CTG	Cardiotocograph
CO_2	Carbon dioxide
CV	Coefficient of variation
DAO	Diamin Oxidase
EC	Energy Charge
ECG	Electrocardiography
EDA	Epidural analgesia
EFM	Electronic fetal monitoring
FBS	Fetal blood sampling
FHR	Fetal heart rate
HCG	Human choriogonadotropin
HCO_3	Bicarbonate
H^+	Hydrogen ions
H_2O	Water
IGFBP	Insulin-like growth factor binding protein
IUP	Intrauterine pressure
LDH	Lactate dehydrogenase
L/P	Lactic acid/Pyruvate
LR	Likelihood ratios
MMG	Magnetomyography
MCT	Monocarboxylate transport proteins
NAD^+	Nicotinamide adenine dinucleotid
NPV	Negative predictive value
OR	Odds ratios
1PAMG-1	Placental alpha microglobulin –1
Pi	Inorganic phosphate
pT	Pico Tesla
PPV	Positive Predictive value
PROM	Prelabour rupture of membranes
PPROM	Preterm Prelabour rupture of the membranes
ROM	Rupture of the membranes
RR	Relative risk
WHO	World health organization

INTRODUCTION

If exhaustion or muscle fatigue is discussed in a general conversation, usually people will refer to lactate accumulation as a primary cause. Lactate accumulates in blood and tissues during exercise, particularly when oxygen is lacking. The concentration is highest at or just following exhaustion. Lactate has historically been considered as a dead-end waste product of anaerobic metabolism due to hypoxia and the primary cause of fatigue (Berzelius 1808; Araki 1891; Hartree & Hill 1921; Hill 1922). Lactate has also been considered as a key factor in acidosis-induced tissue damage; however the role of lactate in metabolism has changed during the last decade (Brooks 1986; Brooks 2002; Brooks 2002). Lactate is no longer considered as a harmful end-product, but mainly one of the central players in cellular and whole body metabolism.

The breakdown of glycogen during anaerobic conditions leads to intracellular accumulation of lactic acid. Lactic acid is a strong monocarboxylic acid (Pka 3, 86) and it dissociates easily at physiological pH into lactate and hydrogen ions (H^+). The lactate itself has been considered to have little effect on muscle contractions. However, increased production of H^+ and reduced pH with acidosis has classically been considered as the cause of muscle fatigue. The role of reduced pH as an important cause of fatigue has been challenged (Karlsson et al. 1975). Present day knowledge is that anaerobic metabolism with the production of lactic acid might also lead to increased production of other factors, like phosphate (Allen et al. 2002; Westerblad & Allen 2002; Westerblad et al. 2002) which is likely to have a more prominent role in muscle fatigue. One important finding, which has influenced the hypotheses for this thesis, is that the myometrium produces lactate with myometrial contractions (Taggart & Wray 1993;

Taggart et al. 1996; Taggart et al. 1997; Taggart & Wray 1998; Wray et al. 2003; Quenby et al. 2004).

The knowledge that amniotic fluid (AF) contains high concentration of lactate has been published since the 1970s (Fadel et al. 1979; Brace 1997). Some publications have suggested that the source of lactate in AF is the fetus itself, mainly through urine and lung excretion (Perks et al. 1991). Several reports have suggested the myometrium as a potential lactate producer (Taggart & Wray 1993; Taggart et al. 1996; Taggart et al. 1997; Taggart & Wray 1998; Quenby et al. 2004). The lactate concentration in amniotic fluid is reported to be 4 - 6 times higher as compared with fetal and maternal blood. However, from the literature it is not clear from where the high AF lactate concentration is derived.

Fetal surveillance during labour is often based on fetal heart rate monitoring using the cardiotocograph (CTG). A normal CTG is reassuring for a well - oxygenated fetus (Vintzileos et al. 1995). However, a non-reassuring trace occurs in up to 50% of all recordings, but only a small proportion of these fetuses are at risk of hypoxia (Ingemarsson 1993). A diagnostic test in cases with non-reassuring or ominous fetal heart rate traces is therefore needed. Fetal scalp blood sampling for pH analysis has been the `gold standard´ to identify hypoxia during labour (Bretscher & Saling 1967; Westgren et al. 1998; Tuffnell et al. 2006). A shortcoming of this method is high sampling/analysis failure rate and that the method does not discriminate between respiratory and metabolic acidemia. Lactate analysis on fetal scalp blood during labour might be an alternative method in the management of intrapartum fetal distress.

ENERGY METABOLISM

The main substrate for energy metabolism is glucose (Meyerhof 1920). Under normal conditions, with sufficient oxygen supply, aerobic metabolism occurs. Here glucose is broken

down along the glycolytic pathway, and the resulting pyruvate enters the citric acid cycle (Fig.1) (Hill 1922). Energy is produced along the glycolytic pathway, together with carbon dioxide (CO_2) and water (H_2O).

Nicotinamide adenine dinucleotid (NAD^+) is a powerful hydrogen ion acceptor. In the citric acid cycle NAD^+ accepts an H^+ to produce NADH. In the reaction O_2 is consumed, and a large amount of energy is released (36 ATP).

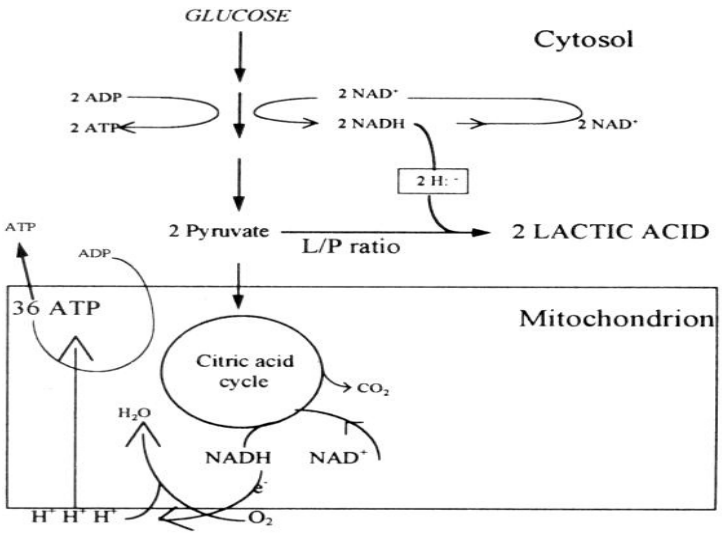

Fig.1 from: Intrapartum Fetal Hypoxia and Biochemical Markers; a review (Nordstrom, Arulkumaran 1998).

If oxygen supply reaches a critical low level, the metabolism will change to become anaerobic. Here, instead of entering the citric acid cycle, pyruvate is reduced to lactic acid and H^+. This reaction is catalyzed by the enzyme lactate dehydrogenase (LDH), and also involves the oxidation of NADH to NAD^+. NADH is generated in glycolysis, and re-oxidised into NAD^+. Under anaerobic conditions, this oxidation is impaired, resulting in accumulation of NADH, promoting the conversion of pyruvate to lactate.

In normal conditions there is a steady state relation between lactic acid/pyruvate (L/P). If oxygen supply is limited, a progressive lactate acidemia (metabolic acidosis) develops.
Anaerobic metabolism produces less energy (2 ATP/glucose) compared with aerobic conditions (36 ATP/glucose).

CELLULAR ENERGY PRODUCTION

With prolonged lack of energy due to anaerobic metabolism, there is difficulty in maintaining cellular integrity. Cellular functions rely on ion gradients across cell membranes. Ion pumps require ATP to function. Regeneration of sufficient amount of ATP can no longer be sustained if anaerobic metabolism continues. In this catabolic situation, the basic cellular functions start to fail.

Three different cellular energy statuses are described (Nordstrom & Arulkumaran 1998). The first one is aerobic when there is sufficient amount of oxygen and a lot of energy is produced in the form of ATP. This is an efficient way of energy production. The two others are dependent on the level of oxygen supply, and if the situation is compensated or not. Lack of oxygen forces the cell into an anaerobic metabolism with production of lactate and H^+. Energy is produced but to a limited amount. If the demand of energy is still sufficient, the cellular energy status is *compensated*. This can continue as long as energy demand and production is in balance. If the situation is progressing, regeneration of ATP can no longer be keep up with demands and the cellular energy status will be *decompensated.*

BUFFERING SYSTEMS

In a normal state, the buffering systems of the organism have the capacity to maintain pH within a physiological range. It is important for the organism to maintain stability in pH, i.e. H^+

concentrations. A fluctuation of H^+ is dangerous for the cell. If the concentration of H^+ rises it may disturb cellular function and affect the activity of cellular enzymes.

There are different buffering systems within the organism. The two most important systems are the bicarbonate and the protein buffering systems. These two systems main functions are to neutralize H^+ which has been produced through anaerobic metabolism. The role of the bicarbonate buffer is to establish equilibrium between CO_2, H_2CO_3, bicarbonate (HCO_3) and hydrogen ions (H^+), via the equation shown below (Siggaard-Andersen 1971)

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO3^-$$

In this reaction CO_2 is passing through and at the end is converted to bicarbonate, which leaves the red blood cells by means of an exchange of chlorides. The equation goes from left to right and back again several times until a steady state condition is established. At steady state total cellular CO_2 production equals CO_2 elimination.

LACTATE IN MUSCLES

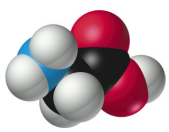

Fig. 2 Lactate acid molecule. The picture is taken from `chemical and biological uses´ (2006).

History

Knowledge about lactate is a rapidly changing field, and our understanding of the role of lactate metabolism has changed dramatically from the classical views held in the 19th century. The lactic acid era began in 1808 when Berzelius at the Karolinska Institute discovered elevated

concentrations of lactate in `the muscles of hunted stags´ (Berzelius Djurkemien 1808). Araki showed in 1891 that lactic acid concentration in exhausted animal muscles was proportional to the amount of exercise and was associated to 0_2 availability (Araki 1891).

Some 100 years after Berzelius, Fletcher and Hopkins showed that lactic acid appeared in response to muscle contraction in human muscles (Fletcher & Hopkins 1907). They also showed that accumulated lactate disappeared when oxygen became available. Later on the `lactic-acid-cycle´ was described, and showed two distinct pathways in metabolism, the aerobic and the anaerobic. The coming period was called `the revolution in muscle physiology´. From the 1930s to the early 1970s lactic acid was largely considered to be a `dead-end metabolite of glycolysis after muscle hypoxia´ (Meyerhof 1920; Hill 1922). Lactic acid was also believed to be the major cause of muscle fatigue. Since the early 1970s, a `lactate revolution´ has occurred (Hermansen 1981; Wasserman 1984). At present we are in the midst of a `lactate shuttle era´ with the introduction of the `lactate shuttle hypothesis´ by George Brooks (Brooks GA.1986; Brooks 2000; Brooks 2002).

Lactate shuttles

Lactate exchange is a dynamic process with simultaneous muscle uptake and release between cells at rest and during exercise (Brooks 1986; Brooks 2000; Brooks 2002). At rest muscles slowly release lactate in to the surrounding fluids on a net basis, but muscles may also show a small net uptake. During exercise, muscle tissue produces lactate rapidly. This results in an increased intracellular concentration of lactate and an increased net output of lactate from the muscles to the surrounding fluids. During recovery there is a net uptake of lactate from the ambient fluid by resting muscles, or other muscles that are exercising at low or moderate intensity. During prolonged exercise of low to moderate intensity, the muscles that originally released lactate on a net basis at the onset of exercise may actually reverse it to net lactate uptake.

The conclusion from many recent studies is that lactate is a useful metabolic intermediate which can be exchanged rapidly between tissue compartments (Brooks 2002). Lactate can also be used as a substrate in aerobic condition.

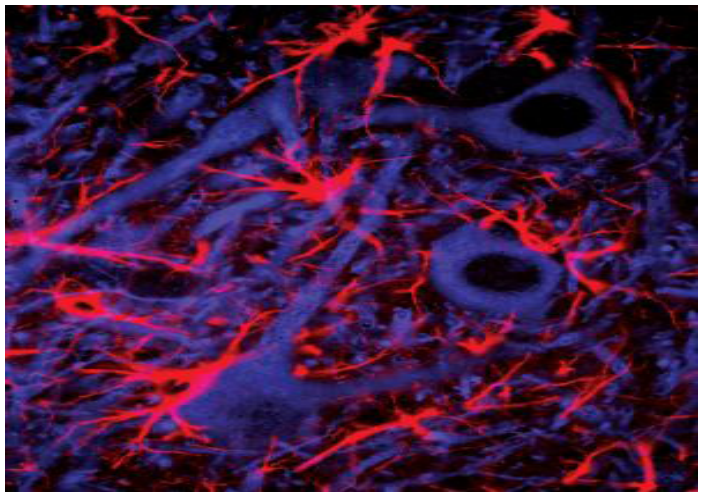

Fig.3. Astrocytes (red cells) and neurons (blue cells). Astrocytes are providing extra lactate "fuel" to neurons, confirming the astrocytes-neuron lactate shuttle hypothesis (from `developmental Resource for Biophysical Imaging Opto-Electronics´ 2006, published with permission from the author).

The transport of lactate has historically been considered as a system of passive diffusion; depending on a pH gradient (Crone 1963; Brooks 2002; Philp et al. 2005). Recent publications have suggested an active lactate transport system beside the passive one. An entire family of monocarboxylate transport proteins (MCT), which facilitate the transport of lactate in and out of the cells, has been described. The MCT proteins are suggested to have a primary role of the lactate transport in skeletal muscles and heart muscle but also in other tissues (Bonen et al. 1997; Brooks et al. 1999; Bonen 2000; Bonen 2001). During exercise lactate and H^+ move in and out of tissue primarily via MCT1 and MCT4, diffusion of undissociated lactate constitutes a smaller component of the transport.

Studies have also shown that from interstitial fluids, lactate and H^+ gain access to, blood through endothelial clefts, and probably also across endothelial cells (Juel 1997). The extent to which lactate and H^+ move from interstitial fluid through endothelial cells in most tissues is, however, unknown.

Muscle fatigue

It is well known that muscle performance may declines with prolonged or intense muscle activity, especially if there is a shortage of 0_2 (Allen et al. 1995; Westerblad et al. 2002). This decline is known as muscle fatigue. The causes of fatigue are probably multiple, but the consequence is that the power output may be drastically reduced. The consequence of lost power is obvious during sporting activity, for example, in endurance sports. It is almost impossible to maintain a marathon race if the muscles are exhausted.

When a muscle goes from rest to high-intensity exercise a marked acidification occurs because of the shortage of 0_2. The energy demand exceeds the capacity from available aerobic metabolism. The metabolism will enter the anaerobic pathway and the ATP required will come from anaerobic metabolism. Anaerobic breakdown of glycogen leads to intracellular accumulation of inorganic acids such as, for example, lactic acid. Lactic acid is a strong acid and dissociates easily to lactate and H+ at physiological temperature (Westerblad et al 1997). Lactate might therefore have limited effect of its own on the muscle contractions. The traditional thinking was that H^+ is produced together with lactate, and H^+ created the pH change and was the important cause of fatigue.

Data presented by Westerblad et al (Westerblad et al. 1991; Westerblad et al. 1998; Westerblad 2002; Westerblad & Allen 2003) provide substantial support for that increased inorganic phosphate (Pi) having a key role in muscle fatigue, especially at physiological temperature. For

acidosis, on the other hand, most recent data indicate that its depressive effect on muscle contraction is limited. Other studies express doubts about the effect of $P_{i,}$ and indicate that it is too early to dismiss H^+ as an important factor in muscle fatigue (Fitts 2003). Fatigue has many sources that may be present in different sites in the muscle cells (Taggart & Wray 1998). Many constituents of muscle metabolism change during fatigue and for each of these metabolites we need to know which role they have in the regulation of the muscle contraction. Despite nearly 200 years of muscle function research, the question of muscle fatigue still remains partly unresolved.

THE UTERINE MUSCLE

The uterine muscle has a dualistic function. First it has to shelter the growing fetus during pregnancy within the uterine cavity. To fulfil this demand of pregnancy/parturition the human uterus has a unique construction. The uterine cavity is surrounded by smooth muscle - the myometrium- whereas the cervix – the exit of the uterine cavity - is composed mainly of connective tissue. This construction gives the fetus a space to grow during pregnancy. The uterine muscle has a relatively relaxed state at this time. Second, when labour starts the uterus becomes a strongly coordinated working muscle with a high level of activity.

Earlier studies of contractile myometrial activity are mostly concerned with the hormonal control. We have knowledge about the effect of oxytocin (Rezapour et al. 1996; Rezapour et al. 1996), gestagens and estrogens (Roy & Arulkumaran 1991; Spencer et al. 2005), as well as the prostaglandins during labour (Challis 1974). Their ultimate effects are assumed to be modified by local factors in the tissue, e.g. metabolites. Extended knowledge about these metabolites seems to be of importance, especially in the light of the clinical expression of labour dystocia (Steingrimsdottir et al. 1995).

During the late 80s and the 90s several studies have been published on myometrial activity by Ulmsten and associated (Wedenberg et al. 1990; Wedenberg et al. 1991; Ronquist et al. 1993; Steingrimsdottir et al. 1995; Wedenberg et al. 1995). They have shown that the pregnant myometrium has a low energy charge (EC), described as an index of energy status, and compared with striated and cardiac muscles. The difference was considered to be due to the very special demand of the uterine muscle, compared to other muscles. The cardiac muscle has to work continuously, with only short periods of rest (diastole). Striated muscles must work instantly on command. The uterine muscle remains relaxed for long periods of time and then, only for short periods, has to transfer to a state in which strong contractions are required. This situation demands energy (Steingrimsdottir et al. 1993; Steingrimsdottir et al. 1995; Steingrimsdottir et al. 1997; Steingrimsdottir et al. 1999). The research group cited has shown an increased content of glucose in the pregnant smooth muscle in term pregnancy, compared with early pregnancy and the non- pregnant uterus (Wedenberg et al. 1990). This finding along with a positive artriovenous difference in blood-glucose across the uterus (i.e. net uptake), indicates glucose to be the principal nutritive metabolite for the pregnant uterine muscle (Steingrimsdottir et al. 1999).

The anaerobic pathway seems to be more active in the myometrium than in striated muscles. The L/P ratio, an indicator of anaerobic metabolism, is reported to be higher in the pregnant myometrium compared with other muscles (Steingrimsdottir et al. 1995). The lactate content of pregnant uterine muscle has been reported to be doubled compare with the skeletal muscle, probably reflecting a vigorous glycolytic flow when the uterus is active.
The uterus undergoes a general metabolic preparation for a hypoxic condition in late gestation. A significant physiological alkalinization of the muscle over the last few weeks of pregnancy has been shown (Parratt et al. 1995). This might therefore contribute to the mechanisms ensuring

that strong and efficient contractions occur during labour, when acidity is added during normal myometrial contractions.

AMNIOTIC FLUID

Amniotic fluid production

The essential function of AF is to cushion the fetus (Williams et al. 1980). The fluid gives the fetus space to grow, and allows it to undergo a `physical´ development. The AF function is also to protect the fetus from trauma and to maintain temperature. It also has a minimal nutritive function.

In the first half of pregnancy AF has a composition similar to fetal extracellular fluid (Sinha & Carlton 1970; Modena & Fieni 2004). The volume is closely related to fetal weight, and the skin of the fetus offers no resistant to movement of fluid. AF at this stage may be regarded as an extension of fetal extra - cellular fluid. Beyond mid - pregnancy (about 20 weeks) the fetal skin keratinizes (Modena & Fieni 2004), and continuity between the fetal extra - cellular fluid and AF is lost. AF becomes completely external in the sense that it can now no longer equilibrate with either the fetus or the mother. After keratinization of the fetal skin, the AF osmolarity decreases. A part of the changing composition reflects the increasing maturity of the fetal kidneys. The fetal kidneys begin to produce urine at about 12 weeks gestation (Wlodek et al. 1994; Brace 1997; Topuz et al. 2004) Low osmolarity provides a large potential osmotic force for the outward flow of water across the intra- and transmembraneous pathways.

The regulatory mechanisms to achieve an adequate AF volume operate at three levels; placenta control of water and solution, transfer regulation of inflow and outflow by the fetus, and maternal effects of the fetal fluid balance. The most contributing proportion of the AF balance is the fetus and its urine production (Wlodek et al. 1994; Brace 1997; Topuz et al. 2004), and the

AF ingested by the fetus through swallowing. A smaller contribution of AF is distributed by the fetal pulmonary fluid production (Perks et al. 1991; Brace 1997), and fluid filtering through the placenta and the membranes (Lingwood et al. 1980; Lingwood & Wintour 1983; Lingwood & Wintour 1984; Gilbert & Brace 1989; Daneshmand et al. 2003; Modena & Fieni 2004).

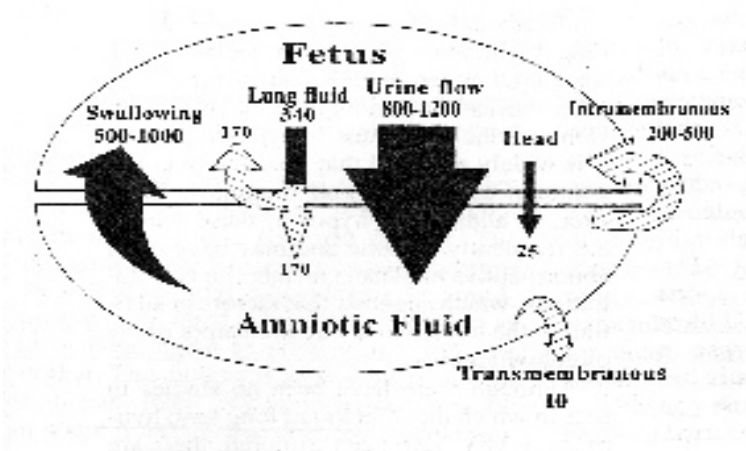

Fig.4. Estimates of inflow and outflows of AF in the near-term fetus (From: Williams's Obstetrics 21st edition, 2001).

The volume of amniotic fluid each week of gestation is quite variable (Queenan et al. 1972; Modena & Fieni 2004). In healthy pregnancies the AF volume has its maximum at 32-34 weeks, averaging 800 ml. Thereafter it declines, and the decline will be most marked post term (Magann et al. 1997; Cunningham 2001).

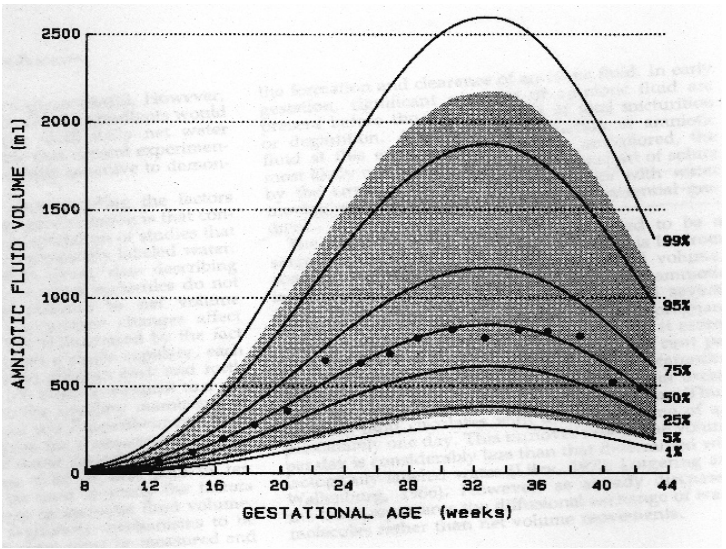

Fig.5. AF volume as a function of gestational age. Dots represent measured volumes with 2 week intervals (mean) in 705 women. Shaded area represents 95% confidence interval. (From: William's Obstetrics 21st edition, 2001).

PRELABOUR RUPTURE OF THE MEMBRANES (PROM)

Prelabour rupture of membranes (PROM) is defined as `spontaneous leakage of AF prior to onset of labour´ with a gestational age of 37 weeks or more (WHO definition). Preterm PROM (PPROM) is ruptured membranes before 37 weeks of gestation. PROM is a relatively common event in obstetric practice, and the prevalence is reported to be 5-19% of all pregnancies (Hannah et al. 1996; Ladfors et al. 2000).

Clinical management

The management of PROM has been considered controversy since the 1950s. The modern era of this field began in 1966 with several reports that showed increased risk for both the mother and the fetus, when expectant management of PROM was undertaken. PROM without immediate

onset of labour was considered to carry a high potential risk of incurring intrauterine infection (Shubeck et al. 1966; Webb 1967; Gunn et al. 1970; Mozurkewich 2006).

In the 1950s the perinatal mortality associated with PROM was estimated to range from 2.6% to 11%, and increased with the duration between PROM and delivery. The maternal mortality related to PROM, was reported to be 0.2‰ (Gunn et al. 1970). On the basis that PROM without immediate onset of labour was considered dangerous an aggressive approach to PROM was advised in the 70s and 80s. Early induction and operative intervention were suggested, especially if labour had not started within 24 hours. One problem with this aggressive approach was failed inductions with concomitant cesarean sections.

In 1979, Kappy et al published retrospective studies of women with PROM and unfavourable cervix status (Kappy et al. 1979). They described a spontaneous onset of labour within 24 hours in 85% of the women with established PROM. They also reported a reduced cesarean section rate with expectant management, and no evidence of increased neonatal infections.
In a trial by Ekman-Ordeberg and co-workers, they randomly assigned 20 nulliparous women with established PROM and unfavourable cervices to immediate induction of labour with oxytocin or PGE_2-gel. They noted fewer instrumental deliveries in the PGE_2-group (Ekman-Ordeberg et al. 1985).

In a large randomised trial of 5041 women with PROM were randomly assigned to immediate induction of labour or expectant management (Hannah et al. 1996). The women were randomised to induction with oxytocin, vaginal PGE_2-gel or expectant management up to four days after PROM. If labour had not started within four days, the women were induced with oxytocin or PGE_2 gel. The primary outcomes were neonatal infection and women's evaluation of their treatment. They found no significant differences between the study groups, and

concluded that in both management groups a similar rate of neonatal infections (2-3%) and cesarean sections (10%) were found. Women evaluated early induction of labour more positively than expectant management.

In a Swedish PROM study conducted by Ladfors et al (1996) 1385 women was included (Ladfors et al. 1996). They found a 13% prevalence of PROM after 34 weeks of gestation. They compared obstetric and neonatal outcome between two different expectant management groups, expectancy for 48 or 72 hours. The result showed a higher rate of spontaneous deliveries among nulliparous in the `late´ induction group compared with `early´ induction. The rate of instrumental delivery was lower in the `late´ induction group, but the rate of cesarean sections was similar. They concluded that expectant for 72 hours was to be recommended. Digital vaginal examination before onset of labour was not allowed in this trial. Low frequencies of maternal and fetal infections were found, and there were no differences between the groups. False negative diagnosis with visual inspection at speculum examination was found to be 12% (Ladfors et al. 1997; Ladfors et al. 1998) No disadvantage, i.e. infections, was found for mother or child if the woman was sent home after a false negative speculum examination. They questioned the value of using biochemical tests in the management of women with suspected PROM. No comments were made on the assumed false positive diagnosis in women with suspect PROM. All women included in the trial had visible AF at examination, but 43 (3.1%) of them had signs of intact membranes at delivery.

Historical review of PROM tests

In 1927 (Gold 1927), Gold found that vaginal pH turned from acid to neutral or alkaline when contaminated with amniotic fluid. In 1938, Baptisti and Abe introduced a ***nitrazine test*** **(**Baptisti 1938), which measured pH in vaginal secrete within a narrower range. This method has been widely used all over the world. The crystallisation pattern of AF was first described by Kardos

and Tamási in 1955 (Kardos & Tamasi 1955). The crystallisation phenomenon, also called ***ferning* or *arborisation test*** (Kovacs 1962; Smith & Callagan 1962; Tricomi et al. 1966; Smith 1976) is dependent on the relative concentration of electrolytes, proteins and hydrocarbonates in AF. The crystallisation test is nowadays still one of the most commonly used methods in clinical practice worldwide.

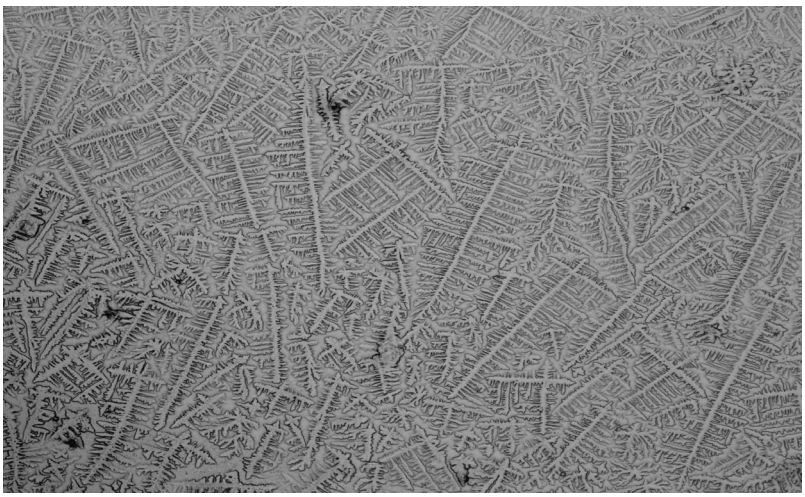

Fig.6. A photo taken at microscopy (x 40) of AF from one woman included in paper I.

Nile blue sulphate staining of the neutral lipid in cells from fetal sebaceous glands was described in 1965 by Brosens and Gordon (Paavola 1958; Brosens & Gordon 1965). The cells turn orange as a consequence of the oxazone in Nile blue. The cells are single or grouped in clusters. Other cells, like vaginal squamous, and pus cells or erythrocytes stain blue. A limitation of this test is that these fat-containing cells are only present after 32 weeks of gestation.

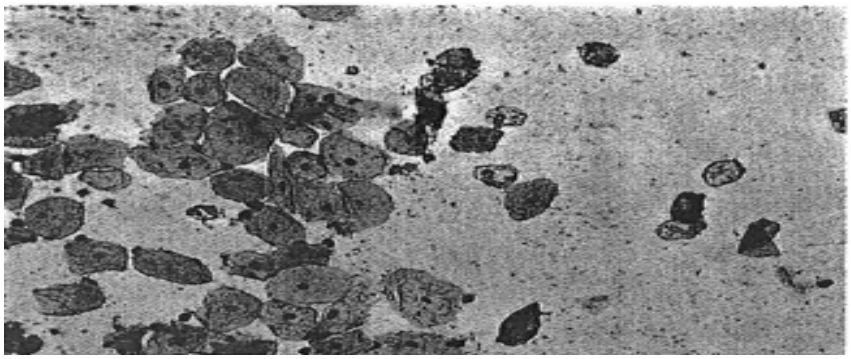

Fig.7. "The cytological diagnosis of ruptured fetal membranes using Nile blue sulphate staining" (Brosens 1965).

In selecting a spectrum of tests to be used in doubtful instances of ruptured membranes, it was determined that a combination of these three tests described above would produce an accuracy of diagnosis approximating 93% (Friedman & McElin 1969).

Present day tests

DAO test (DiAmine Oxidase activity)

The DAO test was one of the first biochemical tests for PROM, and was developed during the 1970s. DAO is present in high concentrations in AF but is absent in normal vaginal secretions and urine. DAO is produced by placental decidual cells and increases during pregnancy. The first publication of diagnosing PROM with the DAO test was made by Elmfors and co-assistance in 1974 (Elmfors et al. 1974; Wishart et al. 1979; Gahl et al. 1982; Bank et al. 1991; Gaucherand et al. 1995). The method is reported to have a sensitivity of 84-100% and a specificity of 74-100%. The test was carried out with 10 ul of AF absorbed on a paper strip, and the test requires a scintillation counter. This method is not available today because of the toxic chemicals that are used in the analysis.

AFP test (Monoclonal antibody test kit.)

AF also contains high concentrations of alpha feto protein (AFP) especially in preterm pregnancy. A monoclonal antibody assay method with high specificity was presented by Huber in 1983 (Huber et al. 1983; Rochelson et al. 1987; Garite & Gocke 1990; Gaucherand et al. 1995). The sensitivity was reported to 98%. However, they also reported that a false positive test may occur as AFP may cross weakened membranes in cases with chorio- amnionitis or heavy blood contamination. This test is not used in clinical practice any more.

Fetal Fibronectin (ROM-check).

Fibronectin is a large plasma glycoprotein. Three sub-types are available, of which one is fetal-derived. The concentration of fetal fibronectin in amniotic fluid is 5-10 times higher than in maternal plasma. In the 90s many papers were published about fetal fibronectin and its usefulness to detect AF in women with suspect PROM (Lockwood et al. 1991; Eriksen et al. 1992; Hellemans et al. 1992). They concluded were that fetal fibronectin is a sensitive test (97%) for detection of AF in the vagina but with a very low specificity (27%). Additionally, in patients without rupture of the membranes, they found that the interval between sampling and delivery was significantly shorter if fetal fibronectin was present. They concluded that the presence of fetal fibronectin in cervicovaginal secretions may be a good marker for impending labour rather than a good test for ruptured membranes (Lockwood et al. 1991; Reus et al. 1992).

Insulin-like growth factor binding protein-1 (PROM-test™)

Insulin-like growth factor (IGF) is a peptide and is bound to a binding protein (IGFBP) in the blood circulation. IGFBP-1 is a placental protein and is present in much higher concentrations in AF as compared with serum, cervical mucous, urine or seminal plasma. A commercial kit, with monoclonal antibodies to IGFBP-1 attached to a small wand has been available since 1993

(actim PROM-test™). During the last decade, many papers have been published on the actim PROM-Test™ (Rutanen et al. 1993; Rutanen et al. 1996; Darj & Lyrenas 1998; Erdemoglu & Mungan 2004). The sensitivity of the test is reported to be 71-100% and specificity 88-100%. It has been concluded that actim PROM-test™ is one of the most accurate diagnostic tests today in the diagnosis of suspected PROM. However, contamination of maternal blood or leakage of IGFBP-1 through stretched fetal membranes may cause false positive tests (Rutanen et al. 1993). A false negative result may occur if there is an inadequate sampling, intraamniotic infection, vaginal discharge, maternal blood loss, or prolonged time from rupture of membranes to application of the test. Gestatational age should not influence the test.

B-HCG in vaginal washing fluid

B-HCG is a glycoprotein produced exclusively by syncytiotrophoblasts in the placenta. Several studies have investigated β-HCG as a useful test for the diagnosis of PROM in the third trimester. Theses studies have shown a sensitivity of 68-100% and a specificity of 95-97% (Anai et al. 1997; Esim et al. 2003).

Amnisure®

In 1975, the placental alpha microglobulin–1 (PAMG-1) protein was isolated from AF. Antibodies were obtained against the protein and Amnisure® is an immunochemical method, used to measure the content of PAMG-1 protein in AF. Amnisure® has been available on the market since 2005. In a study which included 203 women with suspected PROM, a sensitivity of 98.8% and a specificity of 100% were found (Cousins et al. 2005).

PREDICTION OF LABOUR

Fibronectin

Different mechanisms may promote onset of labour. Increasing evidence suggests that these processes share a common pathway characterised by changes in extracellular matrix, which leads to cervical modification and disruption of the chorion-decidua. Lockwood and co-workers suggested that damage to the fetal membranes might lead to separation of chorion from the decidua. This phenomenon releases fetal fibronectin and gives rise to a biochemical marker for imminent start of contractions (Lockwood et al. 1991). The time between sampling and delivery is significantly shorter if fetal fibronectin is present (Rust et al. 2005; Tsoi et al. 2005; Tsoi et al. 2006).

Sonographic measurement of the cervical length

Measurement of cervical length and detection of funnelling at the internal cervical os by transvaginal ultrasonograpgy has been shown to be helpful in the prediction of spontaneous onset of labour within 7 days, in pregnancies with high risk of preterm delivery (Rust et al. 2005; Tsoi et al. 2005; Tsoi et al. 2006). Recently studies have been carried out also in near term pregnancies (Bayramoglu et al. 2005). They reported that about 74% of the women in term pregnancy (40 weeks of gestation) with a cervical length less than 24.5 mm delivered within 7 days (Bayramoglu et al. 2005). Sonographically measured cervical length together with fetal fibronectin assessment in cervicovaginal secretion can be an even better marker of onset of labour (Rizzo et al. 1996; Mercer et al. 2000). Even in term pregnancies, this prediction of labour might be useful, especially in complicated pregnancies.

There is a strong association between a short cervical length, an engaged fetal head, and labour. Transvaginal ultrasound might be helpful in the prediction of spontaneous onset of labour, but the method may increase the risk of infection in women with PROM. Transperineal ultrasound

might help the clinician in the prediction of labour by measuring the engagement of the fetal head, but with a reduced risk of infection, as a non-invasive method. (Eggebo et al. 2006).

Transabdominal magnetomyography (MMG)

Magnetomyography (MMG) is a non-invasive transabdominal method. The purposes of these studies were to characterise the electrophysiological activity of the uterine muscle in women reporting contractions. 15 women participated in the study, 11 at term and 4 preterm. Cervical dilation and outcome were recorded. Out of 8 having a peak MMG activity exceeding 8 pT (pico Tesla), all but one delivered within 48 hours. Of the 4 preterm women 1 had a peak MMG >8 pT and delivered within 48 hours. Seven women had peak activity below 8 pT, and five of these failed to deliver within 48 hours of the recordings. The aim of these studies was to show that an increase in the electrophysiological activity of the myometrium could be used as a predictor of labour in term and preterm pregnancies (Eswaran et al. 2004).

PROGRESS OF LABOUR

Labour dystocia

"In Africa the sun should never rise twice during labour, then it's dangerous", an
Old African saying was recounted by an African obstetrician at `Federation International Gynecologie Obstetrique´ (FIGO) 2006.

Labour dystocia is a common worldwide obstetrical problem, and is one of the main indications for operative intervention during parturition. Labour dystocia is clinically defined as slow/arrest of progress during labour, i.e. cervical dilatation and descent of the presenting part. It is estimated that labour dystocia occurs in about 20% of all deliveries worldwide (Quenby et al. 2004). However, it is difficult to find a precise definition. The usual metod to identifying labour dystocia is to use a partogram with an `alert line´ representing cervical dilation of 1 cm per hour

and an `action line´ drawn 2-4 hours to the right of the `alert line´. The clinical method of identifying dystocia is when the graphically plotted rate of progress crosses the action line or if no progress is made over the previous 2 hours (Studd & Duiagnan 1972; Studd & Philpott 1972). Labour dystocia is associated with increased risks, such as labour abnormalities, increased risk of instrumental/operative intervention, depressed Apgar score at 5´minutes and extended need for newborn care (Roemer et al. 1976; Cohen 1977; Katz et al. 1987; Saunders et al. 1992). Dysfunctional labour is also associated with a higher frequency of postpartum infections, higher estimated maternal blood loss and lengthened maternal and newborn hospital stay (Cohen 1977).

A number of papers have been published on myometrial acid-base balance, and correlation to inefficient contractions and dysfunctional labour. One finding is that acidification of the myometrium with accumulation of lactate, and a decrease of myometrial pH during contractions, could depress uterine contractions and thereby contribute to dysfunctional labour (Taggart & Wray 1993; Parratt et al. 1995; Taggart et al. 1996; Taggart & Wray 1998; Khan et al. 2001; Monir-Bishty et al. 2003; Wray et al. 2003). Quenby at al have shown that lactate concentration of myometrial capillary blood is significantly higher in women having a cesarean delivery due to dystocia than in women having an elective cesarean section or being operatively delivered with normal contractions (fetal distress) (Quenby et al. 2004). Furthermore, reduced pH and raised lactate concentrations in myometrial strips change regular contractions to irregular ones with reduced amplitude in in vitro studies. One of the suggested clinical explanations for this process was that during labour blood vessel supply might be occluded while the uterus is contracting. The irregular contractile pattern in dysfunctional labour might lead to extended occlusion of the uterine vessels. Extended occlusion might lead to a lowering of the myometrial oxygen levels and accumulation of lactic acid. Thus, despite the inefficient contractions, there is an inadequate reoxygenation of the uterus. Quenby has suggested that that there is a variation in response to

intermittent hypoxia in different women. The recovery period from the low oxygen episode after occlusion might differ.

Active management of labour

In the late 1960s 0´Driscoll and co-workers at National Maternity Hospital in Dublin carried out some pioneering work on normal/dysfunctional labour (O'Driscoll et al. 1969; O'Driscoll et al. 1973). They approached the management of labour in nulliparous women, which is nowadays referred to as `active management of labour´. The method includes strict criteria for the diagnosis of labour, early rupture of the membranes, prompt intervention with oxytocin stimulation in the event of abnormal progress of labour (inefficient myometrial contractions), and a commitment to never leave a labouring women unattended during the period of labour. Most of these studies have, however, been based on normal labour and not on dystocic ones. Some criticism has been made of the aggressive approach which constitutes `active management of labour´. Trials have been conducted with some of the strict diagnostic criteria such as early amniotomy, early oxytocin administration, attending midwife, and a combination of these interventions (Akoury et al. 1988; Turner et al. 1988; Boylan et al. 1991; Lopez-Zeno et al. 1992; Frigoletto et al. 1995). There have only been a few randomised studies with `the total package of management of labour´, and only one of these (Lopez-Zeno et al. 1992) showed significantly reduction in odds ratio (OR) for cesarean birth associated with active management. In contrast continuous professional support in labour has been shown to reduce the rate of operative interventions.

The aim with intervention in dysfunctional labour should be to reduce the proportion of maternal and fetal complications, with limited increase in cesarean sections or instrumental deliveries. It is also of a great important that the women in labour is given a feeling `of control´ because this is one of the major contributors to the woman’s satisfaction during childbirth.

INTRAPARTUM FETAL SURVEILLANCE

A French physician by the name of Marsac described in 1650 for the first time the fetal heart beat (Schwartz 1870). He likened the sound to the “clapper of bells”. The tubular wooden “stethoscope” was invented in 1821. It amplified the sound of the fetal heart beat and excluded other sounds. As early as 1870 an abnormal fetal heart rate pattern was described as a predictor of 'poor fetal well-being’ (Schwartz 1870). Schwartz recommended that the fetal heart rate (FHR) should be counted as frequently as possible during labour, both during and in between contractions (Schwartz 1870). Criteria of intrapartum fetal distress were described by Von Winckel in 1893. He included tachycardia, bradycardia, “irregularity” of FHR, meconium stain liquor in the vertex presentation and gross alteration of fetal movements as the most important signs of fetal distress during labour. The mechanism for FHR bradycardia was understood and reported as a result of vagus nerve activity in the beginning of the 20th century (Delee 1913).

Modern intrapartum fetal surveillance is mainly focused on detecting fetal asphyxia, in order to prevent perinatal death or future neurodevelopment handicap. The surveillance during labour is often based on fetal heart rate monitoring using the cardiotocograph (CTG). A problem with electronic fetal monitoring (EFM) is its poor predictive value. A normal CTG is reassuring for a well oxygenated fetus. However, a non - reassuring trace occurs in up to 50% of all recordings, but only a small proportion of these fetuses are at risk of hypoxia.

Cardiotocography (CTG)

Henley described in 1931 for the first time an electronic fetal monitor, using phonocardiography (Goodlin 1979). In 1958 the first studies of fetal electrocardiography (ECG) were presented. This method detects the electrical energy from the fetal heart. The first doppler ultrasound monitor, a kind of cardiotocograph (CTG) was described and clinically introduced by Hon et al

in 1977 (Hon & Hess 1957; Hon & Petrie 1975; Hon et al. 1975; Hon et al. 1975) This method measures the mechanical activity of the fetal cardiac cycle by indirectly using ultra sound. Today continuous fetal monitoring is achieved via an external doppler transducer, which is placed on the maternal abdomen. A computer averages three consecutive beat-to-beat intervals to give the FHR.

A disposable spiral electrode was developed by Hon in 1972 (Hon et al. 1972). This electrode obtains direct contact with the fetus. It is a bipolar spiral electrode which can be placed on the fetal scalp and which detects the fetal ECG and measures the R-R intervals to determine the heart rate.

To evaluate the efficacy and the safety of intrapartum fetal monitoring several trials has been performed comparing continuous monitoring with CTG and intermittent auscultation (MacDonald et al. 1985; Grant et al 1989; Vintzileos et al. 1993; Vintzileos et al. 1995). At introduction the hypothesis was to reduce the perinatal neurological damage or death by introducing continuous monitoring. The conclusions of the studies were that intrapartum EFM, as the primary and only method of intrapartum fetal surveillance, was associated with decreased neonatal seizures at the cost of a higher rate of surgical intervention. In a high risk population EFM has also been shown to reduce perinatal mortality (Vintzileos et al. 1993).
FHR monitoring has not been shown to prevent cerebral palsy (CP), but should prevent intrapartum fetal loss. The incidence of cerebral palsy remains unchanged after introduction of EFM at approximately 2/1000 births, but only a small part of the incidence is due to intrapartum asphyxia (MacDonald et al. 1985). The majority of CP cases are due to antepartum events.

Fetal scalp blood sampling

pH

In 1962 Saling described a technique for taking blood samples from the fetal scalp (FBS) after ruptured membranes (Saling 1962; Saling 1964; Saling & Schneider 1967). To analyze pH in fetal blood during labour as an indicator of hypoxia has since then been regarded as the `gold standard´ in the identification of intrapartum fetal hypoxia (Bretscher & Saling 1967). A pH >7.25 is considered normal, 7.20 – 7.25 as preacidemia, <7.20 acidemia. A pH <7.20 was suggested as cut-off value to recommend intervention (Huch et al. 1994) The limitation of this method is the invasiveness to the fetus, and is associated with a number of practical problems. pH analyses of fetal blood needs a relatively large amount of blood (30 – 50 µl), and has a sampling failure rate of 11-20% (Westgren et al. 1998; Kruger et al. 1999; Westgren et al. 1999; Ramanah et al. 2005). The disruption of the fetal skin makes vertical infectious transmission a risk, especially in women with HIV and hepatitis. Maternal blood, secretions and amniotic fluid can lead to erroneous pH values. Analysis of pH does not discriminate between respiratory and metabolic acidemia, the latter known to be the parameter associated with neonatal morbidity.

Lactate

Lactate is a metabolite in anaerobic metabolism and reflects tissue hypoxia. Studies analysing lactate in FBS have been published since the 1970s (Yoshioka & Roux 1970; Eguiluz et al. 1983; Smith et al. 1983). These reports showed that lactate concentration in the fetal scalp blood correlate with fetal acidosis in a significant way, but the same technical problems as with the analysis of pH was shown. These methods required large volumes of blood (150µl). Lactate is good in the identification of depressed newborns, well comparable with pH analyses, but was not regarded as clinically useful because of the large amount of blood. Further observational studies showed, however, that lactate had similar or better predictive properties compared with pH analysis in the identification of short term neonatal morbidity. Since the 90s , a new method

has been evaluated, a simple micro volume technique for measuring lactate in fetal scalp blood (Shimojo et al. 1993; Nordstrom et al. 1995; Nordstrom et al. 1998; Westgren et al. 1998; Kruger et al. 1998; Ramanah et.al. 2005). This electrochemical test strip devise needs only 5 ul of blood, and gives the result within 60 seconds. One of the potential advantage with lactate over pH in fetal scalp blood measurements is the smaller amount of blood needed (5 ul vs.30-50 ul), which leads to a higher success rate in obtaining an adequate sample for analysis. With this new lactate method (Lactate-Pro^{TM}) a lactate value <4.2 mmol/l is considered as normal value, 4.2 – 4.8 mmol/l as preacidemia, and >4.8 mmol/l as acidemia (Kruger et al. 1998). In cases with preacidemia, a repeat FBS is recommended within 20-30 minutes, if no other indication for intervention is present. In cases with acidemia the clinician should consider delivery. Studies suggest that lactate levels may be more predictive of subsequent neurological morbidity (Yoshioka & Roux 1970; Eguiluz et al. 1983; Smith et al. 1983; Nordstrom et al. 1995; Kruger et.al 1999).

Apgar score

It has been said that `Every baby born is first seen through the eyes of Dr. Virginia Apgar´. More than 50 years ago (1953) Dr Apgar described for the first time a new method which evaluates the status of new born infants according to `5 signs´ (Apgar 1953). The status was taken at 60 sec and 5 minutes after the baby was born. In the original report (1021 infants), she concluded that there was a correlation between Apgar score and neonatal outcome. Ten years later they confirmed the relationship of a poor Apgar score with neonatal mortality as well as the acid-base status of the newborn (Apgar & James 1962). By using this method, their goal was to teach the staff in the delivery room to "observe several physical signs at once, evaluate them rapidly and act accordingly".

It is doubtful whether Dr Apgar was aware of that a similar evaluation system had been proposed 18 centuries earlier, by a Greek physician by the name of Soranus, who practised in

Rome (AD 98-138). Heart rate was the only indicator not included in "Dr. Soranus score", because the circulatory system had not been discovered at this time.

Subsequent publications have shown a poor correlation between Apgar score, neonatal acidosis and neurological outcome (Sykes et al.1982). Dr Apgar´s goal of focusing attention on the infant immediately after birth has however succeeded. The method is used worldwide.

PERINATAL ASPHYXIA

The definition of asphyxia is derived from a Greek word which means "a stopping of the pulse", and it describes a condition with a lack of O_2 and an excess of CO_2 in the body. Ischemia is characterized by reduced blood perfusion in a tissue bed. Hypoxia refers to tissue concentrations of oxygen less than normal, and implies that there is insufficient oxygen supply to the tissue for their metabolic requirement. Hypoxia and ischemia occur simultaneously or in sequence. Anoxia indicates a complete lack of oxygen and of such severity as to result in permanent damage to the tissue.

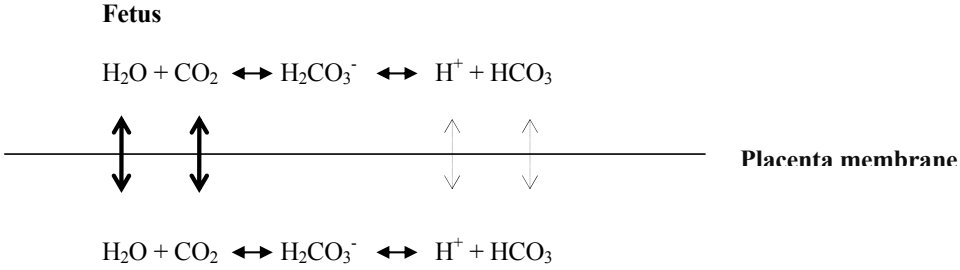

Fig.8. Taken from `Perinatal acid-base balance´ (Rooth, Studentlitteratur 1988).

During asphyxia gas exchange is reduced for some reason, resulting in an anaerobic metabolism with an excess of CO_2, lactate and thereby metabolic acidosis. Possible cause of asphyxia during labour might be; interruption of umbilical cord circulation, placental abruption or placental

insuffiency. Maternal causes of asphyxia might be; hypotension, hypertension, abnormal uterine contractions (dystocia) or impaired maternal oxygenation for any reason (Parer 1980).

The purpose of fetal acid – base balance is to maintain pH within a physiological range by the elimination of undesired metabolites through the placenta. It is important for the fetus to maintain stability. CO_2 and H_2O have a free passage across the placental membranes, H^+, HCO_3 and lactate equilibrate slowly, and only in exchange with similarly charged ions or together with those which have an opposite charge. When hypoxia for some reasons occurs, initially CO_2 accumulate because of reduced blood flow, pH fall and pCO_2 increase. If hypoxia continues, the fetus is dependent on anaerobic glycolysis for energy requirements. In this state glucose can only be oxidased from pyruvate to lactate. Lactate accumulates together with an excess production of H^+, and creates a further fall in pH. Studies have shown that the lactate acidosis, while reflects tissue hypoxia, is more harmful to the baby than respiratory acidosis. The lower metabolic rate and a good glycogen reserve will help the fetus to withstand a shorter period of asphyxia without complications.

If the fetus is exposed to hypoxia, several compensatory mechanisms will develop in an endeavour trying to maintain intracellular steady state (Peeters et al. 1979). Increase heart rate and to shunt blood to provide sufficient oxygen to vital organs such as the liver, the heart, the brain and the adrenal glands is one mechanism. Decreasing breathing and gross body movements is another way to lower the oxygen demand. Brain injury occurs only when asphyxia is severe enough to impair cerebral blood flow, and the defence mechanisms have been strong enough to maintain stability. Unfortunately no specific FHR pattern predicts subsequent neurological impairment. Speed, onset, degree and duration of oxygen deprivation are important in assessing the severity of hypoxia. Severe fetal hypoxia of short duration may result in a

profound cord artery acidemia, but can easily be compensated by the newborn, when the exchange is restored.

The mechanism of neuronal cell damage in the fetal brain after a hypoxic/ischemic event has generated interest in experimental research over the last few years. It has been established that the cell damage occurs in two phases. A primary loss takes place at the time of the hypoxic event, due to deterioration of the cellular steady state including acidosis, disturbed ion distribution and altered tissue perfusion. The secondary loss during the reoxygenation phase is due to metabolic changes, neurotoxicity, and circulatory changes (Pulsinelli et al. 1982; Blomgren & Hagberg 2006).

Careful intrapartum surveillance is recommended to prevent asphyxia during labour. Asphyxia before or during birth is an important cause of neurological morbidity and perinatal mortality. Neonatal encephalopathy is a serve complication of asphyxia. Sarnat and Sarnat described hypoxic-ischemic encephalopathy (HIE) as a specific condition, during the neonatal period (Sarnat & Sarnat 1976). They described three stages of HIE: mild, moderate, and severe. The presence of this condition was shown to predict future neurodevelopmental handicap. Later it became obvious that not all babies with a clinical picture of HIE were, or had been, hypoxic during labour. It is reported that 3 out of 4 asphyxia related neuropathologies in the fetus occur in the antepartum period, before labour has started (Perlman 1997; Meberg & Broch 2004; Himmelmann et al. 2005; Graham et al. 2006). This clinical picture could also occur in cases of infection (Wu & Colford 2000; Wu 2002; Hagberg 2003), hypoglycaemia, intracranial haemorrhage or metabolic disorders. Despite intrapartum fetal monitoring of present day, the rate of CP is still 1 or 2/1000 births (Perlman 1997; Derrick et al. 2007).

Aims of the studies

The studies upon which this thesis is based were designed to fulfil the following aims:

- To determine the optimum "cut-off" lactate concentration in vaginal fluid to discriminate between ruptured and intact membranes.

- To compare performance of the 'Lac-test' and a commercially available test based on analyses of IGFBP-1 (actim PROM-test) in the diagnosis of PROM.

- To investigate the association between lactate concentrations in vaginal fluid and time to spontaneous onset of labour for women with suspected PROM, and to examine whether lactate in vaginal fluid can be used to predict time to onset of labour in these cases.

- To assess whether measuring the lactate concentration in amniotic fluid during labour could be used in clinical practice to identify dystocic labour at an early stage.

- To assess whether lactate analysis in fetal scalp blood with the electrochemical test strip method, Lactate-Pro^{TM} is at least as good as pH analysis in the prevention of birth asphyxia.

Materials and Method

ETHICS

The studies have been approved by the regional ethics committee (Karolinska Institute, D no: 99/02, 01/351, 109/02). Oral informed consent was obtained from all the women in study I - III. In paper IV, information about the study was given to the pregnant women in late pregnancy at the antenatal clinics, and consent was acquired at this time or when the woman was admitted in labour.

MATERIAL

In paper I – III, all women were attending the department of Obstetrics and Gynaecology at General South Hospital, Stockholm. In paper I-II, the women had a singleton pregnancy, a suspected history of PROM (scanty leakage of fluid from the vagina) after 34 weeks gestation and without uterine contractions. In paper III, the inclusion criteria in the study were women with a singleton fetus in cephalic presentation, gestational age >34 completed gestational weeks. They all had a cervical dilatation ≥4 cm, ruptured membranes, no meconium staining of the AF, regular painful uterine contractions lasting at least 20 seconds, and no evidence of fetal distress. In paper IV, the women were recruited from ten different labour wards in Sweden. The inclusions criteria were; singleton pregnancy, cephalic presentation, gestational age ≥34 weeks, and a clinical indication for fetal scalp blood sampling.

METHODS AND DESIGN

Study I and II were designed as prospective observational studies. The clinical management was based on whether AF was visible or not. If AF was observed, induction of labour was planned after two days if labour had not started spontaneously. If AF was not seen and the

pregnancy was otherwise uneventful, the woman was sent home with no further follow-up planned. Visible AF at speculum examination was regarded as `true´ ruptured membranes. In study I -III, the lactate concentration was analysed and registered by an independent nurse, and the value was concealed from the clinician in charge of the delivery ward.

Paper I

Two hundred women with a history of suspected PROM were recruited. The end-point was whether or not there was visible AF. Half the cases (n = 100), had an actim PROM-test™ (Medix Biochemical OY AB, Finland) carried out.We wanted to compare performance of the 'Lac-test' with PROM-test™, a test which has previously been evaluated as reliable in a clinical setting (Eriksen 1992; Darj E & Lyrenas S 1998; Erdemoglu E & Mungan T 2004). The outcome of study I was to evaluate the performance of the `Lac –test´ after a cut –off value had been derived, and to compare it with a commercially available test (PROM-test™).

Paper II

Three hundred women were recruited, of whom 179 had spontaneous onset of labour at South General Hospital These women constitute the material of the study. Women excluded from the study were as follows; 47 delivered at another hospital in Stockholm, 62 with labour induction, and 12 who were delivered by emergency caesarean section before the end of the latent phase (i.e. before the cervix was ≥4 cm dilated). End-point was time from speculum examination to spontaneous onset of labour.

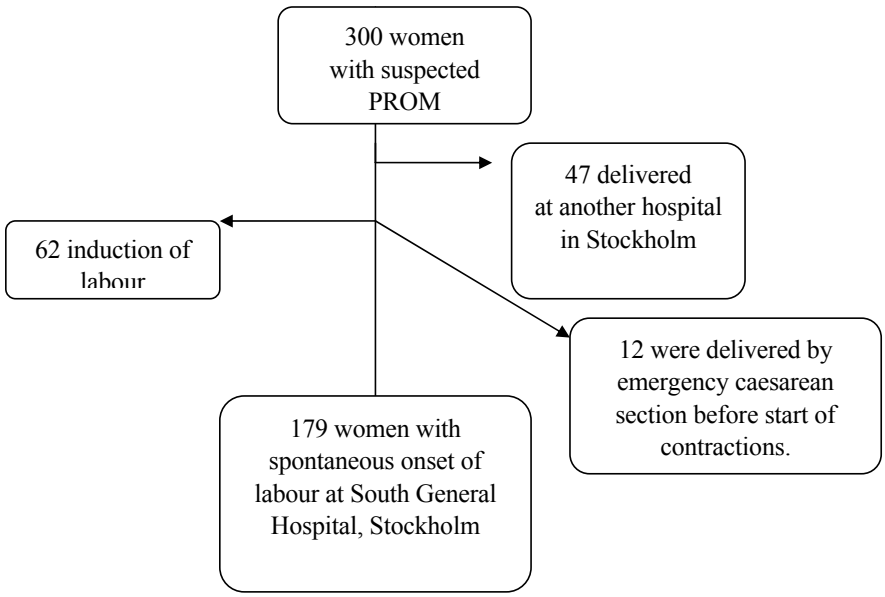

Fig.9. Flow chart for women recruited in paper II.

In study II, 86 women from study I were included. As we had different end-points in the two studies, visible AF at speculum examination or not and time from examination to spontaneous onset of labour, we found the cases to be independent in relation to the previous study. Due to the different end-points we found no reason to exclude these 86 cases. This has been explained in correspondence with the journal (Wiberg-Itzel 2007).

The main outcome in study II was to explore whether there was an association between lactate concentration in vaginal fluid and time to spontaneous onset of labour for women with suspected PROM, and to examine if lactate in vaginal fluid can be used to predict time to onset of labour in cases with suspected PROM

Paper III

The study was designed as a prospective observational study. Seventy-five women had an intrauterine pressure (IUP) catheter inserted from which 2 ml sample of AF was collected at insertion. Thereafter the aim was to sample AF every 30 minutes for lactate concentration determinations. We used IUP to make the sampling procedure more standardised. We excluded 21 women from the study as they were instrumentally delivered due to other reasons than labour dystocia. The remaining 54 women had at least two measurements of lactate in AF collected 60 minutes apart.

The aim of the study was to asses whether measuring the lactate concentration in amniotic fluid during labour could be used in clinical practice to discriminate between normal and dystocic labour at an early stage. Main outcome was spontaneous or instrumental/operative delivery due to dystocia.

Paper IV

Three thousand and seven women were randomised to either fetal scalp blood pH or lactate analysis when the clinician decided to perform an FBS due to a non-reassuring or ominous fetal heart rate traces. Fifteen cases were excluded due to multiple pregnancies or gestational age <34 weeks, leaving 2992 cases which constitute our material, 1496 were randomised to pH and 1496 to lactate determinations. The primary end-points of the study were metabolic acidemia in cord artery blood at birth (pH <7.05 and base deficit$_{blood}$ >12 mmol/l) or pH <7.00. The aim of the study was to compare pH with lactate analysis, regarding prevention of acidemia at birth. Randomisation and data entry were performed using an internet-based system. If sampling or analysis failed, management had to be carried out based on other clinical information. No change to the alternative method for FBS analysis was allowed, and if cross-over occurred, it was regarded as protocol violation.

At randomisation stratification was performed for each participating labour ward.

As some of the departments used the fetal electrocardiogram (the STAN-monitor™) in clinical practice, stratification for the use of this method was also performed.

BIOCHEMICAL MEASUREMENTS

Lactate measurements

An assay system for measuring lactate (Shimojo et al. 1993; Nordstrom et al. 1995; Nordstrom et.al 1998) was used. The Lactate-Pro™ consists of a single-use test strip of an enzyme-coated electrode and a small meter. The system requires 5 ul of whole blood/AF. The electrode strip is made up of three plastic films: a cover sheet, a spacer, and an insulation layer printed with electrodes that are coated with lactate oxidase and ferricyanide as an electron mediator. The meter measures the magnitude of the anodic current of the reduced mediator by the enzymatic reaction and displays the lactate concentration 60 s after a sample (5 ul) is applied. This assay provides a rapid and convenient test for measuring lactate concentration. The coefficient of variation (CV) for blood is less than 4% in the intercept of clinical interest (3-10mmol/l) (Nordstrom et al. 1998). Lactate samples of AF had a CV of 1.7–3.0% for AF (Wiberg-Itzel et al. 2005)

Acid-base balance

The women in study IV were recruited from 10 different labour wards in Sweden. Different blood gas analyzers were used in different departments. Base excess is generally defined as a amount of acid required to restore pH to 7.40 at PCO_2 8.3 kPa at 37ºC, at the actual saturation of blood, expressed in mmol/l (Rooth, 1988). Full acid-base analysis means that pH and PCO_2 are measured and BD is calculated from these two parameters. In paper IV, base deficit was calculated for the blood compartment with the algorithm used by Radiometer™ blood gas analyzer, recently reported to show a higher association with neonatal depression than base deficit calculated for the extracellular fluid compartment (Wiberg et al. 2006). Since hemoglobin

concentration in cord blood was not registered, we used the general approximation of a hemoglobin concentration of 150 g/l.

The cut off value for defining pathological fetal acidemia is discussed, i.e. the threshold which is associated with neonatal morbidity and mortality. Pathological fetal acidemia is defined as a pH <7.0 and a BD>16mmol/l (Goldaber et al. 1991; Winkler et al. 1991) according to `The American College of Obstetrics and Gynecology´. Goldaber and co-workers tried to define the pH cut-off more precisely (Goldaber et al. 1991). They looked at 3506 term newborns, with an umbilical artery pH <7.20. They divided them in 5 groups, and discovered that significantly more newborns with a pH <7.00 had low 1`and 5`-minute Apgar score, and neonatal death was significantly more common in this group. The significant pH cut off for *all seizures* was found to be <7.05.

A study made by Low and co-workers in 1994 showed that severe respiratory acidosis at delivery does not lead to newborn complications. However, intrapartum fetal asphyxia with severe metabolic acidosis at delivery accounts for complications in the central nervous system, cardiovascular system, respiratory system and kidneys (Low et al. 1994; Low 1997). The magnitude of the complications is dictated in part by the severity and duration of the metabolic acidosis. The criteria for metabolic acidosis used in their study was an extracellular base deficit of >12mmol/l. The average equivalent of pH was 7.08 (6.95-7.27) due to the range of PCO_2.

In our study IV, the primary end-points were metabolic acidemia in cord artery blood at birth. We defined metabolic acidemia as pH <7.05 and base $deficit_{blood}$ >12 mmol/l, and we used pH <7.00 as another primary end-point, according to the referred earlier studies.

Blood/amniotic fluid sampling

In studies I, II and IV the woman was in a supine position with her legs in stirrups during sampling of vaginal fluid or fetal scalp blood. The samples of vaginal fluid for lactate analyses

(paper I and II) were collected from the top of the speculum after vaginal inspection. The lactate test strips were introduced in the lactate meter and then dipped into the fluid pool remaining on top of the speculum. The lactate analysis was carried out at the bedside. In study III, samples of AF were collected in a 2 ml syringe from the IUP. The test strips were introduced into the lactate meter and the end of the test strip thereafter dipped into a pool of AF which was applied on the folium hosting the test strip.

The procedure for the fetal scalp blood (FBS) sampling has been described in detail by Saling (Saling 1962; Saling 1964; Saling & Schneider 1967). Blood samples were collected in preheparinized glass capillary tubes. For lactate concentration determination, the test strips were introduced into the lactate meter and the end of the test strip thereafter dipped into the pool of blood which was applied on the folium hosting the test strip. Lactate analysis of fetal blood was carried out at the bedside with the lactate meter. The pH analyses were performed immediately using equipment in the labour ward.

In study IV, cord blood samples were collected immediately after delivery, before the baby's first cry, by puncturing the cord artery and vein with a fine needle and aspirating blood into a pre-heparinised syringe. A segment of cord was instrumentally or manually (temporary) clamped. Approximately 2 ml of blood was drawn into a plastic syringe. The syringe was prepared with a heparin solution (100 IU/ml) which has been shown not to alter the pH value (Kirshon & Moise 1989).

Statistics

Receiver operator characteristic (ROC) curves were constructed to determine `cut-off` lactate concentration to distinguish between visible or non-visible AF (paper I), and to discriminate between dystocic and normal labour (paper III). We also used ROC curves to evaluate a practical decision rule for women who had one, two and three or more consecutive samples with

high lactate determinations during labour (paper III). A ROC curve shows the possible values of sensitivity (true positive test) and specificity (true negative test) for all possible 'cut- off' values. An ideal test has both a high sensitivity and specificity.

Cumulative event rates of not being in labour for different concentrations of lactate were calculated using the Kaplan-Meier method (study II). The Kaplan-Meier method, display the cumulative probability of an individual remaining free of the end-point at any time after baseline (Kaplan & Meier 1958).

We used uni- and multivariable logistic regression to estimate the association between lactate concentration in vaginal fluid and time to onset of labour (paper II), and the association between lactate concentrations in AF and dysfunctional labour (paper III), (Kass 1980; Bagley et al. 2001). In logistic regression the question is which explanatory variables influence the outcome, and which can be used in the equation to predict the outcome category into which an individual will fall from values of her explanatory variables. We used the following model strategy to enter the explanatory variables. First, crude associations of each explanatory variable with the odds were studied in univariable models. Second, the crude associations were adjusted by entering variables into a multivariable logistic model in a backward procedure. The criteria for inclusion and removal of a variable were set to $p < 0.05$ and $p < 0.10$, respectively. The associations were presented as odds ratios (OR) with 95% confidence intervals (CI). Interactions between the explanatory variables in the models were tested by including a multiplicative term in the model. Variables and interactions were tested with the Wald test, and considered significant if $p < 0.05$

To describe a practical prediction rule to categorise whether a woman will be in labour or not, classification trees were built using the CHAID algorithm (Kass 1980). A CHAID analysis starts with all data in one group. Each possible split on each explanatory variable is considered, to find

the split that leads to greater significance on the dependent variable. The resulting split groups were further divided until one of the following criteria were reached; tree depth was limited to four levels, no group with less than 50 patients was formed and no split, with a Bonferroni adjustment of less than 0.05 was executed.

To evaluate the predictive capabilities of lactate concentration in vaginal fluid/AF sensitivity, specificity, positive (PPV), negative (NPV) predictive values with 95% confidence intervals (C.I.) and likelihood ratios (LR) for positive and negative tests (paper I, II, III) were calculated. LR for a positive result is the ratio of the likelihood of a positive result if the patient has the disease to the likelihood of a positive result if she does not have the disease. A high LR (>2 is described as somewhat useful, >5 moderately useful,>10 very useful.) suggests a high likelihood of the clinical outcome after the result of the test is known. LR can also be generated for a negative test result (Chien & Khan 2001). The kappa indices were also calculated (paper I, II), which provides a measure of the degree to which two diagnostic tests performs a sorting on the basis of specified criteria (Khan & Chien 2001).

In paper IV; a sample size analysis was calculated. We estimated the prevalence of metabolic acidemia to be 1.6% in a group requiring FBS for pH analysis. Our intention was to correctly identify a 100% increase of acidemia between the two groups (from 1.6 to 3.2%), with a precision of 80% power at 5% significance level, two-tailed. A total of 2872 participants (1436 in each arm) were required to detect this difference. Regarding pH <7.00 as the end point and with an estimated prevalence of 4%, to detect a 50% decrease or increase (to 2% and 6%, respectively) 1141 cases had to be included in each arm.
Study IV was analysed on an `intension to treat´ basis. It means that all women on whom we have information are analysed in the groups to which they were originally allocated; irrespective of whether they followed the treatment regime or not. The chi-square test and

relative risks (RR) with 95% confidence intervals (C.I.) was used to compare the pH and lactate groups. The relative risk measures the increased (or decreased) risk of event associated with exposure to the factor of interest. A relative risk of 1.0 indicates that the risk is the same in the two groups. A risk >1.0 indicates an increased risk in the exposed group compared with the unexposed group. A p-value of less than 0.05 was considered significant.

An independent steering committee was established to conduct interim analyses after 1400 and 2400 randomised cases. After the second analysis (in November 2005) it was recommended to close the study after 3000 cases, which was estimated to be by the end of 2005.

The statistical analyses were performed in SPSS 14.0 (SPSS Inc. Chicago, Illinois, USA), and the statistical Package Statistica for Windows, version 7.0 (Stat Soft, Tulsa, Oklahoma, USA).

Results and discussions

PRELABOUR RUPTURE OF THE MEMBRANES (PROM)

Clinical management of PROM (paper I)

In study I, we looked for a good, reliable and useful clinical test for PROM with both a high sensitivity and a high specificity. We wanted a test which would be easy to use in the clinical situation with an answer immediately available at the bedside. Previous studies have shown that lactate concentration in AF is high (Fadel et al. 1979; Brace 1997). We found that a vaginal fluid lactate concentration of ≥4.5 mmol/l in women having a history of suspect PROM was the best cut-off value to discriminate between visible/non visible AF at speculum examination.

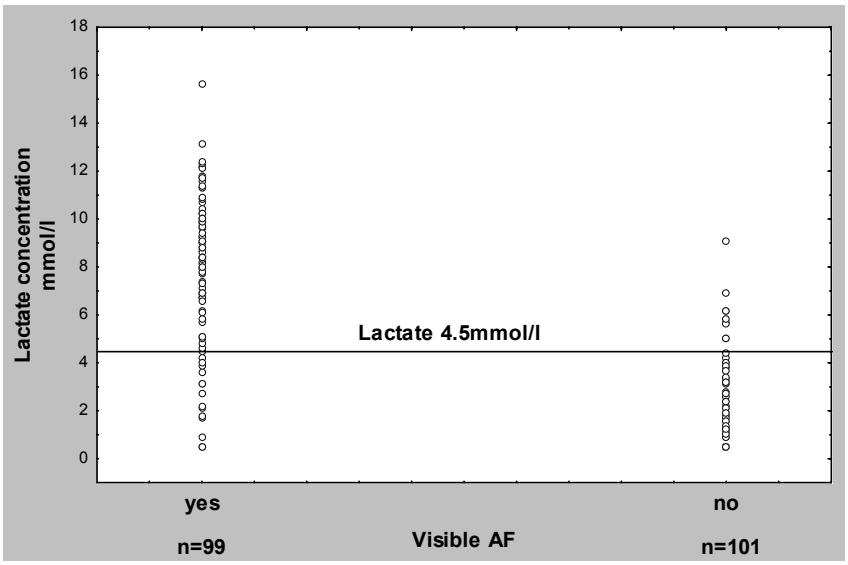

Fig.10. This scatter plot shows cases with and without visible AF and their measured lactate concentration in vaginal fluid.

Many clinical trials have tested biochemical methods to detect AF when PROM is suspected. The DAO test has been shown to be a good test with a sensitivity of 84-100% and a specificity

of 74-100% (Wishart et al. 1979; Gahl et al. 1982; Bank et al. 1991). The method was rather impractical, as laboratory service was needed which delayed the result for 2 days. The DAO test is not available today because of its use of toxical chemicals. Another test available o the market is the actim PROM-test which has been evaluated in many studies (Rutanen et al. 1993; Darj & Lyrenas 1998; Erdemoglu & Mungan 2004). The sensitivity of the actim PROM-test™ is reported to be 71-100% and specificity 88-100%. In our study (paper I), we found that the main difference between the 'Lac-test' and the actim PROM-test™ was the sensitivity. When there was visible AF at speculum examination (our end-point), the sensitivity of the 'Lac-test' (i.e. the prevalence of a positive 'Lac-test') was 86%, while sensitivity for the actim PROM-test™ was 60 %. However, all cases with positive actim PROM™ tests also had positive 'Lac-tests'.

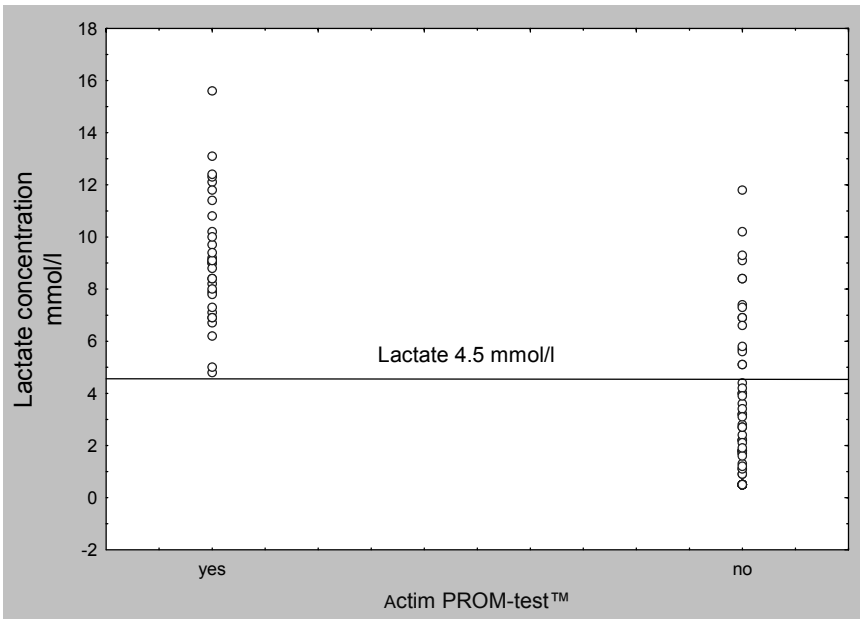

Fig. 11. This scatter plot shows cases with a positive actim PROM-test™ a negative one and their measured lactate concentration in vaginal fluid.

A likely explanation of the low sensitivity for actim PROM-test™ in our study as compared with others (Eriksen 1992; Erdemoglu E & Mungan 2004) was the differences in the study designs. The trial quoted had a more 'experimental design', analysing test performance in cases with obviously ruptured membranes (artificially or spontaneously). Our series were cases with suspected PROM, excluding cases with poring water. In a study made by Darj and Lyrenäs (Darj & Lyrenas 1998), they reported a sensitivity of 71% for cases attending with only a history of suspected PROM, which is a better comparison with our data, and more in agreement with our results for the PROM-test™.

In most cases, the diagnosis of PROM is obvious. The woman describes having experienced a history of limited water-like secretions from the vagina, and water is seen streaming down the legs or in pads. However, there still remain cases in which the history is strongly suggestive of ruptured membranes but at physical examination no AF can be seen. In these situations a speculum examination is recommended to confirm or exclude ruptured membranes. In the study by Ladfors and associates (1997), a false negative diagnosis with visual inspection of speculum examination was found to be 12% (Ladfors et al. 1997) (DAO test was used as true PROM). No increased morbidity (i.e. infection) was found in this group. No comments were made in that trial on the assumed false positive group of women with suspected PROM., i.e. those where AF was seen but the DAO test was negative. However, in the trial quoted 3.1% of the women were reported to have signs of intact membranes at induction of labour, and could represent cases with false positive diagnosis at inspection.

Even when a speculum examination is performed, our experience suggests that all ´water seen´ is not always ruptured membranes. Consequently, no visible AF can be a false negative observation, and visible AF can be false positive one. We have in our study 55 women with visible AF, 44 of these had positive 'Lac-tests 'and 31 had positive actim PROM-test™. If the

woman has not started labour spontaneously within 48 hours after PROM is diagnosed, she will normally be exposed to induction of labour. In paper I, 62 women were induced because they had not commenced labour within 48 hours. Only 28/62 of these women had a lactate value ≥ 4.5mmol/l at examination but 42/62 women had `visible AF´. We had a 44% intervention rate (instrumental or emergency caesarean delivery) in the induction group. A particularly high frequency of intervention occurred in the group of women with visible AF but low lactate concentration. This is an important finding, as reliable diagnosis might prevent unnecessary intervention, the `Lac-test´ is shown to be such a reliable test, which also is simple and handy in the clinical management.

To summarise paper I, the `Lac-test´ was found to be a reliable test with both a high sensitivity and a high specificity (86%, and 92% respectively). In our series it performed better than the commercial available actim PROM-test™. Its ease of application makes it attractive in clinical practice.

Myometrial lactate production

The knowledge that amniotic fluid (AF) contains high concentration of lactate has been published since the 1970s (Fadel et al. 1979; Brace 1997). Some publications have suggested that the source of lactate in AF is the fetus itself, mainly through urine and lung excretions (Perks et al.1991).

In a series of 30 planned cesarean sections we analysed concentration of lactate in AF and the lactate concentration of the newborn's first urine production immediately after delivery (n=23 non published data). The concentration of lactate in the urine was low, with a mean value of 1.5 mmol/l (range 0.5-3.3 mmol/l). The mean lactate concentration in AF determined simultaneously was 8.9 mmol/l (range 6.6 – 10.8), and there was no significant correlation between concentration of AF and urine lactate concentration. Lactate is reported to be secreted

to AF with fetal intrauterine expiration (Perks et al. 1991). However, this possible source of AF lactate is limited by the fetal blood lactate concentration, which under normal conditions is low (<4.2 mmol/l). Hence, our conclusion is that the fetus contributes to the lactate concentration in AF, but only to a minor degree. There has to be another main producer of lactate in AF.

The lactate concentration in amniotic fluid is reported to be 4 - 6 times higher compared with fetal and maternal blood. A number of papers have been published on the myometrial acid-balance, lactate and pH changes and the correlation to changes in myometrial activity (Taggart & Wray 1993; Taggart et al. 1996; Taggart et al. 1997; Taggart & Wray 1998; Quenby et al. 2004). In paper I, we measured lactate concentrations in vaginal fluid in women with suspected PROM, between 0.8 and 15.6mmol/l. With this wide range of lactate concentrations we questioned whether the different lactate concentration in vaginal fluid could reflect the activity of the uterus during the latent phase.

It has been shown in sports medicine that lactate exchange in muscles is a dynamic process with simultaneous lactate uptake and release at rest and during exercise (Brooks 1986; Brooks 2000; Brooks 2002). A pregnant uterus before the onset of contractions might have a slow release of lactate into the surrounding fluid, i.e. into the AF, on a net basis. This might lead to a balance in the production and release of lactate during pregnancy. We hypothesised that when labour contractions start, the uterine muscle increases its production of lactate. As no other obvious producer of lactate in high concentrations then the myometrium has been described, we speculate that this is the source of AF lactate. There are publications about a vigorous glycolytic flow when the uterus is active (Steingrimsdottir et.al. 1995; Steingrimsdottir et.al 1997).

Prediction of onset of labour (paper II)

At term pregnancy PROM is often a part of normal parturition and most of the women with PROM will have a spontaneous onset of labour within a limited period of time. PROM occurs in 5-19% (Hannah et al. 1996; Ladfors et al. 2000) of all patients at term and is followed by spontaneous onset of labour in 60% within 24 hours and in 95% within 72 hours (Hannah et al. 2000). However, it is crucial to diagnose ruptured membranes (paper I). Some 10% of pregnant women at term attend hospitals with suspected PROM, and to have the possibility to predict those who will start labour spontaneously would clearly simplify management. A good prediction is also appreciated by the parturient.

In our study II time to onset of labour was essentially similar among women with lactate concentration of ≥4.5mmol/l. Median time to onset of labour was in the group of women with lactate concentrations of between 4.5 – 9.0 mmol/l was 10 hours, and among those with lactate concentrations of >9.0mmol/l, 8 hours. In contrast, women with lactate concentrations <4.5 mmol/l appeared to have longer time to spontaneous onset of labour (median time 54 hours). There is a significant difference in lactate concentration in vaginal fluid in women with suspected PROM, and time to onset of labour, but only among the groups ≥4.5 mmol/l and <4.5mmol/l.These findings lend support to the view that it is the rupture of the membranes (ROM) which is crucial to diagnose, when estimating the probability of spontaneous onset of labour within one or two days.

In clinical practice there is a lack of any adequate predictor to identify women with spontaneous onset of labour within a certain time limit. Transvaginal ultrasonographic measurement of cervical length is one method which has been used (Rust et al. 2005; Tsoi et al. 2005; Tsoi et al. 2006). However, this method is mainly used in cases with a risk of pre-term labour. No clear, rational and evaluated strategy for daily practical use has emerged. To make it possible to use lactate determination in vaginal fluids as a predictor of onset of labour

in term pregnancies, we designed a `decision tree´ (Kass 1980). The tree describes women with spontaneous onset of labour within 24 hours, based on lactate concentrations, visible amniotic fluid or not, and gestational age. We believe this could be of help in the clinical management of women with suspected PROM. For example, 96 (54%) of the 179 women were in labour within 24 hours. Among those with a lactate concentration ≥ 4.5 mmol/l, 76 (88%) had spontaneous onset of labour < 24 hours, compared with 20 (21%) among those with lower lactate. However, in the group where amniotic fluid was not visible and the lactate level was low (<4.5 mmol/l), only 10 (15%) had started labour within 24 hours. By using lactate concentration ≥ 4.5 mmol/l as cut-off, a total number of 149 (83%) would be correctly classified as to whether they were going to be in labour within 24 hours or not. This corresponds to a sensitivity of 79% (76/96) and specificity of 88% (73/83).

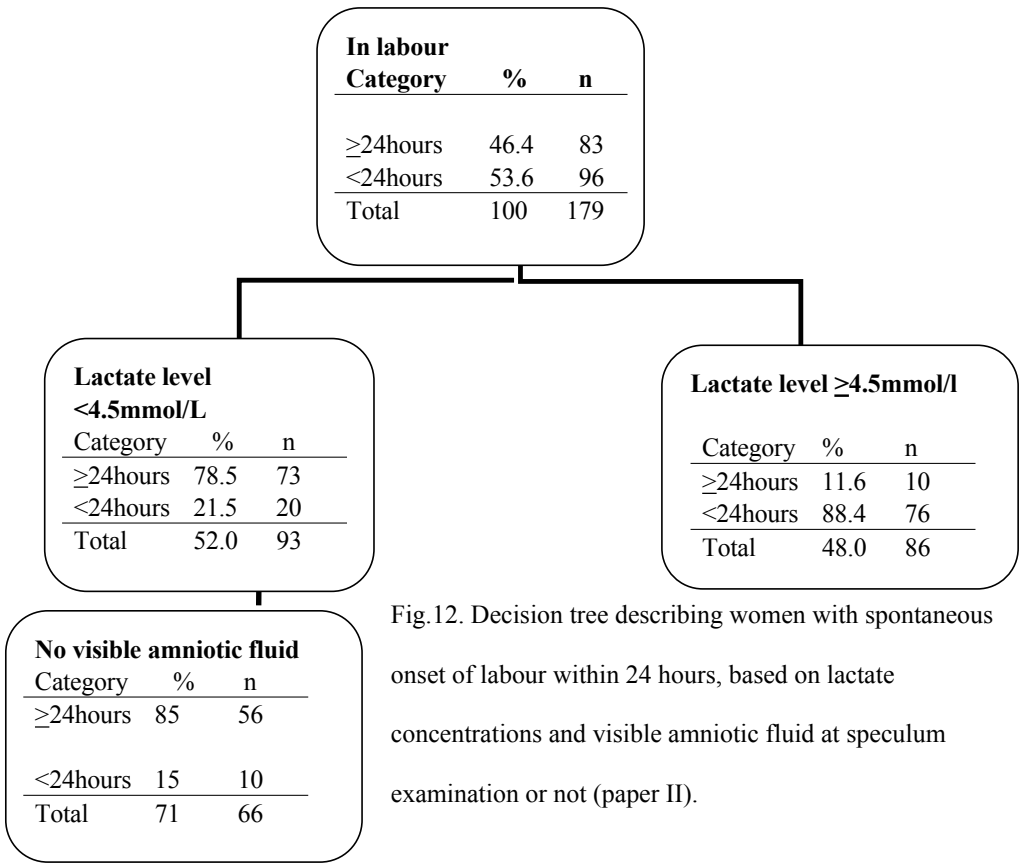

Fig.12. Decision tree describing women with spontaneous onset of labour within 24 hours, based on lactate concentrations and visible amniotic fluid at speculum examination or not (paper II).

Earlier studies from Hanna et al (Hannah et al 1996, Hannah et al. 2000) showed that women with PROM will in 60% of cases start labour within 24 hours as compared with 54% (96/179) in our study. We could correctly predict onset of labour within 24 hours in 83% of the cases if vaginal fluid lactate value is ≥4.5 mmol/l, and predict no onset of labour in 79% in the cases if vaginal fluid lactate value is <4.5 mmol/l. We found no significant correlation between different lactate concentrations in vaginal fluid ≥4.5 mmol/l, and spontaneous onset of labour. We suggest that the strong association between lactate concentration ≥4.5 mmol/l and onset of labour rather reflect that lactate is a very good marker of PROM and that broken membranes physiologically are followed by onset of labour.

Summarising paper II suggests that cases with suspected PROM (not water streaming down the woman's legs) should primarily be correctly diagnosed with the `Lac-test´, to avoid false positive tests at inspection and unnecessary intervention, and to obtain a good prediction of onset of labour.

Dysfunctional labour (paper III)

Prolonged labour is a common obstetrical problem world wide, and is one of the main indications for operative intervention during parturition. It is estimated that labour dystocia occurs in about 20% of all deliveries world-wide, and that labour dystocia has been one of the major contributors to an upward caesarean trend world- wide (Quenby et al. 2004). Prolonged labour frequently leads to physical and mental fatigue of the mother, the fetus, the midwife and the obstetrician. On the other hand, limiting the duration of labour inevitably leads to a higher incidence of instrumental/operative delivery, and it is sometimes a difficult decision to make. Few areas in obstetrics are less precisely defined and more subject to individual preference of the attending obstetrician than the time management of dystocic labour.

The common way of identifying labour dystocia is to use the partogram (Philpott 1972; Friedman 1973; Friedman 1978). In paper III we decided to use the outcome; i.e. spontaneous/operative delivery due to dystocia, instead of the usual use of `passing the alert line´ in the partogram. The duration of normal labour has a wide range and it is of great importance not to interfere during the progress of normal labour. We therefore choose operative intervention due to labour dystocia as the end-point, which should be a more robust definition of outcome than defining labour dystocia using information from cervicometry plotted in a partogram.

In papers I and II we found a wide range of lactate concentrations measured in vaginal fluids when PROM was diagnosed. Of the179 women 44 (25%) had a lactate value in vaginal fluid above the range of 7-9 mmol/l, which has been reported as normal concentration of lactate in AF during pregnancy (Fadel et al. 1979; Sims et al. 1993). We challenged the questioned whether `high lactate concentrations measured in vaginal fluid/ AF could describe uterine myometrial activity, and if `too high´ lactate concentration is associated with labour dystocia. It is well known from sport medicine that prolonged or intense muscle activity, especially when there is a shortage of 0_2, might lead to declined muscle strength (Tesch & Karlsson 1977; Kuitunen et al. 2007). This decline is known as muscle fatigue, and the consequence is a decreased power output from the muscle. This is obvious during sports activity. The basic question in paper III was whether `high lactate concentration´ in AF displays an inefficient contracting uterine muscle with an increased lactate production, and if this could be used in clinical practice to identify dystocic labour at an early stage.

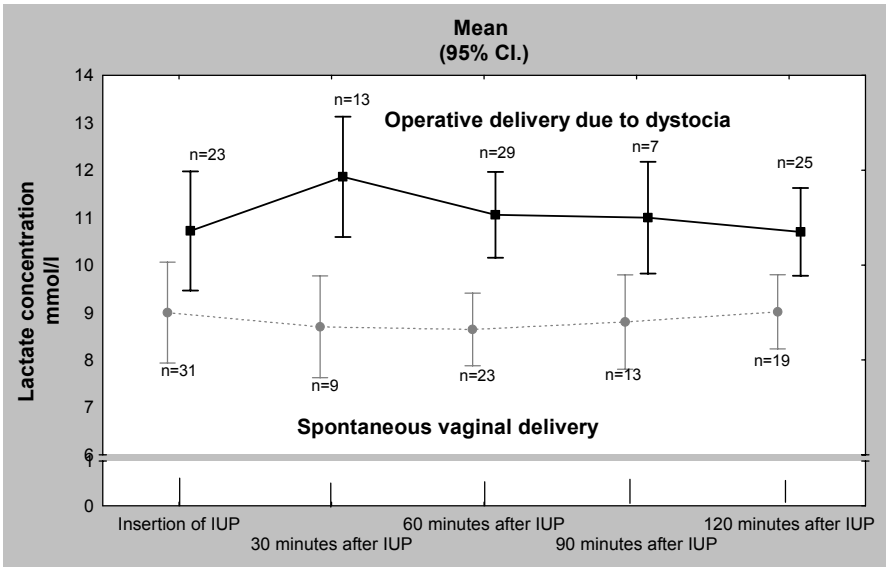

Fig.13. Mean lactate concentrations and 95% confidence intervals at the first five sampling occasions grouped according to normal (filled dots) and dystocic (filled squares) labour.

(From paper III)

The result of paper III showed a significant difference ($p<0.01$) in mean lactate concentrations in AF between women with spontaneous vaginal delivery (mean 8.9 mmol/l, range 6.6– 10.8) and women with operative delivery due to dystocia (mean 10.9 mmol/l, range 8.0 – 16.1).

Constructing ROC curves is a way of finding out the best `cut-off˝ value between best sensitivity (true positive test) and best specificity (true negative test) in the material. We constructed several ROC curves where we discovered that the best cut-off concentration to discriminate between normal labour and labour dystocia, was a lactate concentration ≥10.1 mmol/l. If we used at least two consecutive high lactate samples, with this method we will correctly identified 82% (44/54) of normal/dystocic labour in the study, with a false positive rate of 14% (4/29). The main purpose of a new diagnostic test for labour dystocia is not only to identify the condition at an early stage, but also to have a low proportion of women with false positive tests. This is justified

from the fact that such a test could introduce intervention in a labour which could have had a perfectly normal course.

Confounding factors of dystocia

Of the 54 women, 47 had oxytocin augmentation at any time during labour (87%). The mean time of use of oxytocin in the dystocic group was 9 hour (range 0-18) compared with 2, 5 hours (range 0-9) in the group delivered spontaneously, a significant difference ($p<0.001$). The use of oxytocin during labour leads usually to an increase of the uterine activity and a greater explosive force in the contractions. However, our speculation is that our common use of oxytocin can be ineffective in dystocic labour with an acidified uterine muscle. According to previous publications (Quenby et al. 2004), oxytocin might initially stimulate contractions, even in cases with dystocia. This might further worsen the acidification of the muscle and the myometrial oxygen supply in selected cases. The local oxygen supply might be even lower, and lactate production/accumulation will increase. The force of contractions might ultimately be reduced (Quenby et al. 2004). We know that an adapted dose of oxytocin might yield a better labour outcome, but an overdose of oxytocin, in a combination with labour dystocia, might be a potential danger to the fetus. Therefore, it is an urgent need to study lactate determinations in AF and augmentation with oxytocin.

In paper III, 41/54 (76%) of the women had epidural analgesia (EDA) during labour. In the group of dystocia, 28/31 (90%) had EDA, compared to the spontaneously delivered group where the figures were 13/23 (57%). We found a significant difference in the use of EDA between women with low lactate concentration in AF and spontaneous delivery and those who had a high lactate concentration in AF and were delivered due to dystocia ($p<0.01$). A meata analysis of studies comparing `epidural´ with `no epidural´ showed a 10% increased rate of cesarean section due to dystocia if epidural was provided (Sharma et al. 2004) . On the other hand, consensus

among others is that epidural has little, or no, effect on the dystocic- related cesarean section rate (Bofill et al. 1997). Further studies will show whether there is any association between lactate concentration in AF, labour dystocia and epidural analgesia.

One-third (18/54) of all women in the trial already had high AF lactate concentrations at first sampling. Of these 18 women 14 later fulfilled the criteria for dystocia (78%). Within this group of women with high AF lactate concentration already at first sampling, we might find early presenting inefficient uterine activity (primary dystocia), or women with prolonged latency phases. This is in comparison with secondary arrest after a time period with normal myometrial activity. Our speculation is that women with high AF lactate concentration at first sampling might already have inefficient uterine activity, and are likely to have an increased risk of primary labour dystocia, i.e. will never establish normal uterine contractions. We have a limited number of precipitating women in our study, too few to be able to draw more firm conclusions on this issue. This should be addressed in structured, longitudinal studies of AF lactate determinations, starting with AF sampling upon admission in early labour, in future research.

To summarize study III this is the first time lactate concentration in AF has been used as a diagnostic tool during labour. We have in this pilot study shown the usefulness of lactate in AF in the diagnosis of labour dystocia.

Prevention of birth asphyxia (paper IV)

Our trial is the largest published randomized study (2992 labouring women) comparing pH and lactate analyses of fetal scalp blood in the clinical management of intrapartum fetal distress. It is the first study of these methods powered to assess the ability to prevent acidemia at birth.

The result of the study showed no significant differences in neonatal outcome between the two management groups (pH vs. lactate) in metabolic acidemia (RR: 0.96. p=0.08), or pH <7.00 in cord artery blood at birth (RR: 0:84, p=0.65). Operative interventions were also similar in the two study groups, in total (RR: 1.02, p=0.74) as well as for the indication of fetal distress. We concluded that lactate analysis on fetal scalp blood prevented acidemia equally as well as pH, whereas blood sampling and assessment were more successful for lactate than for pH measurement (protocol violation RR 1.13, p<0.01).

Suggested cut-off lactate concentration to recommend intervention, 4.8 mmol/l, corresponds to the 75th percentile in a population where FBS is needed (Kruger et al.1998). Correspondingly, a pH value of 7.21 represents the 25th percentile. This implies that with both methods, one out of four analyses will end up in a pathological result. If both methods were equally good in the bedside management one could expect similar intervention rate due to fetal acidemia.

Protocol violation

A significant difference in blood sampling and analysis failure was found between the two methods, pH vs. lactate (protocol violation RR: 1.13, p<0.01). A higher failure rate with pH determinations was statistically significant, and led to protocol violations in 10.4% of the cases , i.e. the clinician decided to do a lactate analysis when pH blood sampling or analysis failed, comparing with 1.2% in the lactate group. A previously published randomised study (Westgren et.al 1998) have shown similar results, i.e. unsuccessful fetal blood sampling procedures occurred significantly more often in pH measurements than in the measurements of lactate (OR 16.1 with 95% CI 5.8-44.7).

Sampling failure rate was not specifically reported in paper IV. This event was probably a part of the group mentioned in the protocol as `no FBS collected´ or `protocol violation´. We also

believe that there were a number of failures among those cases where FBS was not collected and no reason was given. One could therefore speculate that there should be higher frequencies of metabolic acidemia among fetuses and/ or operative interventions with pH analysis, if the alternative method (lactate) was not available.

Fig.14. Comparing protocol violation between the pH and the lactate groups.

Primary end-points in a trial like paper IV should ideally be HIE. However, even in this large, selected group of 2992 high risk labouring women, this condition is rare, with a prevalence of HIE of 4/1000. This makes it impossible for HIE to be used as an end point to be able to show a significant difference. In the present trial these cases were evenly distributed between the management groups: six cases in the pH group and five in the lactate group.

STAN

To monitor term fetuses with cardiotocography combined with automatic analysis of ST waveform (STAN-monitor™) during labour involves a search for further improvement in fetal

surveillance during labour (Amer-Wahlin et al. 2001; Olofsson 2003; Ojala et al. 2006). The aim is to reduce the neonatal metabolic acidemia (umbilical artery pH <7.05 and base excess <-12 mmol/l) and operative interventions during labour. In paper IV a subanalysis was performed, as 785 of the women in the study were monitored with the STAN-monitor™ and stratified for FBS with pH or lactate analyses. In the ST analysis subgroup, metabolic acidemia occurred in 3.8 % of the cases randomised to management by pH compared with 2.0% in the lactate management group (RR=0.53, p=0.14). Corresponding values in the CTG only group were 2.8 % vs. 3.3 % (RR=1.16, p=0.53).

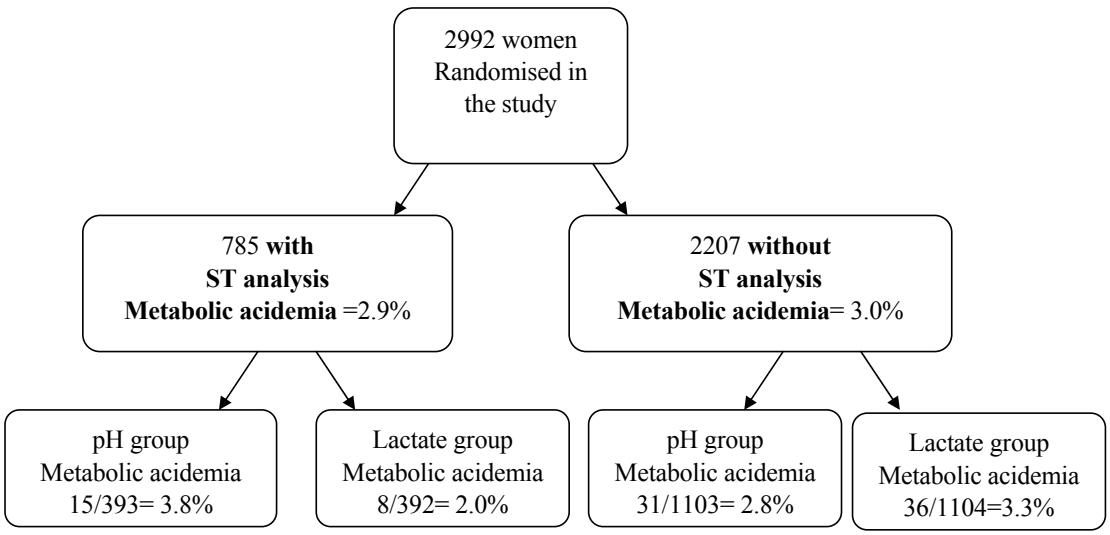

Fig.13. Frequencies of metabolic acidemia in the subgroup analysis of use of the STAN monitor™ compared to the CTG only subgroup.

The primary end point pH <7.00 in the ST analysis subgroup had a prevalence of 2.8 % in the pH management group compared with 1.8 % in the lactate management group (RR=0.6 p=0.34). Corresponding values in the CTG only group were 1.2 % and 1.3 %, respectively (RR=1.08, p=0.84).

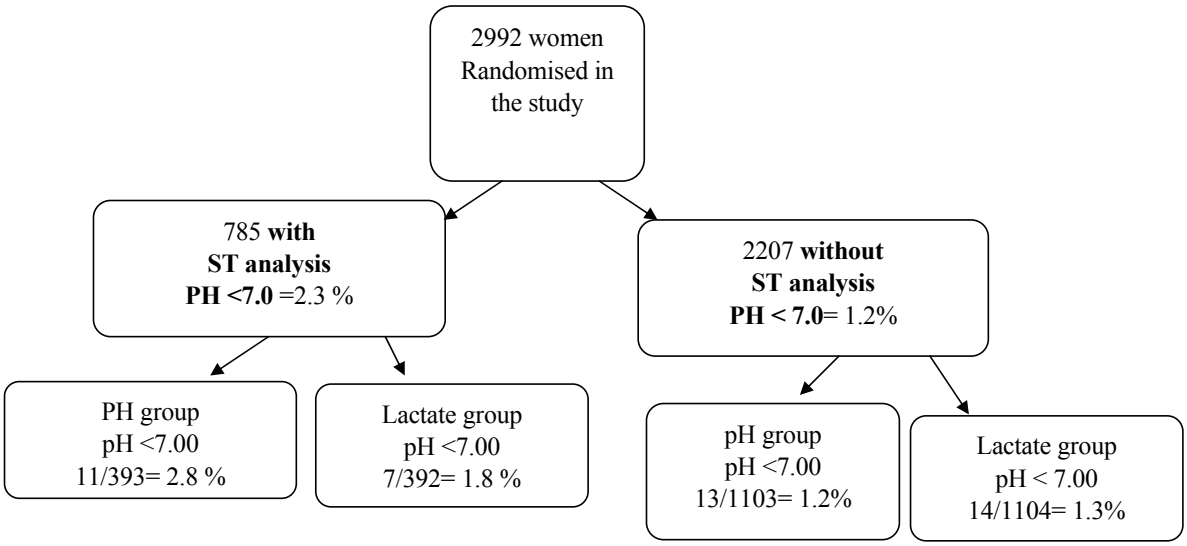

Figure 14. Show frequency of PH <7.0 in the subgroup analysis of use of the STAN-monitor™compared to the subgroup without STAN-monitor™.

If the non-significant higher prevalence of birth acidemia in the pH as compared to the lactate managed group among STAN-monitored cases is attributed to power, i.e. number of included cases, or appeared by chance is not known. However, one can speculate that high risk cases monitored with STAN more rapidly ends up in a severe birth acidemia. Therefore, the simplicity and rapidity of the Lactate Pro™ might be in favour for this method and should be recommended when these groups of high risk patients are monitored. Future research should highlight this issue.

To summarize paper IV we found that lactate analysis of fetal scalp blood was at least as good as pH analysis in the management of intrapartum fetal distress. We found no significant differences in birth acidemia, operative interventions, low five minute Apgar scores, or admissions to NICU. However, sampling failure was more than six times as common in the pH group. We suggest that lactate analysis with the Lactate Pro™ can replace pH analysis in intrapartum fetal monitoring

Swedish summery (svensk sammanfattning)

Berzelius upptäckte det han kallade ‘köttmjölksyra’ (Karolinska institutet 1807). Han fann att denna syra sannolikt hade en relation till utfört muskelarbete samt tillgången till befintligt syre. Under lång tid ansågs mjölksyra (laktat) vara en farlig slutprodukt vid anaerob (syrefattig) förbränning. På senare år har dock laktatet fått en mer central roll i hela kroppens metabolism. Man har bla i den s.k. `laktatskyttel hypotesen´, beskrivit att muskel i vila avger och upptar laktat från omgivande vävnad. Vid ökad aktivitet ökar muskeln sin intracellulära laktat produktion, och avger då i ökad mängd, laktat till sin omgivning. Vid återhämtnings fasen kan sedan muskeln ta upp laktat från omgivningen och omvandla detta överskott till energi.

Livmodern är en av kroppens största muskler och har en dualistisk funktion. Den skall under 9 månaders graviditet skydda fostret. Under graviditeten är livmodern tämligen avslappnad, vid förlossningens start omvandlas den till en starkt koordinerad och hårt arbetande muskel med hög aktivitet. Detta styrs av hormonella faktorer, men också av lokala vävnadsmetaboliter. Studier har visat att energiefterfrågan i livmodermuskeln är mycket stor troligen pga. dess mycket speciella funktion. Metabolismen är i hög grad anaerob. Laktat halten beskrivs närmast dubblerad i den gravida livmodermuskeln jämfört med andra muskler.

Fostret producerar fostervatten i ett nära samarbete med den gravida kvinnan. Huvudsaklig produktion sker via fostrets urinproduktion samt utsöndring via fostrets luftvägar. Absorption av fostervatten sker över livmodermuskeln, moderkakan, fosterhinnorna samt framför allt av fostrets kontinuerliga sväljande.

Handläggning av gravida kvinnor med misstänkt vattenavgång har bekymrat förlossningsläkare sedan många år. Risken för att utveckla infektion om vattnet går men förlossningsarbetet inte startar, har ansetts vara stor. Många arbeten har gjorts för att försöka finna en bra metod att

konstatera vattenavgång. De flesta av dessa metoder haft en god möjlighet att konstatera att fostervattnet verkligen har avgått, men en betydligt sämre förmåga att konstatera att inte så är fallet. Efter en stor svensk studie av kvinnor med misstänkt vattenavgång rekommenderades att vattenavgång ska konstateras vid s.k. spekulum undersökning, dvs. att man vid en gynekologisk undersökning tittar efter synligt fostervatten. Ca 12 % av undersökningarna ansågs vara falskt negativa, dvs. att vattnet hade avgått trots att det inte var synligt vid undersökning. Tre procent av undersökningarna ansågs vara falskt positiva, dvs. att undersökaren tycker sig se fostervatten, trots att det förmodligen inte så var fallet.

Vår erfarenhet är, att det man som undersökande läkare tror sig identifiera som fostervatten vid spekulum undersökning inte alla gånger är det. Missbedömningar görs ibland, och kvinnor utsätts på grund utav detta allt för ofta för onödig intervention i form av induktion, då förlossningen inte startar inom utsatt tid efter konstaterad vattenavgång. Vår kunskap om att laktat halten är hög i fostervatten har lett till utveckling av den test som vi döpt till `Lac-test´. Vår önskan har varit att utveckla en bra, pålitlig och lätt hanterlig test, väl anpassad för klinisk miljö. 'Lac-test' är en test med både hög sensitivitet och specificitet (86 % resp. 92 %), vilket är bättre än andra kommersiellt tillgängliga vattenavgångs tester. Vårt arbete visade att bland 55 kvinnor där synligt fostervatten konstaterades, hade endast 44 st en positiv `Lac-test´samt 31 st en positiv actim PROM™ test.

Vi har funnit en stor spridning av laktatkoncentrationen i vaginalsekret vid undersökning av misstänkt vattenavgång. Vi ville därför undersöka om hög laktat halt i vaginalsekret speglar en aktiv livmoder i analogi med kunskap från idrotts medicin. I denna studie (arbete II) fann vi att kvinnor med en laktatkoncentration i vaginal sekret $\geq$4.5 mmol/l hade 8 timmar (median) till aktiv förlossning, medan kvinnor med en laktathalt < 4.5mmol/l hade 54 timmar (median) till aktiv förlossning ($p<0.01$). Vi fann dock ingen korrelation mellan laktat koncentrationerna i gruppen $\geq$4.5mmol/l och tiden till aktiv förlossning. Kvinnor med en laktat koncentration mellan

4.5-9 mmol/l i sitt vaginalsekret startar sin förlossning inom 10 timmar, medan kvinnor med en koncentration >9mmol/l startar inom 8 timmar, och detta var inte statistiskt signifikant. Vår slutsats är att vattenavgången styr förlossningsstarten, inte det individuella laktatvärdet. Det är alltså betydelse fullt att på ett adekvat sätt konstatera om vattnet gått eller inte. Vi anser att `Lac-test´ är ett mycket kliniskt användbart test, ger ett korrekt och snabbt svar, samt dessutom ge en möjlighet att förutsäga värkstart.

Livmodern utsätts under värkarbete för syrebrist vid varje värk, och detta medför en ökad laktatproduktion i livmoder muskeln. När värken släpper transporteras ansamlade metaboliter bort. Vid värksvaghet sker inte denna borttransport i samma utsträckning, utan större mängd laktat ansamlas i livmodern, och muskelns kontraktionskraft försämras. I arbete III har vi för första gången kunnat påvisa associationen mellan värksvaghet och hög halt av laktat i fostervatten. Vår hypotes är att laktat produceras av livmodermuskeln, och utsöndras till omgivande vävnad (=fostervattnet) i analog med tidigare mer experimentella studier. Vi har funnit att kvinnor med hög laktathalt ($\geq$10.1mmol/l) vid två på varandra följande mättillfällen i 82% av fallen kommer snittas för värksvaghet. I den studien fann vi ett falskt positivt resultat i endast 14% av fallen. Oddset att utveckla en värksvaghet är ca 20 ggr högre om man har två höga laktat värden $\geq$10.1 mmol/l . Fortsatta arbeten pågår, och vår förhoppning är att i en framtid kunna använda denna nya upptäckt som en diagnosmetod för värksvaghet under aktiv förlossning.

Huvudsubstratet för kroppens förbränning är glukos. Under normala förhållanden, när tillräckligt med syre finns, beskrivs metabolismen som syrerik (aerob). Glukos bryts då ned till energi, CO_2 och vatten. Vid syrebrist blir förbränningen istället syrefattig (anaerob), och mjölksyra och vätejoner bildas. När mjölksyran ansamlas uppstår s.k. metabolisk acidos. Detta avspeglar sig som ett lågt pH värde samt ett högt laktat värde i blodet. Energi bildas även vid den anaeroba metabolismen, men i mycket mindre mängd och betydligt mer oekonomiskt. Kroppens

cellfunktioner är beroende av energi. Vid brist på bildad energi sviktar viktiga cellulära funktioner, och medför risk för kvarstående skador och/eller i värsta fall död. I vårt arbete IV har vi genomfört världens största randomiserade studie som jämfört laktat och pH mätning i fosterskalpblod vid misstanke om syrebrist hos fostret under pågående förlossning. I studien randomiserades 2992 kvinnor från 10 olika förlossningskliniker i Sverige. Resultatet visade att det inte är någon skillnad i primärt utfall (metabolisk acidos eller pH <7.00) mellan båda metoderna, däremot är den nya metoden (Lactate Pro™) mycket enklare att använda. Vi har funnit en stor signifikant skillnad i frekvensen `misslyckade prover´. Av pHanalyserna i studien misslyckade 10.4% av proverna vid provtagning/analys, jämfört med 1.2% i laktat gruppen, vilket är en signifikant skillnad ($p<0.01$).

Vi spekulerar i att resultatet förmodligen sett annorlunda ut om byte till laktat mätning inte genomförts i så många fall i studien, när pHmätningen misslyckats. Vi skulle kanske då kunnat se en större skillnad mellan de två grupperna, och förmodligen till laktatmetodens fördel. Att använda laktat i fosterskalpblod under pågående förlossning ger en god och tillförlitlig diagnostik av foster med misstanke om syrebrist. Det ger ett svar omgående, vilket har stor betydelse för den behandlande läkaren, barnmorskan samt den födande kvinnan i denna psykiskt mycket påfrestande situation.

Summering:

Detta är första gången laktat mätning i vaginalsekret/fostervatten har använts som ett diagnostiskt verktyg inom obstetriken. Vi har med dessa teser funnit dess mycket goda användbarhet vid misstänkt vattenavgång, vid förutsägande av förlossningsstart hos kvinnor med misstänkt vattenavgång, samt vid diagnostiken av värksvaghet. Vi har också visat på laktatets förträfflighet vad gäller identifiering av foster med misstanke om syrebrist under förlossning. Laktat mätningens tillförlitlighet samt enkelhet gör den mycket attraktiv i hantering inom klinisk praksis.

Summary

This is the first time lactate concentration determination in vaginal fluids/AF has been used as an obstetric diagnostic tool. We have with this thesis shown the usefulness of lactate in AF in the diagnosis of suspected PROM, in the prediction of spontaneous onset of labour for women with suspected PROM, and also in the diagnosis of labour dystocia. We have proposed the name for this new method to be 'Lac-test'. The simplicity of the test makes it very attractive for clinical practice.

We have also found that lactate analysis of fetal scalp blood was at least as good as pH analysis in the management of intrapartum fetal distress, and we suggest that lactate analysis with the Lactate Pro™ can replace pH analysis in intrapartum fetal monitoring.

Conclusions

- Lactate determination in vaginal fluid (`Lac-test´) is a valid test in cases with a history of suspect PROM, and even betters an actim PROM-test™.
- High lactate concentration (≥ 4.5 mmol/l) in vaginal fluid is strongly associated with spontaneous onset of labour within 24 hours and 48 hours of examination in cases with suspect PROM.
- Lactate concentration ≥10.1 mmol/l in at least two consecutive samples of amniotic fluid during labour collected 60 minutes apart, is strongly associated with labour dystocia.
- Lactate analysis of fetal scalp blood is at least as good as pH analysis in the management of intrapartum fetal distress.

ACKNOWLEDGEMENTS

To begin with I want to thank all of you who have made my research possible in different ways. First of all I wish to give my warmest thanks to all the women who participated in my studies. Without you this work had not been possible.
Then I would like to thank:

Lennart Nordström: My dear friend and principal supervisor, for the privilege of being your doctoral student. Thank you for opening my eyes to the scientific world and research. Thank you for sharing all your enormous knowledge and experience in obstetrics and science with me. For always being there for me, and for your patience and capacity to calm me down when things sometimes are going a little bit to fast. It has been such a pleasure working together. (I promise you, I will never write `preterm rupture of the membranes´ again……).

Sven Cnattingius: My co-tutor and dear friend. Thank you for sharing your enormous experience in the world of research with me. Thank you for all your good advice and support. It has been a pleasure to work with you.

Hans Pettersson: My co-tutor and dear friend. Thank you for your never endless statistic support and bright statistical ideas. Thank you for showing me the great world of statistic!

Margareta Hammarström: Head of the department of Obstetrics and Gynaecology at Söder Hospital. Thank you for giving me the opportunity to do obstetric research. Thank you for all your enthusiastic support and taking a positive interest in all my projects!

Gunlög Lusensky: My dear friend and colleague. Thank you for shearing all you're obstetric and life experience with me. Thank you for never endless support, and for all our laughs and chatting.

Birger Winbladh: For your friendship and great experience. Thank you for all your help, and for being so inventive in all my projects.

Mathias Karlsson: My friend and research companion from Karlstad. It is unusual to meat someone with the same speed in life… Thank you for all our enthusiastic discussions, and all our laughs!

Helena Åkerud: My new research companion from the Department of Obstetrics and Gynaecology in Karlstad. Thank you for your never endless support.

Co- workers in the RCT study, for pleasant collaboration. Claudia L, Margareta N, Andreas H, Dag P, Agneta H, Anna-Lena B, Magnus C, Maria S and Ulla-Britt W. Thank you all, it has been a pleasure working together with you.

Marilyn and Paul Hedges: Thank you for your effort to make my English correct and understandable.

Library staff at Söder Hospital library; for provided such excellent help in searching for references.

Midwifes, colleagues and staff at the maternity ward at Söder Hospital, who stands all discussions about amniotic fluid, labour dystocia and lactate, and who never become tired in finding new patients for me and my research. Tank you all for your great support!!

Finally to the three most important persons in my life, ***Johan, Anna and Klas. I love you*****!**

The work within this thesis was supported by Signhild Engqvists Stiftelse and Allmänna BB´s Minnesfond .

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