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CERVICAL INSUFFICIENCY:

ROLE OF CERCLAGE AND CHANGES

IN FETAL MEMBRANES

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Doctoral thesis in Medical Sciences

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LIST OF ABBREVIATIONS

15-HPGD	15-hydroxyprostaglandin dehydrogenase
17-OHPC	17-hydroxyprogesterone caproate
20α-HSD	20α-hydroxysteroid dehydrogenase
ABCC4	ATP-binding cassette subfamily C member 4
ACOG	American College of Obstetricians and Gynecologists
ALA	alpha-linolenic acid
AP-1	activating protein 1
AKR1B1	aldo-keto reductase family 1 member B1
AKR1C3	aldo-keto reductase family 1 member C3
AP-1	activator protein 1
CAP	contraction associated protein
CBR1	carbonyl reductase 1
CI	cervical insufficiency
COX	cyclooxygenase
CX26	connexin-26
CX43	connexin-43
DAMP	damage-associated molecular pattern
DHA	docosahexanoic acid
DES	diethylstilbestrol
EAPM	European Association of Perinatal Medicine
ECM	extracellular matrix
EMA	European Medicines Agency
EONS	early-onset neonatal sepsis

EPA	eicosapentanoic acid
ERα	estrogen receptor α
FDA	Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
FP	prostaglandin F receptor
GABA-a	gamma-aminobutyric acid
IFN	interferon
IL	interleukin
IM	intramuscular
IPD	individual patient data
IRF-3	interferon regulatory factor 3
LCPUFA	long-chain polyunsatured fatty acid
LA	linolenic acid
LEEP	loop electrosurgical excision procedure
MAPK	mitogen-activated protein kinase
miR-200s	microRNA200 family
miR-199a	microRNA199
miR-214	microRNA214
MyD88	myeloid differentiation primary response 88
NEC	necrotizing enterocolitis
NFκB	nuclear factor kappa B
NFKB1A	nuclear factor kappa B inhibitor alpha, iκBα
NICE	National Institute for Health and Care Excellence
NSDAID	non-steroidal anti-inflammatory drug
OS	oxidative stress

OXTR	oxytocin receptor
PAMP	pathogen-associated molecular pattern
PGD2	prostaglandin D2
PGE2	prostaglandin E2
PGF2α	prostaglandin F2α
PGH2	prostaglandin H2
PGI2	prostaglandin I2
PGES/PTGS	prostaglandin synthase
PGR	progesterone receptor
PLA2G4A	phospholipase A2 group 4A
PR-A	progesterone receptor A
PR-B	progesterone receptor B
PPROM	preterm premature rupture of membranes
PRR	pattern recognition receptor
PTB	preterm birth
PTGS2/PGHS2	prostaglandin synthase2
RCOG	Royal College of Obstetricians and Gynaecologists
RCT	randomized controlled trial
RDS	respiratory distress syndrome
SA β gal	senescence-associated beta-galactosidase
SASP	senescence-associated secretory phenotype
SLCO2A1	solute carrier organic anion transporter family member 2A1
SMFM	Society for Maternal-Fetal Medicine

STAT5B	signal transducer and activator of transcription 5B
SOGC	Society of Obstetrics and Gynecology of Canada
TAC	transabdominal cerclage
TBP	TATA-box binding protein
TLR	toll-like receptor
TNF	tumor necrosis factor
TRIF	TIR-domain-containing adapter-inducing interferon-β
ZAM	zone of altered morphology
ZEB1	zinc finger E-box-binding homeobox 1

1. INTRODUCTION

Preterm delivery before 37 weeks of gestation is the main cause of neonatal morbidity among non-anomalous infants in the United States and developed countries, and is the main cause of death worldwide (1-3). Every year, more or less 15 million babies are born preterm worldwide with prevalence from 5% in most European countries to 18% in Africa (4). The risks of perinatal mortality and morbidity are inversely correlated to gestational age at delivery (5). Preterm birth is a syndrome including multiple pathological processes such as intraamniotic infection, extra-uterine infection, vascular disorders, decidual senescence, uterine overdistension, decline in progesterone, disruption of maternal-fetal tolerance, stress and cervical insufficiency (CI) (6, 7). While the common pathway of parturition is activated physiologically in case of labor at term (Figure 1), several pathological processes cause early activation of the parturition pathway (8). Management strategies (cerclage) targeted to maternal tissue (cervix) are more or less effective in reducing preterm birth (9).

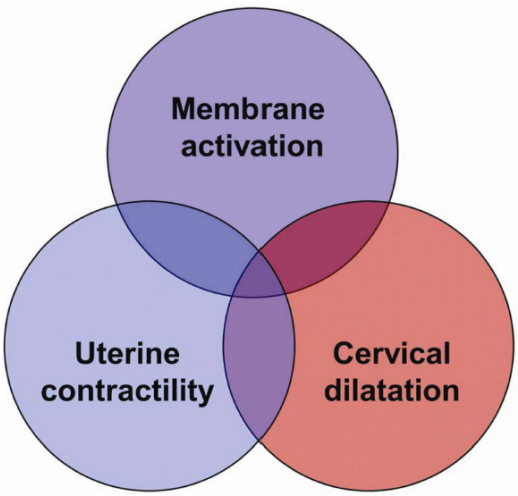


Fig. 1. Uterine components of the common pathway of parturition include: increased uterine contractility, membrane activation and cervical dilation (8).

The present thesis aims to study in cervical insufficiency the role of interventions, management strategies (transvaginal and transabdominal cerclage) and the outcomes in these high-risk pregnancies. In addition to improving our clinical practice, the main objective of our work is to evaluate in vitro fetal or chorioamniotic membranes for understanding the mechanisms and pathways leading to parturition.

The first part of this thesis is a clinical study comparing perinatal outcomes in emergency cerclage between patients with prolapsed and non-prolapsed fetal membranes. We aim to investigate whether some interventions are more effective with better outcomes. What is the contributing role of fetal (membranes) tissue as increasing interest recognizes that fetal membranes experience structural and functional

changes throughout pregnancy and during parturition? Fetal membranes are essential for the protection of the fetus, maintenance of pregnancy and promote ultimately parturition.

The second part of this thesis is based on the histologic changes of fetal membranes in transabdominal cerclage. Special focus highlights the fetal membranes as a model to understand immune, endocrine, mechanical and cellular aspects of pregnancy and pathways of parturition.

2. CERVICAL INSUFFICIENCY

2.1. Definition and etiology

Cervical insufficiency formerly known as cervical incompetence (CI) is the inability of the cervix to support a pregnancy over second or early third trimester in absence of uterine contraction or labor. It is characterized by a painless cervical dilation after excluding other preterm birth etiologies (bleeding, infection, preterm premature rupture of membranes (PPROM). In CI, the cervix starts to shorten and dilates too early, causing either second-trimester fetal losses or early third trimester births.

Cervical insufficiency could be either congenital or acquired (as shown on Table 1). The most common congenital causes are Müllerian Duct anomalies or hereditary connective tissue disorders such as Ehlers-Danlos or Marfan syndromes. Defects of extracellular matrix (ECM) proteins also contribute to cervical insufficiency. The most common acquired causes are previous cervical surgery procedures such as dilation and curettage, hysteroscopy, other procedures needing cervical dilation, previous induced abortion, previous cervical laceration, previous cold knife cone biopsy, previous laser conization and previous loop electrosurgical excision procedures (LEEP). Any history of cervical procedures is a clear risk factor leading to cervical insufficiency.

Tab. 1. Risk factors for cervical insufficiency

Congenital	**Acquired**
• Müllerian Duct anomalies: - *Hypoplasia/agenesis* - *Unicornuate* - *Didelphys* - *Bicornuate* - *Septate* - *Arcuate* - *DES drug related*	• Surgical procedures: - *Dilatation and curettage* - *Hysteroscopy* - *Cold knife cone biopsy* - *Laser conization* - *Loop electrosurgical excision procedure*
• Connective tissue disorders: - *Ehlers Danlos or Marfan syndromes*	• Cervical lacerations: - *Post abortion or post delivery*

Cervical insufficiency incidence is around 0.5% of the general obstetric population and 21% of recurrent miscarriage patients who experienced second-trimester fetal losses (10).

Cervical insufficiency has also been reported to contributing to more than 10% of preterm deliveries (9-12). Wide variation in the incidence of cervical insufficiency has been reported which is likely due to differences among the study populations, the criteria used to establish the diagnosis, and reporting bias between obstetricians and referral centers.

2.2. Diagnosis

The diagnosis of cervical insufficiency is challenging because of lack of clear diagnostic criteria. Many tests have been reported assessing the cervical function outside pregnancy: hysterosalpingography, insertion of cervical dilators (size-9 Hegar dilator, used to measure the size of cervical dilation in millimeters) and hysteroscopy. Ultrasound, magnetic resonance imaging, or hysterosalpingography may diagnose a cervical or uterine abnormality, well-known risk factor but it is not always associated with cervical insufficiency (13).

Diagnosis of cervical insufficiency can be suspected by following three methods (14):

1. Identification of **risk factors in the medical history of the patient**: history of painless cervical dilation after the first trimester with subsequent expulsion of pregnancy in the second trimester, typically before 24 weeks of gestation, or preterm labor in the absence of other risk factors (bleeding, infection, preterm premature rupture of membranes).
2. Detecting cervical changes on **vaginal examination** (dilation of 1 cm or more of the internal os on manual examination or visualization with prolapsed or non-prolapsed membranes on speculum examination) before 24 weeks of gestation.
3. **A transvaginal ultrasound cervical length** (<25 mm, before 24 weeks of gestation) in women with prior pregnancy loss or preterm birth. Ultrasonographic finding of an isolated short cervical length is not a diagnostic criteria for cervical

insufficiency. It is more a marker of preterm birth rather than cervical insufficiency as it is well-known that the risk of preterm birth is inversely correlated to cervical length (15, 16).

Cervical funneling is often associated with short cervical length. Cervical funneling is defined as disruption of the internal os and prolapse of the membranes through a dilated endocervical canal (Figure 2). The funneling depth has to be at least 5 mm to be defined as funneling. The funneling shape (Y, V and U) can also be described but it is somewhat subjective. Cervical funneling is best measured as a categorical variable (present or absent) (17). Funneling may be the first marker of later shortening and risk of preterm birth but cervical length is the standard measurement for evaluation of the cervix for predicting preterm birth (18). Among asymptomatic singleton pregnancies with a short mid-trimester cervical length (less than 30 mm) without prior spontaneous preterm birth, cervical funneling is not associated with an increased risk of preterm birth (19).

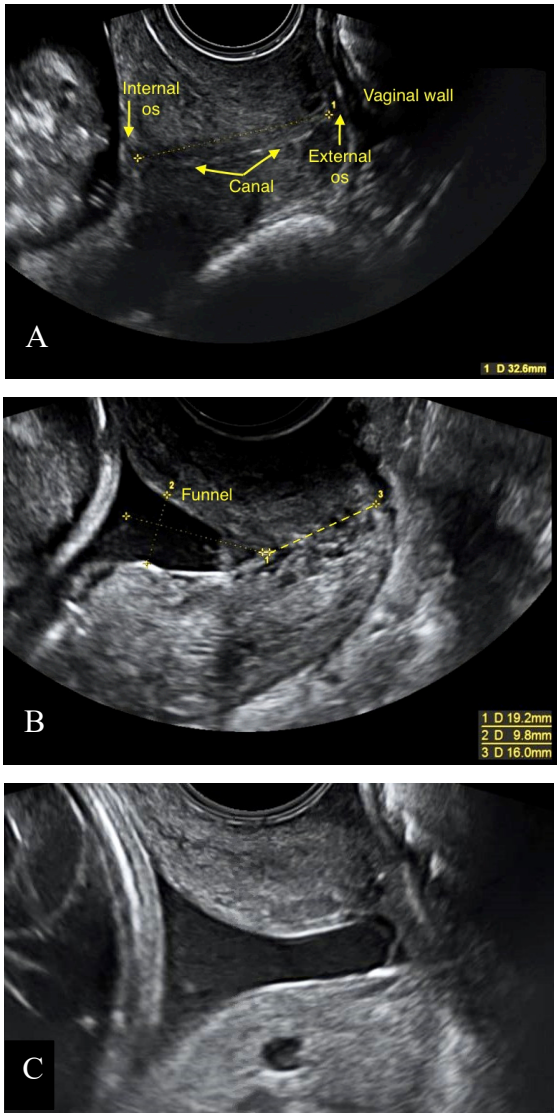


Fig. 2. Transvaginal ultrasound assessment of cervical length. **A**. Normal cervical length. **B**. Presence of cervical funneling and prolapse of the membranes through a dilated endocervical canal. Short cervical length. **C**. Presence of cervical funneling and prolapse of the membranes at the level of external cervical os.

2.3. Management

Cervical cerclage is the gold standard treatment for cervical insufficiency. A suture with a polyester tape or thread is positioned around the cervix aiming to provide mechanical support and keep the cervix closed allowing the pregnancy to reach term (20). Transvaginal cerclage procedures are associated with low rates of complications: membranes rupture, chorioamnionitis, bleeding, cervical lacerations, suture displacement, incomplete suture removal and cerclage failure (14). The incidence of complications is dependent of the indications of cerclage and the gestational age of surgery. A cerclage in the presence of membranes rupture or cervix dilation is generally associated with an increased risk of complications. Life-threatening complications such as maternal septicemia are extremely rare but have been reported with all types of cerclages (21, 22). These risks must be carefully balanced against the benefit of mechanical support of the cervix.

Nowadays, the indications of cerclage in singleton pregnancies are:

- history-indicated cerclage
- physical examination-indicated cerclage
- ultrasound-indicated cerclage

2.3.1. Cerclage

2.3.1.1. Techniques

2.3.1.1.1. Transvaginal cerclage procedures

The standard transvaginal cerclage procedures currently used the Shirodkar and McDonald cerclage procedures (Figure 3). The Wurm-Hefner procedure is usually performed for emergency cerclage. Shirodkar reported first the procedure in 1955 (23). McDonald described the procedure in 1957 (24). In 1961, Hefner reported a new surgical procedure referred to as Wurm's procedure (https://jogi.co.in/articles/files/filebase/Archives/1992/apr/1992_143_146_Apr.pdf).

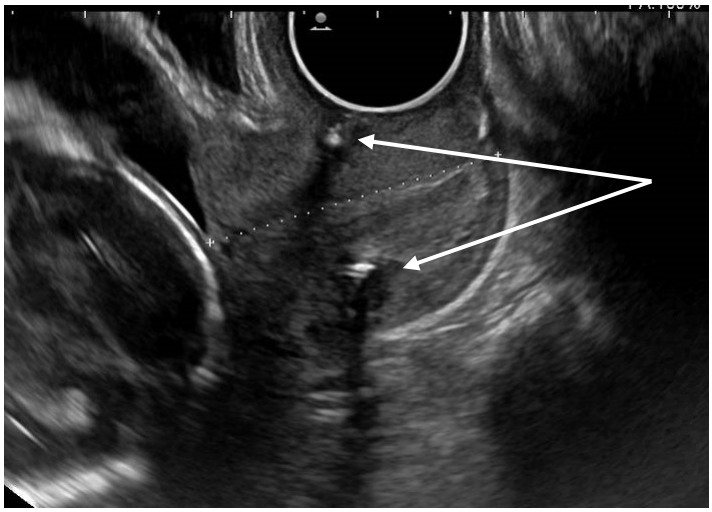


Fig. 3. Transvaginal ultrasound of transvaginal cerclage at mid-length of the cervix. The McDonald cerclage is shown as an echogenic structure.

The Shirodkar procedure involves the incision and dissection of the cervical mucosa in an attempt to place the suture close to the internal

cervical os (Figure 4). The anterior mucosa is incised at the vesico-cervical junction and the bladder is pushed back and upwards. The posterior mucosa is then incised at the level of the pouch of Douglas and the rectum are pushed back and upwards by posterior dissection. The suture is carried out in the cervical stroma from anterior to posterior and from posterior to anterior, joining the areas of mucosal dissections with care to avoid entering the endocervical canal. The suture is firmly tied effectively constricting the cervical internal os. The cerclage stitch is buried by sutures of the mucosal incisions. Multiple technical modifications have been described and published. No significant difference in effectiveness between the procedures was observed (25). Total cervical occlusion is a proposed modification where, in addition to the cerclage, the external cervical os is closed with continuous nylon. The rationale for this procedure is based on the observation that the mucus plug has a double role in preventing preterm labor. The plug is a mechanical barrier between the vagina and uterus, but its intrinsic immune component also makes it a very important element in defending the fetal compartment from ascending infections. Cervical occlusion keeps the plug in-situ, thereby increasing the innate protection of the endocervical canal. In a randomized trial of cervical cerclage with or without cervical occlusion, cervical occlusion with cerclage had no significant additional effect (26, 27).

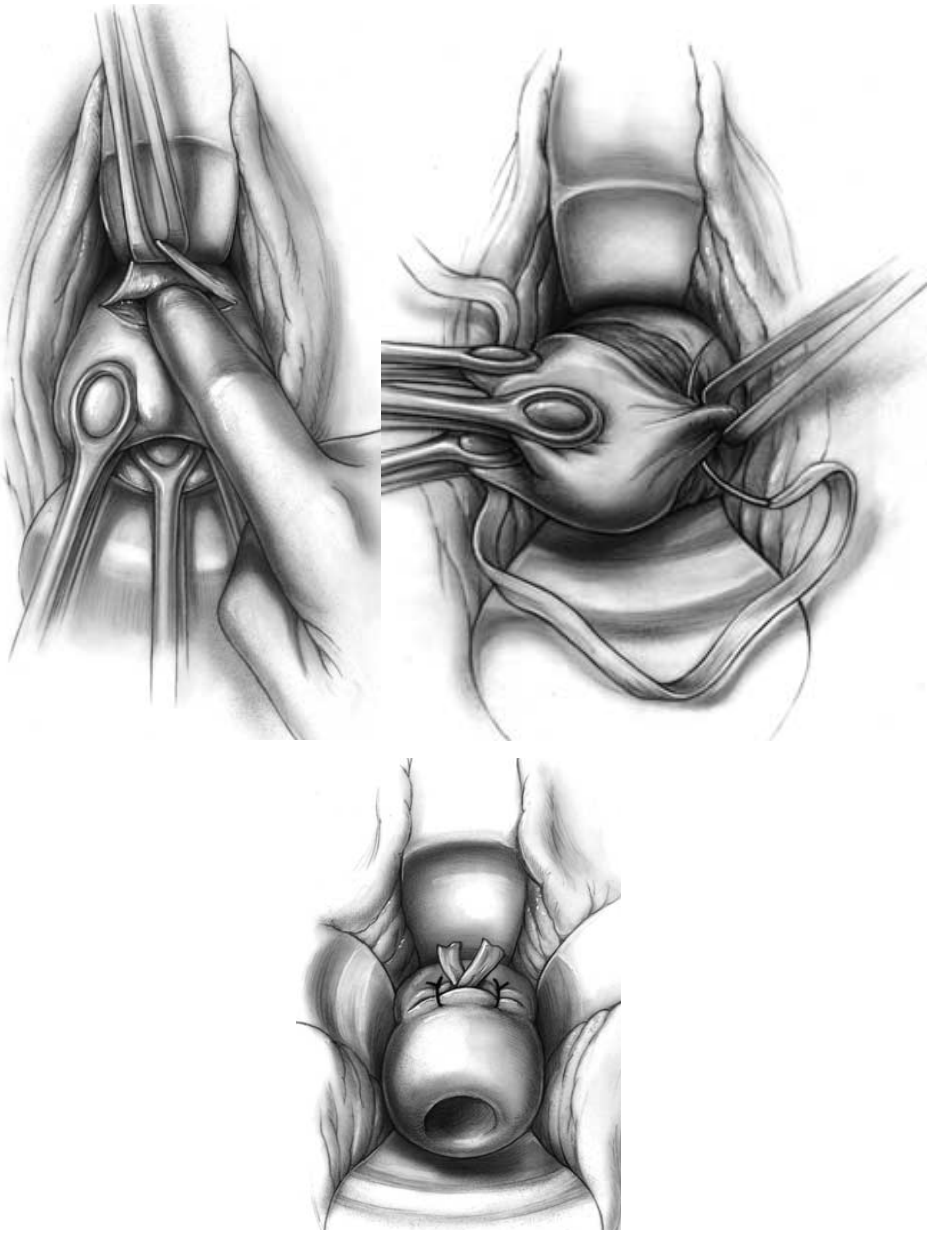


Fig. 4. Shirodkar cerclage technique (25).

The McDonald cerclage procedure does not involve tissue dissection. A single purse-string suture of polyester tape or thread is

inserted at the cervicovaginal junction, thereby encircling the cervix. The suture placement is performed using 4 bites and avoiding the endocervical canal (Figure 5). The cervical internal os should not be reached. The knot is usually placed anteriorly with the easiest access for the removal. The McDonald cerclage procedure is often chosen as it is easy to perform and to remove.

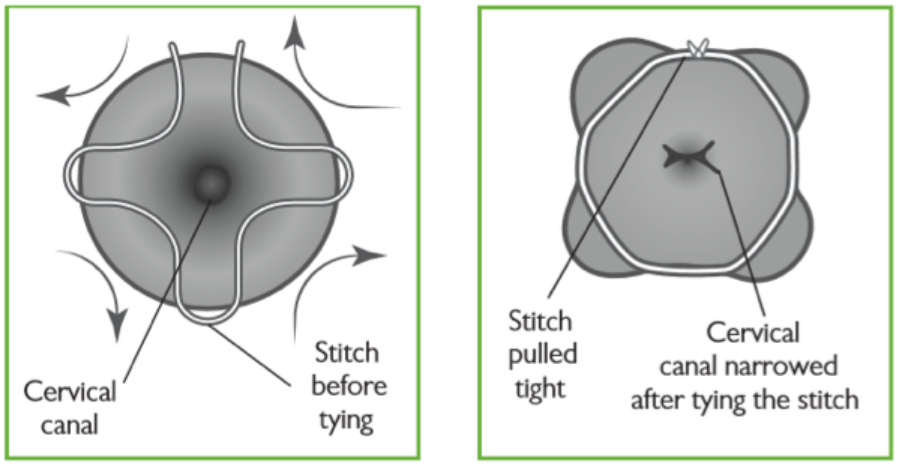


Fig. 5. McDonald cerclage technique
(From cervical stitch patient information leaflet, RCOG).

The Wurm-Hefner procedure is generally used for emergency cerclage with prolapsed membranes. The prolapsed membranes are gently displaced using a finger, Foley catheter balloon, or other methods (28). At the level of internal os, 2 perpendicularly oriented U-shaped sutures are placed through the full thickness of the cervix (Figure 6). One suture enters horizontally at the 2 o'clock position, crossing the canal, and exiting at 10 o'clock position. The suture is

then reinserted at 8 o'clock position, traverses the canal again, and exits at 4 o'clock position. An analogous suture is then placed vertically, entering the stroma at 11 o'clock position, and finally exiting at the 1 o'clock position anteriorly, thus creating a cross pattern. The entry and exit points of each suture at the cervical mucosa are separated by approximately 1 cm, and both are cinched down reinforcing the cervix distal to the reduced membranes (25).

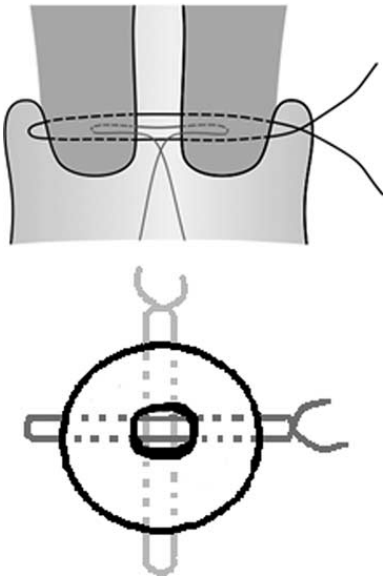


Fig. 6. Wurm-Hefner cerclage technique (25).

Type of sutures: In a recent retrospective study, outcomes were compared by the thickness of suture material: thick 5 mm braided polyester fiber (Mersilene® tape) versus thin polyester braided thread (Ethibond®) or polypropylene non-braided monofilament (Prolene®). For transvaginal cerclage, thick compared to thin suture was associated with longer duration of pregnancy and lower odds of

chorioamnionitis and neonatal intensive care unit admission among all patients regardless of cerclage indication (29).

Vaginally inserted cerclages are either taken out at 36-37 weeks of gestation, or when the women enter in labor. It is usually removed without anesthesia or with epidural or spinal anesthesia if necessary. Cerclage removal is not an indication for delivery. The chance of spontaneous delivery within 48 hours after elective cerclage removal is only 11%. The mean interval between elective cerclage removal and spontaneous delivery is 14 days (30). For women who elect cesarean delivery at or beyond 39 weeks of gestation, cerclage removal at the time of delivery may be performed (14).

2.3.1.1.2. Transabdominal cerclage procedure

Transabdominal cervicoisthmic cerclage (TAC) is chosen in women with cervical insufficiency and anatomical limitations (severe cervical lacerations or after trachelectomy), or in the case of previous failed transvaginal cervical cerclage procedures that resulted in second-trimester pregnancy loss.

The indications of transabdominal cervicoisthmic cerclage for cervical insufficiency were validated in a retrospective review (as shown in Table 2) (31). Recently, a first RCT supported the use of TAC versus transvaginal cerclage in women who have had a failed cerclage (delivery before 28 weeks) (32). The FIGO good practice recommendations confirm the indication of TAC in high-risk women who have undergone an unsuccessful (but not rescue) cerclage (33).

Tab. 2. Indications of transabdominal cerclage

Indications of TAC in well-selected patients:
• congenitally short or extensively amputed cervix • marked cervical scarring • deep cervical defects • wide or extensive cervical conization • one or more previous elective transvaginal cerclage failures (in excluding an emergency cerclage performed for advanced cervical dilation on physical examination)

In 1965, transabdominal cerclage was first described by Benson and was an open procedure (34). Advances in minimally invasive surgery have contributed to the development of laparoscopic TAC (35, 36). The benefits of laparoscopic surgery compared to laparotomy surgery are well described and include: lower incidence of complications, decreased blood loss, lower risk of postoperative deep vein thrombosis, reduced postoperative pain, faster recovery, shorter length of hospital stay, and minimal cosmetic disfiguration (37-39). Currently, TAC can be inserted by laparoscopy or laparotomy and can be performed preconceptionally (interval) or during pregnancy (40).

Laparoscopic and open TAC have their own benefits. Open TAC is usually performed at the end of the first trimester after excluding major structural anomalies with the first trimester ultrasound and screening for major aneuploidies. After this period, the risk of miscarriage (first trimester fetal losses) is almost negligible. Most laparoscopic TAC are

performed before pregnancy due to concerns that laparoscopic manipulation of the pregnant uterus may increase the risk of pregnancy loss (39, 41, 42). Recent papers highlight the surgical differences between pre-conceptional and post-conceptional laparoscopic TAC. The uterine manipulation, the increase in uterine size and vascularization and the knot tightening can make laparoscopic placement more challenging during pregnancy (39, 43). These challenges could be overcome with proper training and technique. With pre-conceptional laparoscopic TAC, an intrauterine manipulator can be used, which facilitates exposure (42). Conversion of laparoscopy to laparotomy has been reported in 5.2% of cases, usually with post conceptional TAC (41). Both laparoscopic and open TAC are associated with intraoperative and postoperative complications: infection, fetal loss, blood loss, bowel, bladder or vascular injury, uterine or bladder perforation, broad ligament laceration, preterm premature rupture of membranes (PPROM) (44). The rate of complications in a series of 300 prophylactic transabdominal cervical cerclage procedures was low (3.7%). Severe complications with maternal morbidity are rare (45). The benefits of laparoscopic TAC placement before pregnancy should be weighed against the number of women who not become pregnant or may have an early miscarriage in the subsequent pregnancy (46). There is little published evidence that interval laparoscopic TAC can lead to fertility problems. An increase in cervical stenosis was reported with cerclage placement following radical trachelectomy (47). The conception rate for pre-conceptional TAC is around 75% (39). A randomized control trial has shown no effect on fertility rates with interval TAC compared

with transvaginal cerclage following conception (48). A recent retrospective study reported that interval laparoscopic TAC does not have a negative impact on the chances of conception (49).

All systematic reviews conclude that both open TAC and laparoscopic cerclage are safe and associated with low rates of complications. With the inherent benefits of laparoscopy over laparotomy, laparoscopic cerclage may have less risk of complications than open TAC. Laparoscopic cerclage may be preferable, in particular when performed before pregnancy. It is unclear whether the placement of laparoscopic cerclage before pregnancy or in the first trimester or both is associated with a lower risk of complications (39, 42, 44, 46). The optimal timing of cerclage (interval or during pregnancy) and surgical technique used (laparoscopy or laparotomy) are both open to debate (50). There is no evidence to support a specific technique or timing (33). More RCT's comparing laparoscopic cerclage with open TAC are needed which would yield more high-quality evidence (44).

The main advantage of the technique is the suture placement higher at the level of the cervical internal os (Figures 7-8) (51). The stitch can be left in situ between pregnancies with subsequent cesarean delivery. Some complications are reported with prolonged retention of transvaginal cerclage such as erosion into the vagina or the bladder (52, 53).

In previous studies of women with TAC, the rate of preterm birth is very low and fetal survival is high (54-57). A recent randomized controlled trial demonstrated that transabdominal cerclage was superior to (high and low) vaginal cerclage (Figure 9) in women with a previous failed

cerclage in the prevention of early preterm birth (<32 weeks of gestation) and fetal loss (32).

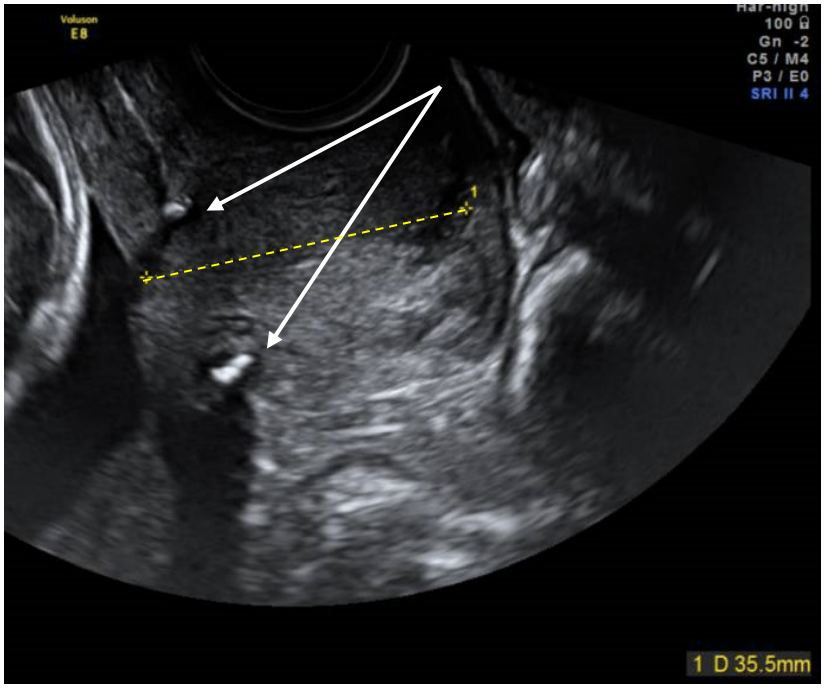


Fig. 7. Transvaginal ultrasound of transabdominal cerclage. Transabdominal cerclage at the level of the internal os by transvaginal ultrasound with cervical length of 35 mm.

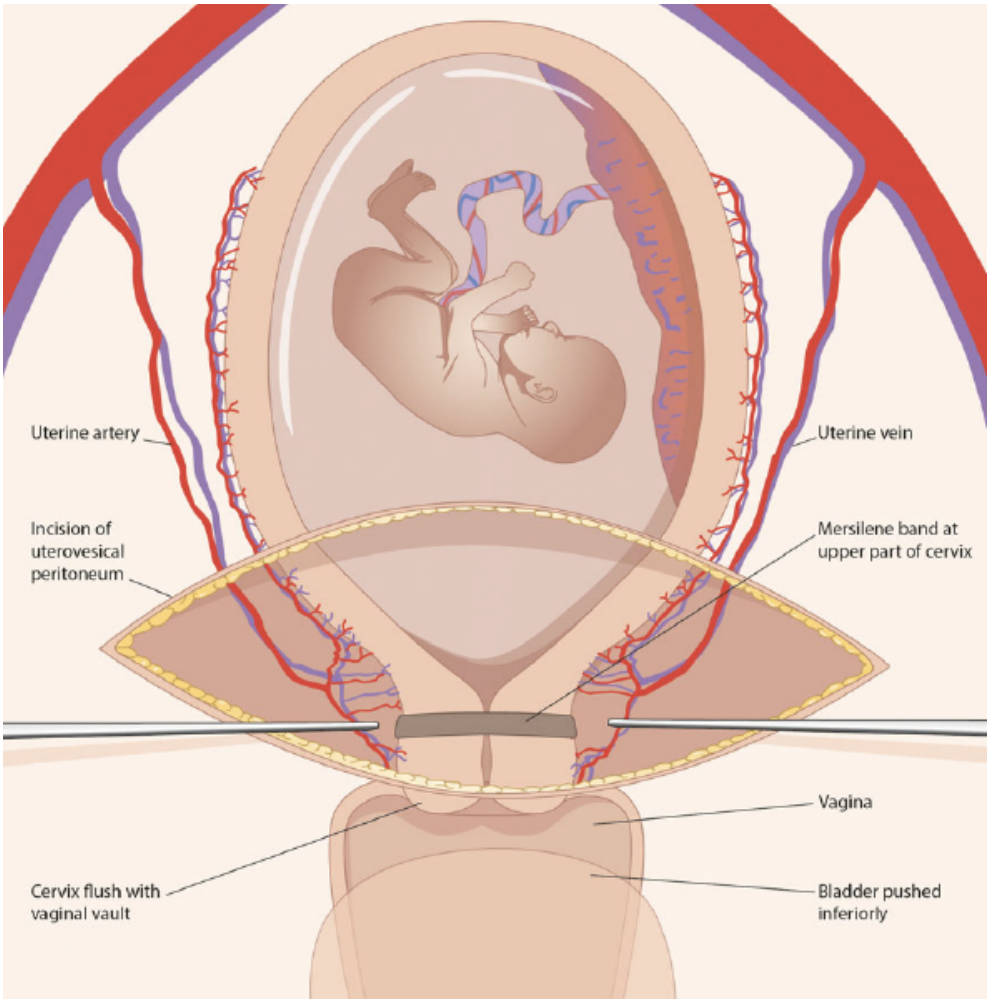


Fig. 8. Transabdominal cerclage (51).

Pfannenstiel incision and view of the cervicoisthmic region with correct position of the cerclage around the cervix, in-between uterine arteries.

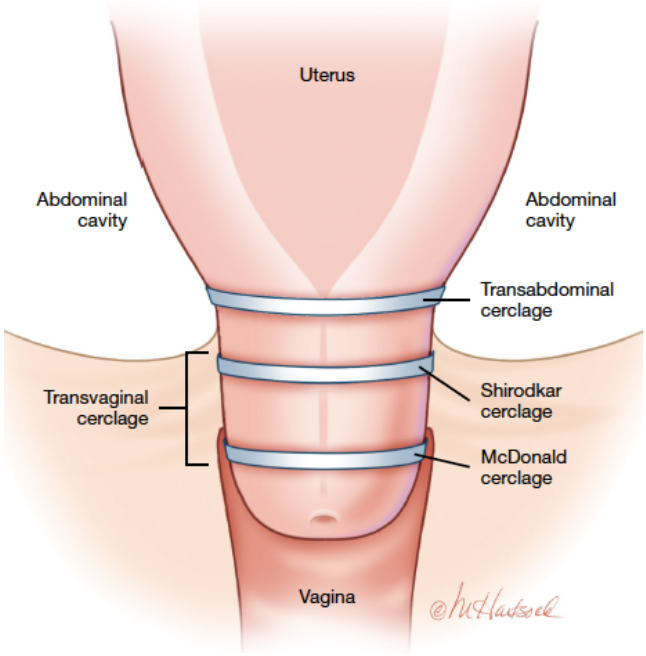


Fig. 9. Suture placement in transvaginal and transabdominal cerclage procedures (mdedge.com/obgyn).

2.3.1.2. Indications

2.3.1.2.1. Prophylactic cerclage

Prophylactic cerclage or history-indicated cerclage was formerly offered to high-risk patients with a history of three or more pregnancies ending before 37 weeks of gestation (21). Now, indications of prophylactic cerclage are well reported in the literature (Table 3). In the last guidelines of the American College of Obstetricians and Gynecologists, cervical cerclage is indicated for women with a history of one or more second-trimester pregnancy losses related to painless cervical dilation (features of cervical insufficiency) or prior cerclage due to painless cervical dilation in the second trimester (14). For these

high-risk patients with a history of cervical insufficiency, some studies reported an alternative option in the management using cervical length assessment by transvaginal ultrasound (58-60). Cervical length screening can be used for the detection of a short cervix (defined as <25 mm before 24 weeks of gestation) in high-risk patients. The use of a 25 mm cutoff in the mid-trimester has been utilized by investigators and clinicians to identify women at the highest risk of preterm birth. This cutoff threshold value is the lowest 10^{th} percentile in both unselected and high-risk patients (15, 61). The 25 mm threshold has also been utilized as the basis for cerclage intervention trials (62-65). Ultrasound-indicated cerclage is recommended for women with a short cervical length on transvaginal examination and prior spontaneous preterm birth at less than 34 weeks of gestation (14). Moreover, the same studies report that more than half the patients do not require cerclage placement despite a history consistent with cervical insufficiency. Unnecessary procedures can be avoided in more than half the patients. So cerclage can be indicated for the minority of women with a short cervical length (58-60). These women are suffering more often of cervical insufficiency than preterm labor. In a randomized controlled trial (RCT) involving cervical cerclage in women with a prior spontaneous preterm birth (PTB) at <34 weeks of gestation, Owen et al. (64) noted that approximately 69% of patients with serial measurement of cervical length remained at >25 mm. Most of these women had probably a prior spontaneous PTB due to a preterm labor but not due to cervical insufficiency. Therefore, they did not require a cerclage for the subsequent pregnancy. When evaluated by randomized controlled trial, vaginal cerclage has limited value,

compared with conservative management. Even without cerclage, most women could have a successful subsequent pregnancy. The challenge is then to identify women whose pregnancy losses are genuinely due to cervical insufficiency and not preterm labor.

NICE guidelines recommend offering either prophylactic cervical cerclage or prophylactic vaginal progesterone for women with a history of spontaneous preterm birth or mid-trimester loss between 16 and 34 weeks, and a cervical length of less than 25 mm between 16 and 24 weeks of gestation (66). They also consider prophylactic cervical cerclage for women with a cervical length of less than 25 mm between 16 and 24 weeks who have had a preterm prelabor rupture of membranes in a prior pregnancy or a history of cervical trauma (66). They always use the cervical length measurement in combination with a previous history of preterm birth, mid-trimester loss and PPROM.

Tab. 3. Summary of the guidelines (14, 33, 66).

ACOG	• History of second-trimester loss(es) and painless cervical dilation • History of preterm birth (<34 weeks) **AND** short cervical length (<25 mm before 24 weeks) • Prior cerclage due to painless cervical dilation	• **Prophylactic cervical cerclage**
NICE	• History of preterm birth (≤34 weeks) or mid-trimester loss (>16 weeks) **AND** short cervical length (<25 mm before 24 weeks)	• **Prophylactic cervical cerclage** **OR** • **Prophylactic vaginal progesterone**
	• History of preterm birth (≤34 weeks) or mid-trimester loss (>16 weeks) **OR** short cervical length (<25 mm before 24 weeks)	• **Prophylactic vaginal progesterone**
	• History of PPROM or cervical trauma **AND** short cervical length (<25 mm before 24 weeks)	• **Prophylactic cervical cerclage**
FIGO	• History of 3 or more preterm deliveries and/or mid-trimester losses	• **Prophylactic cervical cerclage**
	• History of 1 or more spontaneous preterm birth and/or mid-trimester loss **AND** short cervical length (<25 mm before 24 weeks)	• **Prophylactic cervical cerclage**
	• High-risk women with Müllerian abnormalities or cervical surgery without previous preterm birth or mid-trimester loss **AND** short cervical length (<25 mm before 24 weeks)	• **Prophylactic cervical cerclage** can be considered on an individual case basis

History-indicated cerclage is usually planned around 13-14 weeks of gestation. A cervical cerclage placement based on a combination of

history and ultrasound findings may be performed later and until 24 weeks of gestation. Too few data are available to understand if it is better to have a cerclage inserted early in the pregnancy (based on a history) or to wait to perform an ultrasound scan later in pregnancy to see if the cervical length has shortened (9).

Cerclage placement in women with no prior preterm birth and an incidentally very short cervical length (≤ 25 mm) before or at 24 weeks of gestation has not been associated with a significant reduction in preterm birth (67). These women should be offered vaginal micronized progesterone, 200 mg daily (or vaginal progesterone gel, 90 mg daily), to reduce the risk of preterm birth (68, 69). For women in this low-risk population, cerclage placement is not associated with a significant reduction in preterm birth (60, 70). Cerclage might be effective at lower cervical lengths (<10 mm) (71, 72).

2.3.1.2.2. Emergency cerclage

Emergency or rescue cerclage is indicated for women with advanced cervical dilation (≥1 cm) of the internal os on manual examination or with visualization of membranes (prolapsed or non-prolapsed membranes) on speculum examination. Emergency cerclage is considered as a salvage procedure for pregnancies which are at high-risk of second-trimester loss or preterm delivery. Emergency cerclage can be offered to women without signs of infection, rupture of membranes, active vaginal bleeding, and active labor (73). Most data of emergency cerclage have been collected in retrospective studies and have reported the benefits of emergency cerclage in increasing

the latency time between diagnosis and delivery (74-78). There is one randomized controlled trial and it confirmed the benefits of emergency cerclage in the presence of cervical dilation in 23 women (16 singleton and 7 twin pregnancies) (79). Emergency cervical cerclage is not a rational option for women with advanced cervical dilation (>4 cm) associated with prolapsed membranes (80, 81). Another study identified risk factors associated with preterm birth after at least 28 weeks of gestation. Benefits of emergency cervical cerclage are more important when performed at earlier gestational age (77).

According to the Society of Obstetrics and Gynecology of Canada (SOGC) guidelines, emergency cerclage may be considered in women in whom the cervix has dilated to <4 cm before 24 weeks of gestation (82). NICE guideline assessed in studies the role of rescue cerclage in women between 16 0/7 and 27 6/7 weeks of gestation with a dilated cervix and exposed unruptured fetal membranes. Gestational age and the extent of cervical dilation have to be taken into account. Benefits are likely to be greater for earlier gestations. Information on risks and benefits of emergency cerclage should be discussed with both an obstetrician and a pediatrician. Emergency cerclage aims to delay birth and so it increases the likelihood of neonatal survival and with a reduction of severe morbidity (66). Following the FIGO good practice recommendations, in women with exposed membranes prolapsing through the cervical os, a rescue cerclage can be considered on an individual case basis, taking into account the high risk of infective morbidity to mother and baby. Several different therapies have been advocated before or at the time of cerclage. These include tocolysis (usually indomethacin), antibiotics, and amnioreduction. All these

interventions lack evidence of benefit and can be considered on an individual case basis (33, 83). Further research is needed to evaluate the efficacy and synergy of each treatment.

Cerclage indications based on ultrasound- and physical-exam tend to be performed later in pregnancy. Theses cerclages should be limited in the second trimester before fetal viability has been achieved.

In our experience, we evaluated, in a retrospective cohort study, all emergency cervical cerclage performed following physical-exam indication between 15 and 25 weeks of gestation. Emergency cerclage were performed with and without prolapsed membranes. Maternal and neonatal outcomes were analyzed. Emergency cerclage was associated with increased latency time between cerclage and delivery, a decreased incidence of preterm delivery, and improved perinatal outcomes among patients with non-prolapsed membranes. Our data suggest that emergency cerclage can be safe and efficient (as shown in Part 1 – Transvaginal cerclage – clinical study).

2.3.2. Alternative management

2.3.2.1. Progestogens supplementation

The progestogens are steroid hormones with progesterone-like action. It includes natural (bioidentical, micronized) progesterone and its synthetic analogs. Progesterone action is mediated by the progesterone receptor (PGR). PGR are present in the central nervous system, ovaries, breasts, and the female reproductive tracts, including the vagina, cervix, fallopian tubes, and uterine endometrium and myometrium. Progesterone actions are mediated by two intranuclear

proteins, progesterone receptors (PR) A and B. In myometrial cells, PR-A increases the expression of pro-inflammatory genes in response to progesterone. In contrast, PR-B mediates anti-inflammatory actions by inhibiting the expression of pro-inflammatory genes (84) (Figure 10).

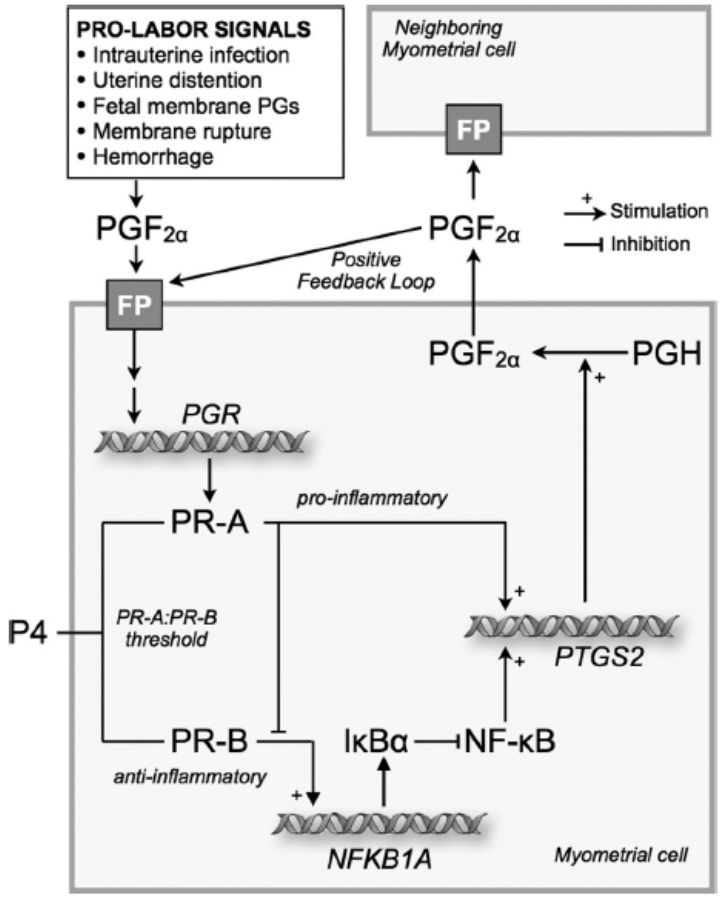


Fig.10. Progesterone actions are mediated by two functionally different but structurally highly related intranuclear proteins, PR-A and PR-B. PR-A and PR-B have opposite effects on pro-inflammatory gene expression in human myometrial cells. Progesterone via PR-B maintains relaxation by inhibiting NF-κB activity (in part by increasing *NFKB1A* expression). Progesterone via PR-A represses the relaxatory actions of PR-B. PGF2$_\alpha$ increases PR-A (84).

During pregnancy, PR-B are predominant in myometrial cells. Progesterone promotes myometrial quiescence through PR-B-mediated anti-inflammatory actions.

At delivery, the rise in PR-A expression promotes labor by inhibiting the anti-inflammatory actions of PR-B and stimulating pro-inflammatory gene expression (8, 84-86). Administration of progesterone receptor antagonists (e.g. RU-486, mifepristone) at any time in gestation leads to cervical ripening, and in some cases they can induce the onset of labor, confirming the relaxant effect of progesterone (8, 87-89). Parturition is associated with an increase of inflammatory response and activation of inflammation-associated transcription factors. They promote an increased expression of myometrial pro-inflammatory (IL-1, IL-8), contraction-associated protein/CAP (connexin-43), oxytocin receptors and cyclooxygenase 2 (COX-2/PTGS2) genes, leading to parturition (90-92) (Figures 11 and 12).

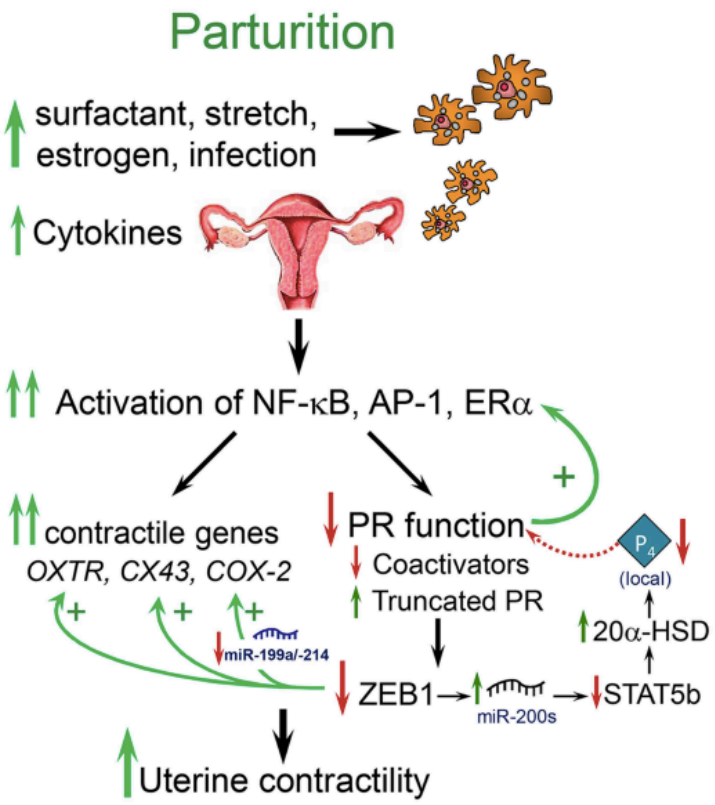


Fig.11. Parturition is associated with an inflammatory response. Term and preterm parturition are initiated by an enhanced inflammatory response, increased levels of proinflammatory cytokines in amniotic fluid and the invasion of the fetal membranes, cervix and myometrium by neutrophils and macrophages. The secretion of cytokines and chemokines by the invading immune cells cause activation of NF-κB and other inflammation-associated transcription factors (e.g., AP-1). These activated transcription factors promote increased expression of myometrial proinflammatory and contractile/CAP (connexin-43 (*CX43*), oxytocin receptor (*OXTR*), and cyclooxygenase 2 (*COX-2/PTGS2*)] genes, leading to parturition. Intra-amniotic infection associated with chorioamnionitis can provide

the stimulus for the inflammatory response leading to preterm labor (91).

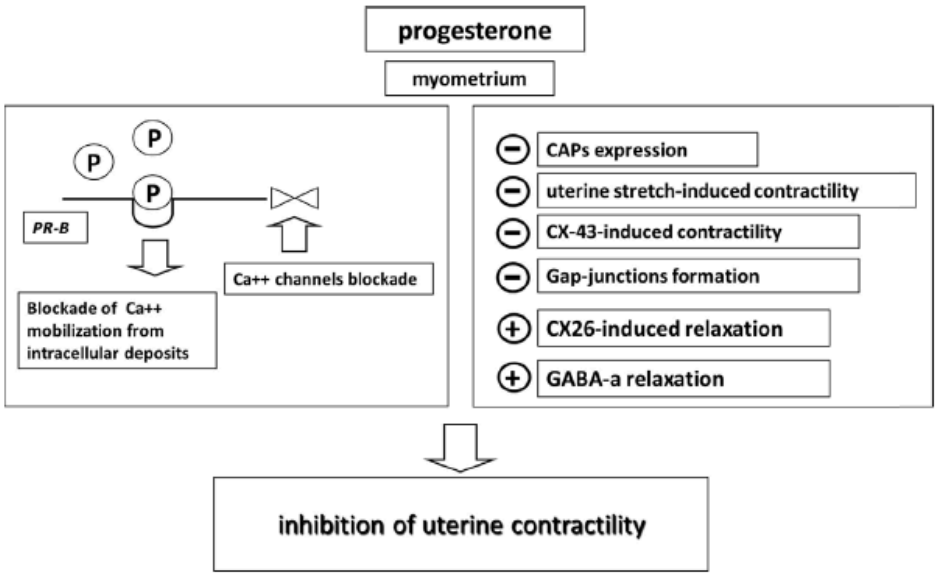


Fig.12. Progesterone activity on myometrium. Progesterone sustains the state of pregnancy and maintains uterine quiescence. Progesterone decreases myometrial contractility and inhibits CAPs expression, uterine stretch-induced contractility, CX-43 induced contractility, myometrial gap junction formation and stimulates CX-26 induced relaxation, GABA-a relaxation. Progesterone down-regulates prostaglandin production, as well as the development of calcium channels and oxytocin receptors (92).

Progesterone supplementation is recommended in order to prevent preterm labor in women with prior spontaneous preterm delivery (8, 90, 93). The exact action mechanism of progesterone is not well

understood (94, 95). Among progestogens, only two have been considered to be safe and effective for preventing recurrent preterm birth:

1. progesterone (natural micronized form) and
2. 17-alpha hydroxyprogesterone caproate (17-OHPC) (synthetic form progestogen).

Progesterone

It is administered by two routes: orally and vaginally. The main advantage of oral administration of progesterone is non-invasiveness and compliance. Oral administration is associated with a first-pass metabolism in the liver. The metabolites have sedative effects. Side effects such as intrahepatic cholestasis, sleepiness, fatigue and headaches are more common when progesterone is given orally (96, 97). With vaginal progesterone, increased vaginal discharge, genital itching and genital irritation are reported (98, 99).

17-alpha hydroxyprogesterone caproate

It has been developed to produce longer-lasting effects than progesterone alone. The half-life of 17-OHPC is 16.2 days (100) compared to 35-55 hours for progesterone (98). The Food and Drug Administration (FDA) approved a regimen of 250 mg weekly. Plasma concentrations continue to rise with repeated weekly administration (100). The 17-OHPC is given exclusively intramuscularly (IM). The 17-OHPC is licensed by the US Food and Drug Administration but not by the European Medicines Agency (EMA).

Regarding the choice of progestogen for prevention of preterm birth in women with singleton pregnancy and prior preterm birth, the Society for Maternal-Fetal Medicine (SMFM) has published a statement: « In women with singleton gestation and a history of prior spontaneous preterm birth between 20 weeks of gestation and 36 6/7 weeks of gestation, we recommend 17-OHPC at 250 mg IM weekly starting at 16-20 weeks of gestation until 36 weeks of gestation or delivery (101). Vaginal progesterone should not be considered as a substitute for 17-OHPC in these patients.» SMFM continues to support the use of vaginal progesterone to prevent preterm birth in women with short cervix (<20 mm) without a history of prior spontaneous preterm birth (101). The NICE guidelines offer a choice of either prophylactic vaginal progesterone or cervical cerclage to women with a history of spontaneous preterm birth or mid-trimester loss between 16 0/7 and 34 0/7 weeks of gestation and a short cervical length less than 25 mm between 16 0/7 and 24 0/7 weeks of gestation. The NICE guidelines offer prophylactic vaginal progesterone to women without history of preterm birth and short cervical length (<25 mm before 24 weeks of gestation) (66). The European Association of Perinatal Medicine (EAPM) recommends 17-OHPC or vaginal progesterone for women with prior history of preterm birth or late second-trimester abortion. The EAPM recommends vaginal progesterone for asymptomatic women with a short cervical length (<25 mm). Women with prior spontaneous preterm birth and a short cervical length (<25 mm before 24 weeks) should be offered cervical cerclage or vaginal progesterone (102). The International Federation of Gynecology and Obstetrics (FIGO) and the American College of Obstetricians and Gynecologists (ACOG) also

recommend vaginal progesterone for women with a singleton gestation and a short cervix without prior preterm birth (103, 104) (Figure 13).

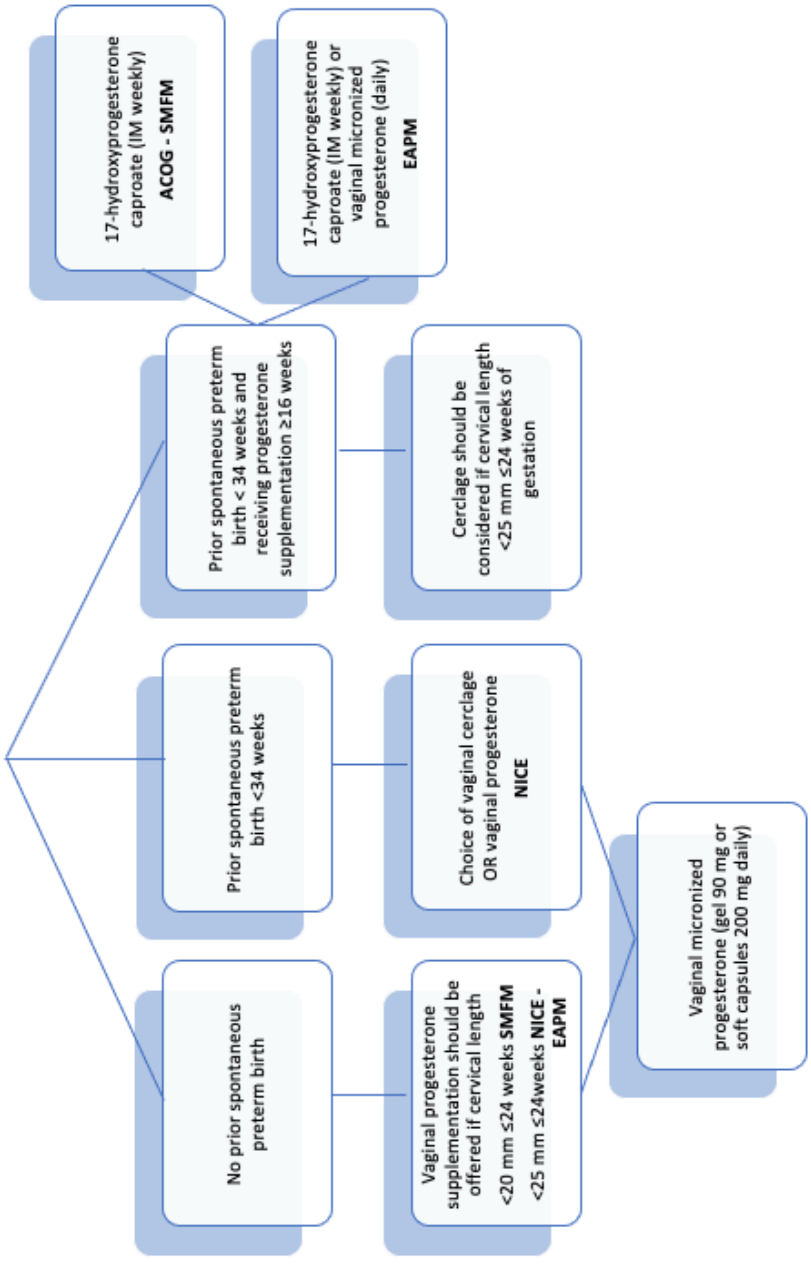


Fig.13. Summary of the guidelines: cerclage and progesterone supplementation.

In women without prior preterm birth, the cervical length screening may be considered between 19 0/7 and 23 6/7 weeks of gestation (105). Randomized trials have investigated the use of vaginal progesterone in women without prior preterm birth but with a short cervix diagnosed through screening strategy. A European trial that enrolled women with a very short cervical length (15 mm or less) demonstrated a lower risk of preterm birth in those treated with vaginal progesterone, 200 mg daily, compared with those treated with a placebo (68). In another randomized trial, the use of vaginal progesterone gel, 90 mg daily, is associated with a significant decrease in spontaneous preterm birth and in composite neonatal morbidity and mortality in women with a cervical length of 10-20 mm between 19 and 23 6/7 weeks of gestation (69). In 2012, a systematic review and meta-analysis of individual patient data (IPD) from randomized controlled trials comparing vaginal progesterone with placebo in women with singleton gestation and a cervical length ≤25 mm in the mid-trimester reported that the administration of vaginal progesterone was associated with a significant reduction of preterm birth from <28 weeks through <35 weeks of gestation, respiratory distress syndrome (RDS) and composite neonatal morbidity and mortality (106). Although randomized controlled trials recommend progestogens (vaginal progesterone and intramuscular 17-OHPC) for women at high risk of preterm birth, the largest trials (PROLONG for 17-OHPC and OPPTIMUM for vaginal progesterone) did not demonstrate efficacy. The PROLONG (Progestin's Role in Optimizing Neonatal Gestation) trial was required by the FDA but the study did not confirm treatment efficacy (107). The OPPTIMUM (dOes Progesterone Prophylaxis To

prevent preterm labor IMprove oUtcoMe?) study is the largest study to compare obstetric, neonatal and childhood outcomes in high-risk women treated with vaginal progesterone to prevent preterm birth. The OPPTIMUM study suggests that the efficacy of progesterone is either non-existent or weak. Progesterone has no demonstrable effect on 2-year neurodevelopmental outcomes (108). Although the evidence on efficacy remains not conclusive, the NICE, FIGO and SMFM organizations all recommend the use of progestogens for women at high-risk of preterm birth (105).

In women without prior preterm birth and with a short cervical length (<25 mm), cerclage might be considered to reduce the occurrence of threatened preterm birth (72). In a small retrospective study, cerclage decreased preterm birth in women with progressive cervical shortening (<10 mm) without prior preterm birth (109). A meta-analysis of five RCT's showed that cervical cerclage did not reduce the rate of preterm birth in women with short cervical length (<25 mm) and without prior preterm birth. A subgroup analysis revealed a significant decrease in preterm birth in women with short cervical length (<10 mm) while on progesterone (71). Cerclage seems to be effective at lower cervical lengths (<10 mm). Vaginal progesterone is effective in the reduction of risk of preterm birth in women with cervical length <25 mm (110), but its effectiveness appears to be decreasing for lengths <10 mm (68, 111).

2.3.2.2. Omega-3 fatty acid supplementation

Omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFA) are essential fatty acids because the body cannot produce them, and therefore must be obtained through the diet or synthesized from their precursor alpha-linolenic acid (ALA) and linolenic acid (LA) (112). Both types of fatty acids are precursors of signaling molecules with opposing effects. The predominant omega-6 fatty acid is arachidonic acid, which is converted to prostaglandins with an inflammatory effect. The omega-3 fatty acids antagonize the pro-inflammatory effect of omega-6 fatty acids (113). The pregnancy is a period of increased risk for omega-3 deficiency as they are used for the developing fetus. Long-chain polyunsaturated fatty acids are critical for the development of the fetal neurological and immune systems (114). Epidemiologic studies have shown significant associations between lower fish consumption in pregnancy and adverse outcomes such as preterm delivery (115, 116). Low omega-3 diet leads to a predominance of prostaglandin substrate and potentially confers a predisposition to preterm delivery (117). Maintaining a balance between the metabolites of omega-3 LCFUPA and the pro-inflammatory omega-6 arachidonic acid is important in maintaining normal gestation length and is a critical element in cervical ripening, and the initiation of labor. For these reasons, omega-3 supplementation is often recommended during pregnancy (118). The World Health Organization recommends an intake of 300 mg of omega-3 long-chain polyunsaturated fatty acids per day in pregnant women. Omega-3 fatty acids such as EPA (eicosapentanoic acid) and

DHA (docosahexanoic acid) are available as over-the-counter nutritional supplements. They aim to improve pregnancy outcomes, without clear recommendations. Several studies and reviews attempted to evaluate pregnancy (maternal and fetal) outcomes such as pre-eclampsia, preterm rupture of membranes, preterm labor, pregnancy-induced hypertension, gestational diabetes, obesity, postpartum depression, prenatal stress, intrauterine growth restriction, congenital malformations or anomalies and birth defects (112, 114, 119-125).

Recently, several studies have been carried out for studying the prevention of preterm birth in asymptomatic high-risk women including omega-3 supplementation. Their results remain not conclusive (121, 126, 127). In the update of Cochrane systematic review including 70 RCT's, preterm birth <37 weeks of gestation and early preterm birth <34 weeks of gestation were reduced in women receiving omega-3 LCPUFA compared with no omega-3 (128). In a recent randomized trial, omega-3 supplementation did not result in a lower risk of early preterm delivery than controls (129). Further studies are needed to determine the benefit of dietary supplementation. As omega-3 supplementation is safe, supplementation should be prescribed in patients with a low dietary intake and risk factors (125).

2.3.2.3. Vaginal Pessary

Another alternative approach for high-risk women with a history of preterm birth or a short cervical length has been to insert a silicone pessary around the cervix (Figure 14).

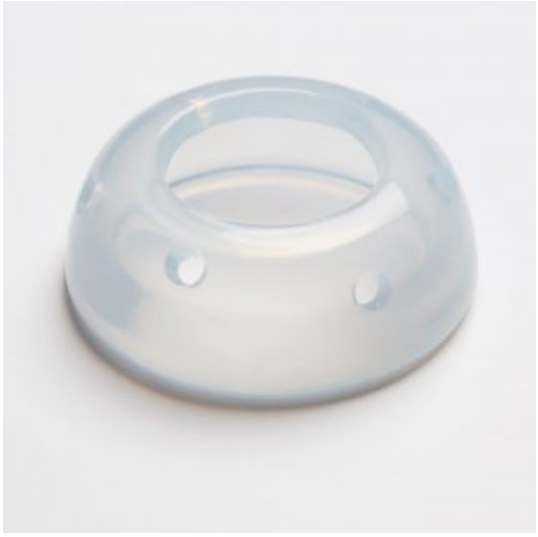


Fig.14. Arabin® Cerclage Pessary perforated (https://dr-arabin.de)

The placement of a pessary seems to be a low-cost procedure, non-invasive, and easy to insert and remove in outpatients unlike cervical cerclage. The mechanism of action of cervical pessaries is thought to bend the cervix backwards. By changing the uterocervical angle, the direct pressure from the uterus on the cervical canal is reduced (130, 131). Another possible mechanism is that the pessary could strengthen the immunological barrier between the fetal membranes and the vaginal microbiological flora, as cerclage has been postulated to do. To date, 4 randomized clinical trials in women with singleton pregnancies and a short cervical length (<25 mm) have been published and have provided contradictory results (132-135). All RCT's investigated whether cervical pessary in women with a short cervix identified by transvaginal cervical length at mid-trimester could reduce the rate of preterm birth <34 weeks of gestation. In the first RCT, spontaneous preterm birth <34 weeks was significantly less frequent in the pessary group than in the expectant management

group. No serious adverse effects associated with the pessary were reported. All women in the pessary group had increased vaginal discharge after the placement of the pessary (132). The next two RCT's did not confirm the reduced incidence of preterm birth <34 weeks but confirmed that additional maternal or perinatal outcomes were not significantly different in the pessary and the control groups (133, 134). The last publication studied women with singleton pregnancy and a short cervix: pessary was associated with a lower preterm birth rate (135). But this trial included women with no history of preterm birth, a different study group compared to the other trials. Finally, an evidence review concluded that further research is needed to investigate whether the effect of cervical pessary is reproducible prior to routine use (136).

Pessary in women with dilated cervix as an alternative to emergency cerclage has not evidenced any improvement in perinatal outcomes compared to expectant management. There was a significant improvement in all perinatal outcomes with cerclage compared to either pessary or expectant management (137).

In conclusion, current evidence does not support the use of cervical pessary to prevent preterm birth in singleton pregnancies with a short cervix (138).

2.3.3. Multiple pregnancies

Although twins and multiple pregnancies represent 1-3% of all pregnancies in Europe and the USA, they account for 17-20% of all preterm births. About half of them deliver before 37 weeks of gestation

(139, 140). In a first meta-analysis, twin gestations do not benefit from any preventive intervention with progesterone, cerclage and pessary to reduce preterm birth (141). In contrast to singleton pregnancies, cervical length screening at mid-trimester is a poor predictor of preterm birth in twin pregnancies (142).

Progesterone

In the first meta-analysis, vaginal progesterone seems to be the most promising intervention to reduce some key secondary outcomes (such as a very low birthweight and mechanical ventilation) in all twin pregnancies (141). In the next two meta-analysis, vaginal progesterone in twin pregnancies did not reduce significantly the risk of preterm birth <33 weeks (106, 143). But it significantly decreased the risk of composite neonatal morbidity and mortality in women with twin gestations and no prior preterm birth (106). Vaginal progesterone may be effective in the reduction of adverse perinatal outcomes (perinatal death, respiratory distress syndrome, intraventricular hemorrhage and necrotizing enterocolitis) in women with a short cervix ≤25 mm but not in women with a prior preterm birth (143).

Pessary

In twins with a short cervix, pessary has been reported to be beneficial in two RCT's (144, 145) and not beneficial in two other RCT's (146, 147). It remains unclear whether a pessary is beneficial in women with short cervix.

Transvaginal Cerclage

In a Cochrane systematic review, there is no evidence that cerclage is an effective intervention in multiple pregnancies for preventing preterm birth and reducing perinatal mortality and morbidity (148). In an older meta-analysis of randomized trials, the authors concluded that twins with short cervix have not been studied sufficiently in RCT's to determine the benefit of cerclage use in this population (149). An earlier meta-analysis indicates that cerclage placement is beneficial for the reduction of preterm birth and the prolongation of pregnancy in twin pregnancies with a cervical length of <15 mm or dilated cervix of >10 mm (150). For twins, the advantage seems more likely at shorter cervical lengths (<15 mm) following the new FIGO good practice recommendations (33). A recent RCT evaluating physical exam-indicated cerclage in asymptomatic twin pregnancies before 24 weeks confirmed a decrease in preterm birth and perinatal mortality (151). The benefit of history-indicated or twin alone-indicated cerclage is less certain in twin pregnancies with normal cervical length according to current literature (150).

Transabdominal Cerclage

An initial retrospective study reported the effect of prophylactic transabdominal cerclage in triplet pregnancies. TAC appeared to reduce the incidence of delivery before 28 weeks. Major limits of this study were: the main indication of TAC placement was the triplet pregnancy (152). In our recent series of 7 cases, perinatal outcomes were improved in twin pregnancies with transabdominal cerclage. Our

results suggested the benefit of transabdominal cerclage for managing cervical insufficiency in twin pregnancies (153).

In conclusion, further RCT's are needed on interventions (cerclage and pessary) for multiple pregnancies in order to determine their efficacy and safety.

2.4. Fetal membranes

2.4.1. Fetal membrane structure and function

Fetal membranes provide structural framework to the uterine cavity and provide immune, antimicrobial, endocrine and mechanical protection to the growing fetus.

Fetal membranes are constituted from the amniotic and chorionic layers consisting of multiple cell types embedded within an extracellular matrix (ECM) network. **Amnion** is the inner layer facing the fetus. It is a single cuboidal epithelial cell layer and the underlying collagen provides the majority of tensile strength. **Chorion** is the outer cell layer with reticular layer and trophoblast cells at the fetal-maternal interface (Figure 15).

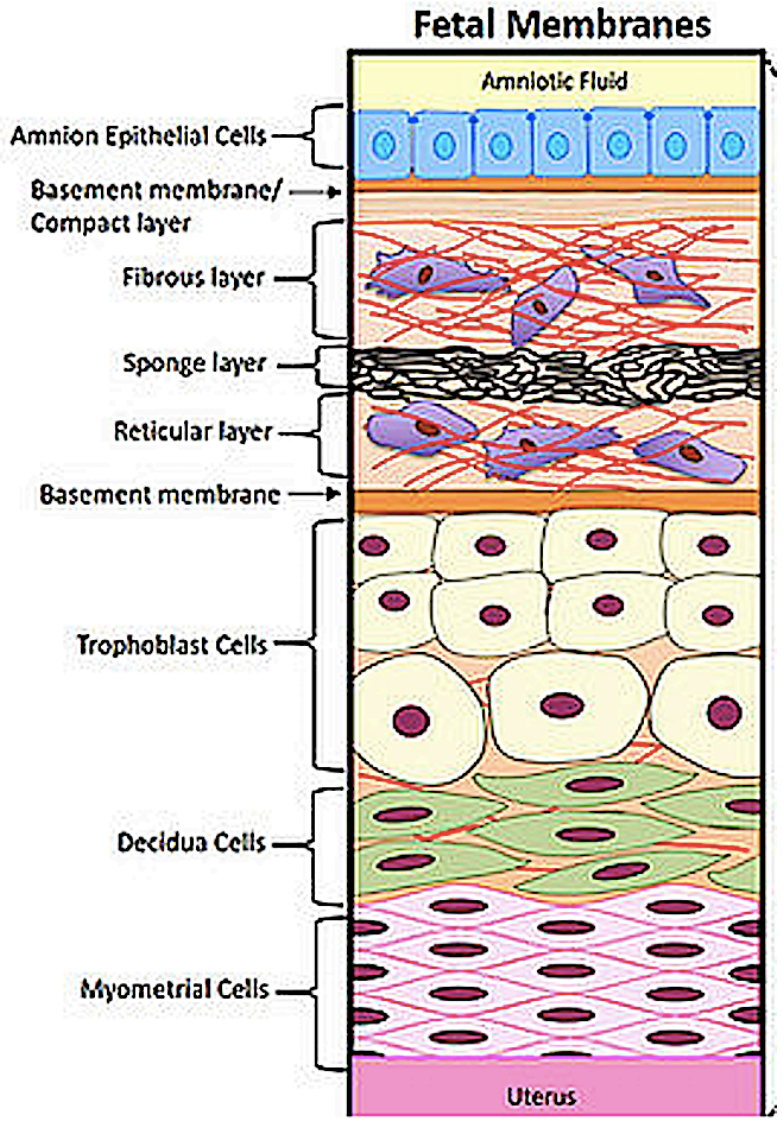


Fig.15. Schematic of fetal membranes
(https://www.fetalmembranesociety.org)

The anatomical structure of normal fetal membranes in late pregnancy was firstly described in 1960 (154). Originally, a zone of altered morphology (ZAM) was identified within the rupture site of term fetal membranes after the onset of labor in vaginal deliveries (155, 156). The morphological changes of this zone were a decrease in the total thickness of membranes with disruption of the extracellular matrix components. These changes weaken the fetal membranes and facilitate their rupture (155). Similar changes were found in the area overlying the cervix within term fetal membrane specimens collected during elective cesarean section before labor (157-159) (Figure 16).

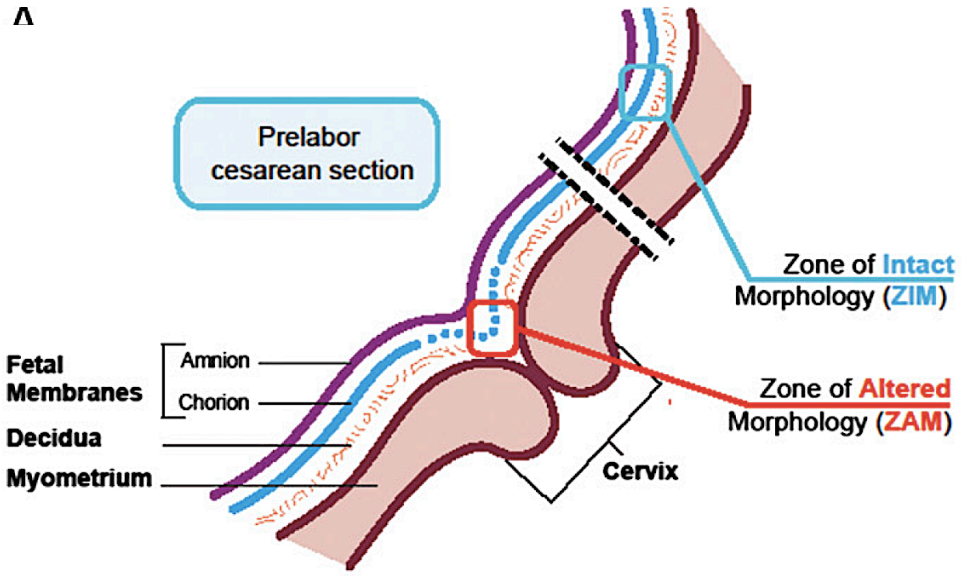


Fig.16. Zone of altered morphology (159).

ZAM are located on the internal os of the cervix and extend over the lower uterine segment (160). Structural changes of ZAM are consistent with collagen matrix remodeling and cell apoptosis prior to rupture of membranes (158). Structural alterations (especially regional

induction of matrix metalloproteinases 2 and 9, the main mediators of extracellular matrix degradation) before labor in the cervical location of membranes can play a role in programing this area to subsequent rupture of membranes (161). It was proposed that the ZAM would play a role in paracrine interactions. Chorion layer has the capacity to metabolize prostaglandins produced in amnion with the help of 15-hydroxyprostaglandin dehydrogenase (15-HPGD). Given the potential significance of altered morphology to paracrine interrelationships between fetal membranes, underlying decidua, myometrium and cervix, further characterization of these membranes may provide insight to their roles and contribution in the pathway of parturition (160).

2.4.2. The 15-hydroxyprostaglandin dehydrogenase (15-HPGD)

Prostaglandins play important roles in the process of parturition by promoting cervical ripening and myometrial contractility (92, 162). Increased production of prostaglandins contributes to initiation of parturition in term and preterm deliveries (163, 164) (Figure 17).

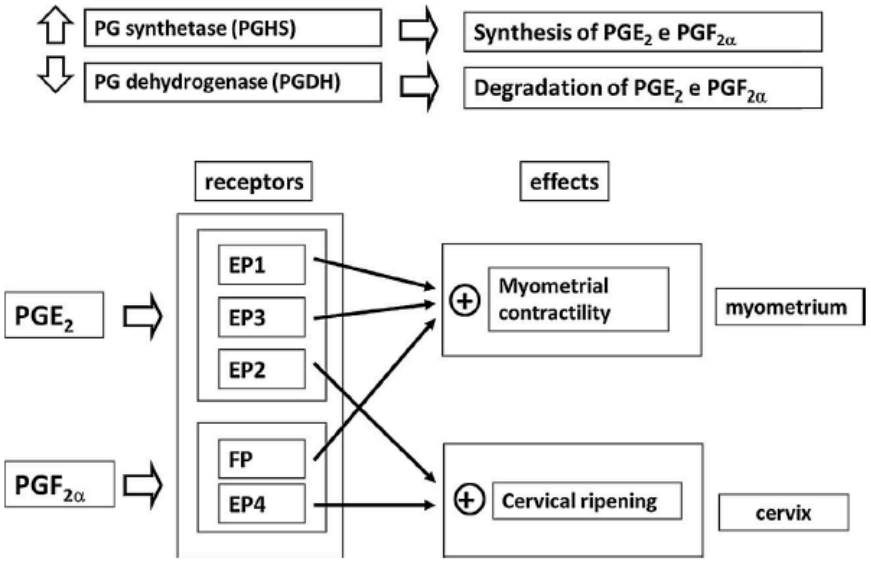


Fig.17. Prostaglandins (PGE_2 and $PGF_{2\alpha}$) play a central role in human parturition, acting to stimulate myometrial contractility and ripen the cervix (92).

In sheep, the placenta is the principal source of prostaglandins production (165), while in humans, fetal membranes are the main site of prostaglandins synthesis and metabolism. Prostaglandins act through prostaglandin or prostanoid receptors in myometrium. Prostanoid receptors are G protein-coupled receptors (166). During pregnancy, fetal membranes have barrier functions and fulfill paracrine signaling functions at the fetal-maternal interface. In fetal membranes, prostaglandins are synthesized in amnion and chorion layers by the prostaglandin synthase (PGHS2). Concentration of active prostaglandins is depending on the balance between synthesis and metabolism, thus finally of chorionic activity of the 15-hydroxyprostaglandin dehydrogenase (167-169) (Figure 18).

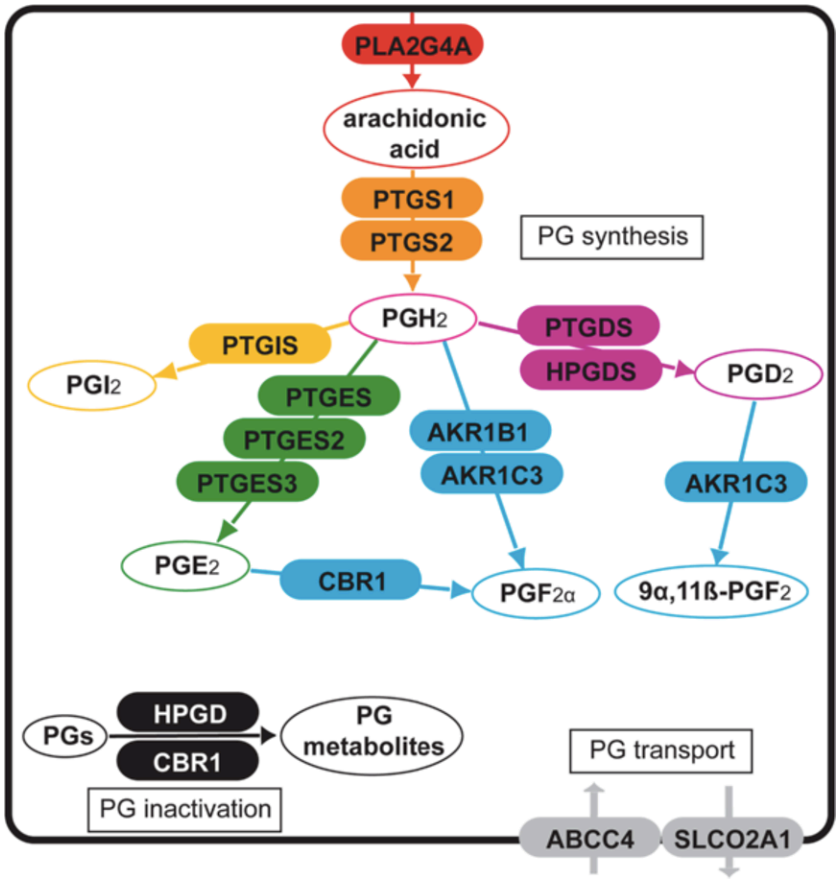


Fig.18. Cellular pathways of prostaglandin metabolism. Prostaglandin synthesis, prostaglandin transport and prostaglandin inactivation. A cell is depicted, showing enzymatic components (colored boxes) involved in precursor prostaglandin synthesis, terminal prostaglandin synthesis, prostaglandin transport and prostaglandin inactivation, with reactions (thin arrows) and products (open circles) (169).

Chorion layer acts as a barrier between maternal and fetal compartments in preventing prostaglandins to reach myometrium (170, 171). Therefore, prostaglandins levels may be controlled by

changes in synthesis and metabolism. Prostaglandins levels increase before and during labor in fetal membranes at term. Thereby, chorionic expression and activity of the 15-HPGD are significantly down regulated with term and preterm labor (172-175). However, mechanisms of regulation of the 15-HPGD remain largely unknown (176). Given that a down regulation of the 15-HPGD is a prerequisite for prostaglandins' action during parturition, the 15-HPGD plays a crucial role in maintenance of pregnancy. A recent study reports mechanisms of prostaglandins action in cervix by using 15-HPGD inhibitors and activators to initiate labor or to prevent cervical ripening and preterm birth (177).

2.4.3. Toll-like receptors

Immune modifications in fetal membranes overlying the cervix in the ZAM are described during late pregnancy before labor (178). These immune modifications clearly precede inflammatory response in parturition (159).

Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system (179). TLRs are transmembrane proteins with three types of ligands: Nucleic acids, proteins, and lipids. In human, ten TLRs (TLR1 to TLR10) have been identified. TLR3, TLR7, TLR8 and TLR9 are involved in viral recognition and are located within endosomes (180). TLRs belong to pattern recognition receptor (PRR) family. These receptors are charged of innate recognition of pathogen-associated molecular patterns (PAMPs). PAMPs are conserved sequences of proteins, lipids, polysaccharides, DNA or RNA present

in the membrane or envelope of pathogenic microorganisms. TLRs are also able to recognize damage-associated molecular patterns (DAMPs), such as heat shock proteins among other proteins released by cells undergoing stress-dependent-apoptosis (181). Signals generated by TLRs are transduced through NFκB (nuclear factor kappa B) signaling and MAP kinases pathway to recruit pro-inflammatory cytokines which promote inflammatory response. Main pro-inflammatory cytokines are TNF-α, IL-6 and IL-8. The nuclear factor kappa B (family of transcription factors) is a key regulator of immune response and inflammation (182) (Figure 19).

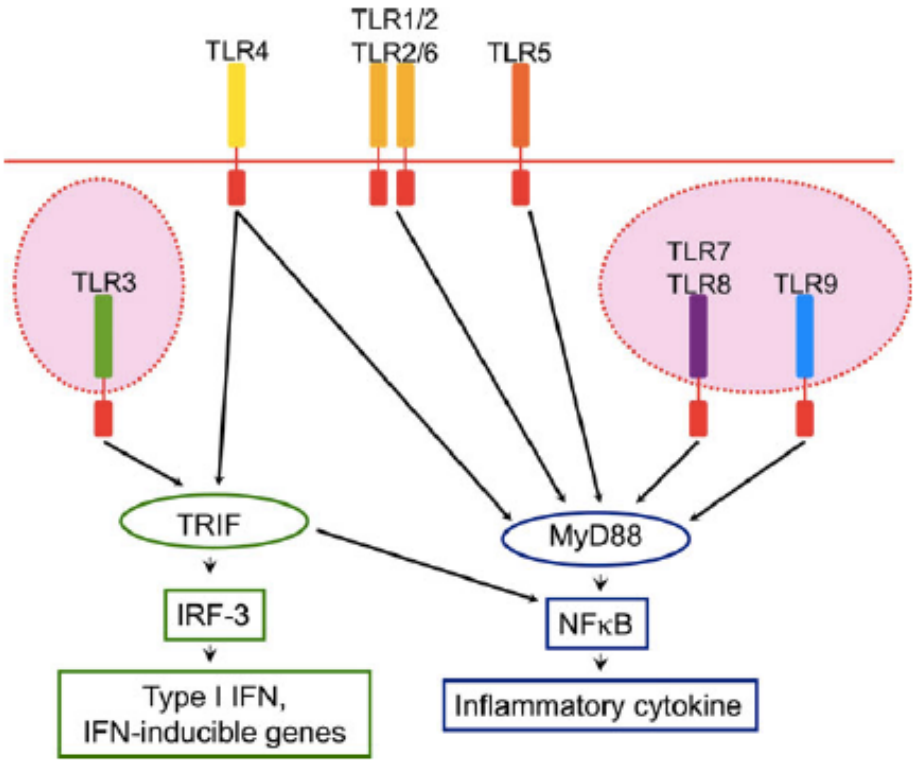


Fig.19. Toll-like receptors at the fetal-maternal interface. Membranal TLRs; TLR1, 2, 4, 5, 6, can recognize external signals, while cytoplasmic TLRs; TLR3, 7, 8, 9 will recognize intracellular signals. Following ligation, the majority of TLRs induce activation of NFκ B pathway, with resultant generation of an inflammatory response. TLR3 and TLR4 can also signal in a MyD88-independent manner. TLR3 and TLR4 can induce activation of TRIF which not only activates the NFκ B pathway, but also results in the phosphorylation of IFN regulatory factor-3 (IRF-3). This alternative pathway generates an antiviral response associated with the production of type I IFNs and IFN-inducible genes (183).

Throughout pregnancy, TLRs are expressed at the fetal-maternal interface (183, 184). TLR2 and TLR4 are expressed in syncytiotrophoblasts with the highest expression at the third trimester, possibly to restrain cervico-vaginal Gram-positive and -negative infections (185, 186). During pregnancy, not only trophoblasts express TLRs as an innate defense tool, but also Hofbauer cells (placental macrophages of fetal origin) and endothelial cells, decidua and fetal membranes (187, 188). Fetal membranes express all ten TLRs during pregnancy (189, 190). In particular, TLR2 and TLR4 expression is increased by chorioamnionitis (191). Relevance of TLRs presence at the fetal-maternal interface may reflect that a genitourinary infection tract could terminate a pregnancy by inducing labor signals, causing premature rupture of membranes associated with intrauterine bacterial infections (192, 193).

2.4.4. Senescence

Senescence or mechanism of aging, is associated with the deterioration process of membranes. It is a normal process in pregnancies (194). Senescence in fetal membranes is well recognized as a contributor of labor inducing signals (195-197). Factors initiating labor are complex and involve maternal, fetal and placental contributions. Hormonal, inflammatory and immune pathways participate to initiation of labor (92).

Contribution of fetal tissues, specifically fetal membranes, is described in initiation of labor. Senescence in fetal membranes is under physiological control and coincides with fetal organ maturation,

thereby indicating fetal readiness for delivery. Prior to the initiation of labor at term, oxidative stress (OS) increase accelerates an already progressing aging process in fetal membranes (198-200). During senescence, cells develop the senescence-associated secretory phenotype (SASP), an inflammatory feature. Therefore, inflammatory signals from senescent fetal membranes could trigger labor and parturition (201). Besides SASP, senescence in fetal membranes increases damage-associated molecular patterns (DAMPs). DAMPs may increase an ongoing inflammatory load in fetal membranes (202). Senescence in fetal membranes generates sterile inflammation mediated by SASP and DAMPs. Localized effects in fetal membranes are insufficient to promote robust uterine contractions, but SASP and DAMPs signals can be propagated across the fetal-maternal interface through direct diffusion to adjacent tissue layers or encapsulated within exosomes, which can be transported to sites of functional activity in myometrium, decidua or cervix. Inflammation and cellular damage are not restricted to membranes. Signal propagation between fetal-maternal tissues can be achieved via extracellular vesicles, specifically exosomes. Exosomes act as transporters of paracrine signals between fetal-maternal tissues (203-207).

In addition to the cell cycle arrest and the SASP, senescent cells also exhibit an enlarged and flattened morphology, expended lysosomal compartment and particular chromatin and epigenetic alterations. In order to validate the presence of cellular senescence with greater confidence, a multimarker approach has been proposed. One of the most widely used markers of senescence is the increased levels of

senescence-associated beta-galactosidase (SA β gal) activity (208-210). SA β gal is a hydrolase enzyme that catalyzes the hydrolysis of β galactosides into monosaccharides.

3. PART 1 : TRANSVAGINAL CERCLAGE – CLINICAL STUDY

This chapter has been previously published as: P. Steenhaut, C. Hubinont, P. Bernard, F. Debiève. Retrospective comparison of perinatal outcomes following emergency cervical cerclage with or without prolapsed membranes. Int J Gynaecol Obstet. 2017 Jun;137(3):260-264. doi: 10.1002/ijgo.12144.

ABSTRACT

OBJECTIVE: To compare perinatal outcomes following emergency cerclage between patients with singleton pregnancies with prolapsed and non-prolapsed membranes.

METHODS: The present retrospective cohort study included data from women who underwent physical examination-indicated emergency cerclage at between 15 and 25 weeks of pregnancy at Saint Luc University Hospital, Brussels, Belgium, between January 1, 2000, and December 31, 2014. Outcomes were compared based on the presence of prolapsed or non-prolapsed membranes. The primary outcome measures were the duration of pregnancy at delivery and the interval between cerclage and delivery. Secondary outcomes included delivery weight, fetal or neonatal death, and neonatal morbidity, including neonatal intensive care unit admission.

RESULTS: Data were included from 140 patients with cervical dilation of at least 1 cm; 85 women had non-prolapsed membranes and 55 women had prolapsed membranes. Among patients with non-prolapsed membranes, the mean duration of pregnancy at delivery was later (P<0.001), the latency between cerclage and delivery was longer (P<0.001), neonatal survival was higher (P=0.036), mean

delivery weight was higher (P<0.001), the prevalence of preterm delivery was lower (P<0.001), and severe neonatal morbidity and neonatal intensive care unit admission were lower (P<0.001).
CONCLUSION: Having non-prolapsed membranes was associated with improved perinatal outcomes following emergency cerclage.
KEYWORDS
Cervical dilation; Cervical insufficiency; Emergency cervical cerclage; Membrane protrusion; Perinatal outcomes
1. INTRODUCTION
Pregnancies complicated by cervical insufficiency during the second trimester of pregnancy are associated with high perinatal morbidity and mortality; chorioamnionitis, preterm premature rupture of the membranes, late spontaneous abortion, and preterm delivery are common adverse events with reported prevalence rates of 0.2%–7% of all pregnancies.[1] Cervical insufficiency has been reported to contribute to 16%–20% of instances of second-trimester fetal losses and 10% of preterm deliveries.[2,3]
Cerclage performed with a dilated cervix is termed rescue, emergency, or urgent cerclage.[4] Only one randomized controlled trial[5] has reported the benefits of emergency cerclage in the presence of cervical dilation and included 23 patients (16 singleton and 7 twin pregnancies). Emergency cerclage was demonstrated to be effective in prolonging the interval between diagnosis and delivery (by 30 days) and in preventing preterm delivery at earlier than 34 weeks of gestation; further, a trend toward improved neonatal survival was observed among patients undergoing emergency cerclage.[5] In a review article, Namouz et al.[6] suggest that emergency cerclage was associated with

a longer latency period and with improved pregnancy outcomes when compared with bed rest. In a recent systematic review and meta-analysis, Ehsanipoor et al.[7] report that physical examination-indicated cerclage was associated with a significant increase in neonatal survival and prolonged pregnancy compared with no cerclage. A recent small retrospective cohort study[8] evaluated pessary as an alternative to cerclage for a dilated cervix with exposed membranes; no improvement in perinatal outcomes was recorded with pessary compared to expectant management among women with a dilated cervix and prolapsed membranes.[8]

A significant concern for clinicians performing emergency cerclage is whether the time interval between cerclage and delivery is safe and if the procedure is efficient in prolonging pregnancy without maternal or fetal complications.

The objective of the present study was to compare perinatal outcomes after physical examination-indicated cerclage between women who had prolapsed and non-prolapsed membranes. The outcomes were evaluated with the aim of providing essential information on the benefits and risk factors of emergency cerclage for clinical practice.

2. MATERIALS AND METHODS

The present retrospective cohort study included data from women with singleton pregnancies who underwent emergency cerclage at Saint Luc University Hospital, Brussels, Belgium, between January 1, 2000, and December 31, 2014. All hospital records from this period were reviewed to identify eligible patients for inclusion. Patients with multiple pregnancies, chorioamnionitis (clinical and subclinical), preterm premature rupture of membranes, active preterm labor, active vaginal

bleeding, or placental abruption were excluded from the present study. Clinical chorioamnionitis was defined by the presence of fever, uterine tenderness, hyperleukocytosis, and/or elevated C-reactive protein. The subclinical chorioamnionitis was defined by amniotic fluid sampling demonstrating a leukocyte count above 50 cells/mm^3, glucose concentration up to 170 mg/L, lactate dehydrogenase above 420 U/L, positive Gram stain, and positive culture. The study was approved by the ethics committee of Saint Luc University Hospital, who waived the need for informed consent.

Cervical dilation was assessed by pelvic and/or speculum examination (by digital examination with a dilated internal os of at least 1 cm and/or by speculum examination with visible membranes). Cerclage was indicated by the detection of cervical changes of the internal os (dilated ≥1 cm, with or without membrane protrusion) by physical examination. The ultrasonography was used to assess the cervix but was not used to determine the application of cerclage.

Based on standard practice at the study institution, cervical cerclage was indicated in women with asymptomatic cervical dilation of the internal os of at least 1 cm who had membranes that were non-prolapsed or prolapsed beyond the external os; to be included, patients had to exhibit no symptoms of labor or chorioamnionitis and to have been at 15–25 weeks of pregnancy at the time of cerclage.

Patients were stratified by the presence or absence of prolapsed membranes beyond the external os. The primary outcomes were duration of pregnancy at delivery and the latency period between cerclage and delivery. The secondary outcomes were late spontaneous abortion, histologic chorioamnionitis, mode of delivery,

delivery weight, fetal or neonatal death, and neonatal morbidity (including admission to the neonatal intensive care unit). Neonatal morbidity was classified using three categories (none, treated with routine neonatal care; minimal, treated through neonatal intensive care unit admission without mechanical ventilation or severe morbidity; and severe, treated through neonatal intensive care unit admission with mechanical ventilation (including respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, sepsis, or life-threatening morbidity)).

McDonald cerclage or Wurm-Hefner cerclage were performed under locoregional anesthesia in the Trendelenburg position using 0.9-mm or 5-mm sutures, respectively (Mersilene; Ethicon, Somerville, NJ, USA). The choice to use the McDonald or Wurm-Hefner cerclage technique was made based on cervical length/dilation, and the presence or absence of prolapsed membranes. The McDonald technique involves the use of a circular suture allowing traction in the entire cervical circumference. The Wurm-Hefner technique involves a double transverse suture; one suture from the anterior to the posterior edge of the cervix and another from the right to the left edge. If prolapsed membranes were present, the amniotic sac was inserted inside the uterine cavity before the procedure to reduce the risk of intraoperative rupture of the membranes during cerclage placement. A smooth-surfaced stick, such as the finger of a glove filled with cotton pads, was introduced via the cervical canal to gently push the prolapsed membranes into the uterine cavity during cerclage placement. It was removed just before the knot was secured. Occasionally, transabdominal amniocentesis with amnioreduction was

performed under ultrasonography guidance to reduce the volume and pressure of the amniotic fluid in the prolapsed sac, thereby allowing the fetal membranes to remain inside the uterine cavity.

All patients were treated with bed rest and prenatal inpatient care or at least 7 days after cerclage. All women received broad-spectrum intravenous antibiotics at the time of the procedure and for a further 48 hours when awaiting cervical culture results. Prophylactic tocolysis with 100 mg of indomethacin was administered rectally twice a day for 2 days. After discharge, a 7-day course of clindamycin vaginal cream was prescribed each month.

Patient data were entered into SPSS version 23.0 (IBM, Armonk, NY, USA). Data were expressed as numbers with percentages, mean±SD, and median values with ranges, as appropriate. Continuous variables were analyzed using the unpaired Student t test, or the Mann-Whitney U test if the data had a non-Gaussian distribution. Qualitative variables were analyzed using the χ^2 test or the Fisher exact test. The study outcomes were evaluated as odds ratios or mean differences, with 95% confidence intervals. Kaplan-Meier curves were generated for the duration of pregnancy at delivery and were compared between patient groups using the log-rank test. For all analyses, $P<0.05$ was considered statistically significant.

3. RESULTS

There were 140 asymptomatic eligible patients identified and included in the present study; all patients underwent physical examination-indicated cerclage. There were 85 patients who had non-prolapsed membranes and 55 with prolapsed membranes. There were no differences in demographic and obstetric characteristics between the

groups, except that cervical length was longer among patients with non-prolapsed membranes (Table 1).

Table 1. Baseline characteristics.[a]

	Patients with non-prolapsed membranes (N=85)	Patients with prolapsed membranes (N=55)	*P* value[b]
Age (years)	31.9 ± 4.8	30.4 ± 5.3	0.089[c]
BMI	24.0 ± 4.3	25.6 ± 4.9	0.107
Past surgical procedure in the cervix (conization, operative hysteroscopy, curettage)	29 (34.1%)	12 (21.8%)	0.132[d]
Gestity	2 (1-10)	2 (1-10)	0.303
Parity	0 (0-6)	0 (0-3)	
Nuliparity/Primiparity	66 (77.6%)	50 (91%)	0.064[d]
Multiparity	19 (22.4%)	5 (9%)	0.064[d]
Gestational age at cerclage (weeks)	20.73±2.81	21.26±2.21	0.425
Cervical length (mm)	19.10±9.23	11.78±11.05	0.003

Abbreviation : BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

[a]Values are given as mean±SD, number (percentage), or median (range), unless indicated otherwise.

[b]Mann-Whitney *U* test, unless indicated otherwise.

[c]Student *t* test.

[d]Fisher exact test.

Kaplan-Meier curves were generated to compare the length of pregnancy at delivery and the log-rank test demonstrated that this differed significantly between the groups of patients (P<0.001) (Fig. 1). The duration of pregnancy at delivery and the time interval between cerclage and delivery were both significantly longer among patients

who did not have prolapsed membranes (Table 2). The rate of vaginal deliveries was similar between both groups but lower rates of late spontaneous abortion, histologic chorioamnionitis, fetal or neonatal death, and severe neonatal morbidity, as well as increased delivery weight were observed among patients with non-prolapsed membranes (Tables 2 and 3). Among the 108 neonates delivered after 24 weeks of gestation, lower odds of preterm delivery at less than 28 weeks of pregnancy, preterm delivery at less than 32 weeks of pregnancy, preterm delivery at less than 36 weeks of pregnancy, severe neonatal morbidity, and admission to the neonatal intensive care unit were recorded among the patient group with non-prolapsed membranes (Table 3). Among live neonates, the incidence of histologic chorioamnionitis was higher among neonates delivered after prolapsed membranes had been recorded. The odds of minimal neonatal morbidity did not differ between the patient groups but the odds of neonates having no morbidity was higher among neonates delivered by patients with non-prolapsed membranes (Table 3).

Table 2. Obstetric outcomes.[a]

Outcome	Patients with non-prolapsed membranes (n=85)	Patients with prolapsed membranes (n=55)	Odds ratio (95% CI)	Mean difference (95% CI)	*P* value[b]
Late spontaneous abortion (< 24 wk)	7 (8)	25 (45)	0.12 (0.05-0.31)	NA	< 0.001
Chorioamnionitis	6 (86)	21 (84)			> 0.99
Vaginal delivery rate	72 (85)	48 (87)	0.81 (0.3-2.18)	NA	0.806
Length of pregnancy at delivery, wk	35.00±5.98	26.54±5.91	NA	8.46 (6.31-10.61)	< 0.001[c]
Latency between cerclage and delivery, d	100.92±43.17	36.98±39.09	NA	63.94 (50.12-77.76)	< 0.001[c]

Abbreviation : CI, confidence interval ; NA, not applicable

[a]Values are given as mean±SD, number (percentage), unless indicated otherwise.

[b]Fisher exact test unless indicated otherwise.

[c]Mann-Whitney *U* test.

Table 3. Perinatal outcomes.[a]

Outcome	Neonates of patients with non-prolapsed membranes (n=78)	Neonates of patients with prolapsed membranes (n=30)	Odds ratio (95% CI)	Mean difference (95% CI)	*P* value[b]
Neonatal mortality	3 (4)	5 (17)	0.20 (0.04-0.90)	NA	0.036
Chorioamnionitis	3 (100)	3 (60)			0.464
Neonatal survival	75 (96)	25 (83)	5.0 (1.11-22.44)	NA	0.036
Histologic chorioamnionitis among live neonates[c]	12 (16)	11 (44)	0.24 (0.09-0.66)	NA	0.006
Birth weight, g	2852.2±839.8	1810.7±1136.5	NA	1041.5 (645.2-1437.8)	< 0.001[d]
Preterm delivery					
< 28 weeks	6 (8)	11 (37)	0.14 (0.05-0.44)	NA	< 0.001
< 32 weeks	10 (13)	18 (60)	0.10 (0.04-0.26)	NA	< 0.001
< 36 weeks	21 (27)	23 (77)	0.11 (0.04-0.30)	NA	< 0.001
≥ 36 w	57 (73)	7 (23)	8.92 (3.34-23.83)	NA	< 0.001
Neonatal morbidity[c]					
Severe	6 (8)	13 (52)	0.08 (0.03-0.25)	NA	< 0.001
Minimal	10 (13)	4 (16)	0.81 (0.23-2.85)	NA	0.739
None	58 (77)	6 (24)	10.80 (3.72-31.35)	NA	< 0.001

Neonates lost to follow-up	1 (1)	2 (8)			0.153
Neonatal intensive care unit admission[e]	16 (22)	17 (74)	0.10 (0.03-0.29)	NA	< 0.001

Abbreviation: CI confidence interval, NA not applicable

[a]Values are given as number (percentage) or mean±SD, unless indicated otherwise.

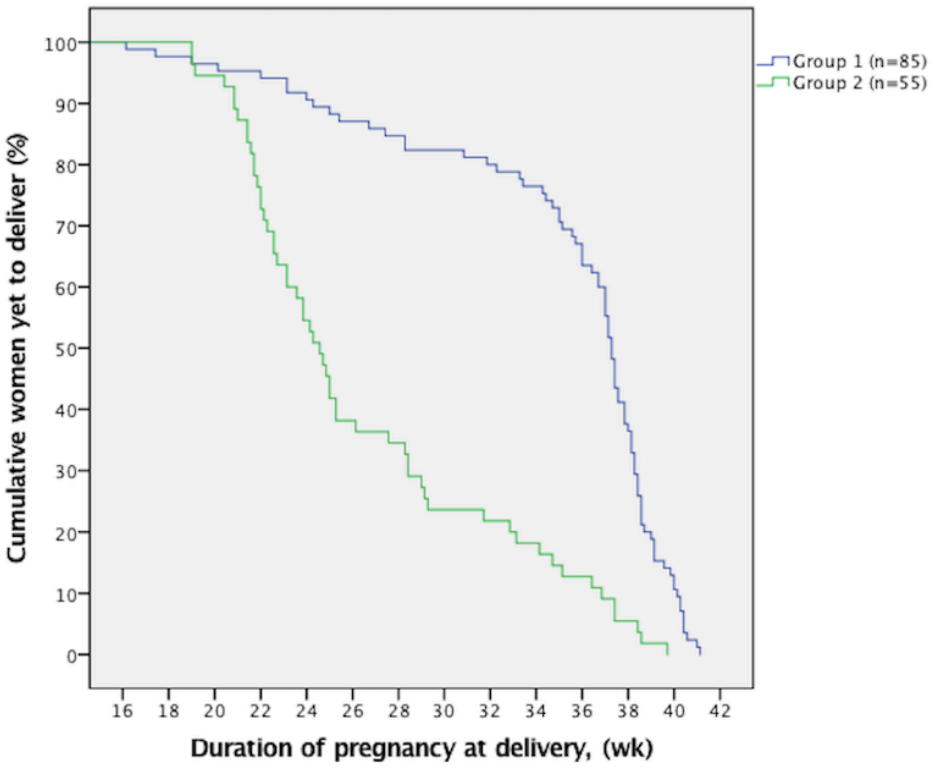
[b]Fisher exact test, unless indicated otherwise.

[c]Percentages calculated based on 75 neonates of patients with non-prolapsed membranes and 25 neonates of patients with prolapsed membranes.

[d]Mann-Whithney *U* test.

[e]Among neonates not lost to follow-up.

Figure 1. Kaplan-Meier curves of the percentages of patients yet to deliver at different lengths of pregnancy. The log-rank test demonstrated a significant difference between the patients who did not have prolapsed membranes (Group 1) and patients with prolapsed membranes (Group 2) at emergency cerclage placement (*P*<0.001).



4. DISCUSSION

The present study investigated differences in maternal and perinatal outcomes of emergency cerclage placement between patients who had prolapsed membranes and those with non-prolapsed membranes; significantly longer intervals between cerclage and delivery, and

significantly longer duration of pregnancy at delivery were demonstrated among patients who had non-prolapsed membranes.

Most data from studies examining emergency cerclage have been collected in retrospective studies.[9] A randomized controlled trial[5] that enrolled 23 women (16 singleton and 7 twin pregnancies) demonstrated significantly longer duration of pregnancy at delivery and decreased incidence of preterm delivery among patients who underwent emergency cerclage compared with patients treated with bed rest; however, the outcomes of the twin pregnancies were not reported separately from the singleton pregnancies.[5] Other publications [6,7,9–11] have supported the efficacy of physical examination-indicated cerclage in increasing the latency between diagnosis and delivery, and in preventing spontaneous preterm delivery. To assess the true benefit of emergency cerclage in clinical practice, predictive factors for successful pregnancy outcomes should be evaluated. Information about risk factors is needed in clinical practice for counseling women susceptible to require emergency cerclage.

The major strength of the present study was the large number of patients. More patients were included in comparison with many previous retrospective studies.[6,7,9–11] The inclusion of only singleton pregnancies gives the results greater clinical strength. The limitations of the present study included its retrospective design; additionally, in practice, some multiparous pregnant patients could have an internal os 1 cm in size without prolapsed membranes and could be at no increased risk of preterm delivery. These patients were not identified in the present study. It is also important to consider the potential

selection bias and the subjectivity of cervical examination. In the present study, a significant difference in cervical length was observed between the patient groups; cervical dilation is more advanced when membranes are prolapsed beyond the external os and this could explain the difference in cervical length recorded.

Terkildsen et al.[10] published a retrospective study that identified risk factors associated with delivery at or after 28 weeks of pregnancy among women with singleton pregnancies following emergency cerclage placement. The prolapse of membranes beyond the external cervical os and a pregnancy shorter than 22 weeks at cerclage placement were associated with decreased odds of delivery occurring at or after 28 weeks of pregnancy. The absence of prolapsed membranes beyond the external os and pregnancy duration longer than 22 weeks at cerclage placement were strong predictive factors for successful pregnancy outcomes.[10] The present study reported similar findings. When the data were stratified based on whether cerclage placement occurred before or after 22 weeks of pregnancy, placement prior to 22 weeks of pregnancy was associated with a lower incidence of delivery occurring after at least 28 weeks of pregnancy (32/43 [74.4%] vs 10/33 [30.3%]; P≤0.001).

Debby et al.[11] reported a retrospective cohort study that included women with a dilated cervix with and without membranes prolapsed into the vagina. The results of this study support the findings of the present study; favorable perinatal outcomes can be achieved after emergency cerclage, even if placement is performed when membranes are prolapsed into the vagina. Major adverse events were more frequent among patients with prolapsed membranes; however,

women can be informed of the risks and benefits of the procedure. The present study data provide important information for counseling women when emergency cerclage placement is considered.

In a study that enrolled patients with singleton pregnancies, Fuchs et al.[12] published a scoring system for predicting early preterm delivery among women undergoing emergency cerclage. The score and associated probabilities could be a tool for physicians making decisions regarding emergency cerclage. Similarly, the present study confirmed that emergency cerclage was associated with favorable outcomes among select patients.

In conclusion, emergency cerclage was associated with increased latency between cerclage and delivery, a decreased incidence of preterm delivery, and improved perinatal outcomes among patients with non-prolapsed membranes at cerclage placement when compared with patients with prolapsed membranes. These data reflect anecdotal experience from the study institution and suggest that emergency cerclage can be safe and efficient. Moreover, the present study has provided important information for addressing questions about the benefits and risks of emergency cerclage; this information could be useful in clinical practice for counseling patients who could require emergency cerclage placement.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design of the study, to the acquisition, analysis, and interpretation of data, and to drafting and revising the article. All authors gave final approval for the article.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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ADDENDUM

1. ULTRASOUND CERVICAL LENGTH

During the study period between 2000 and 2014, the ultrasound cervical assessment was not systematically performed for each patient and was not used as criteria for cerclage. In the group of women with non-prolapsed membranes, the cervical length was known for 50 of 85 patients (59%). In the group of women with prolapsed membranes, the cervical length was known for 23 of 55 patients (42%). As shown in Table 1, the mean (±SD) of cervical length was 19.10 mm (± 9.23) in the group of women with non-prolapsed membranes versus 11.78 mm (± 11.05) in the group with prolapsed membranes (*P*=0.003). In this group with prolapsed membranes beyond the external os, the more advanced cervical dilation could explain the significant shorter cervical length or the absence of cervical length assessment.

In each group, a subgroup analysis of Kaplan-Meier curves was performed between patients with a known and unknown cervical length (unpublished data). The log-rank test did not demonstrate a significant difference between the subgroup of women in the non-prolapsed membranes group (*P*=0.317) and in the prolapsed membranes group (*P*=0.103).

Figure 1A. Kaplan-Meier curves between women with known (n=50) and unknown (n=35) cervical length in the group with non-prolapsed membranes (*P*=0.317).

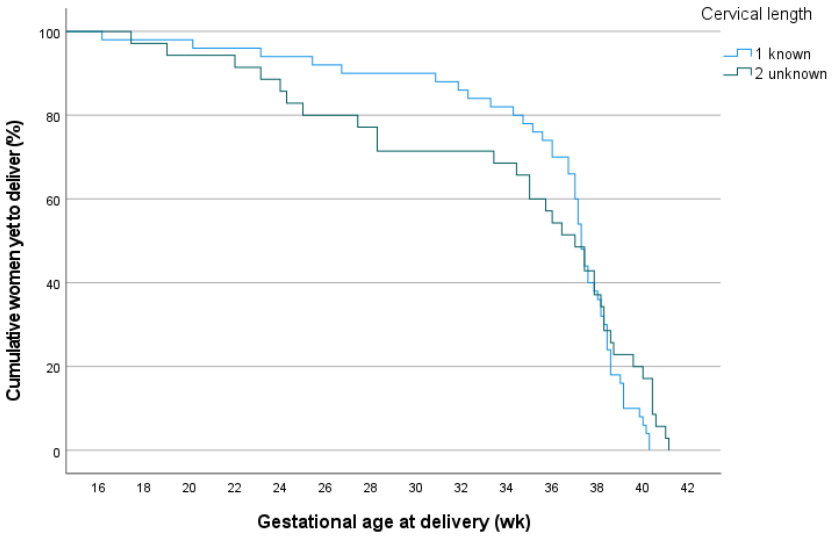
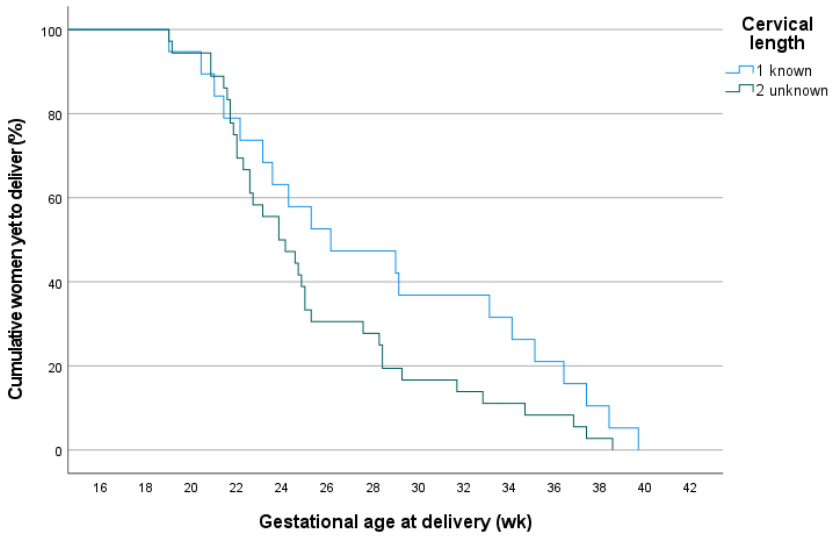


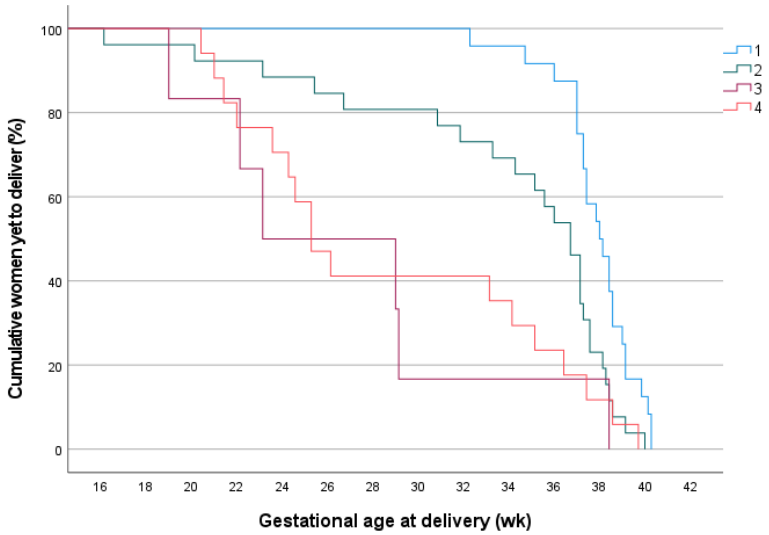
Figure 2A. Kaplan-Meier curves between women with known (n=23) and unknown (n=32) cervical length in the group with prolapsed membranes (*P*=0.103).



In conclusion, outcomes are not significantly different between women with a known and unknown cervical length in the group with non-prolapsed or prolapsed membranes. Women with a known cervical length have the same outcomes than with an unknown cervical length.

A subgroup analysis was also performed in women with a known cervical length between non-prolapsed and prolapsed membranes groups. The cut-off value for cervical length was ≥ 20 mm and < 20 mm. The Kaplan-Meier curves are shown in Figure 3A.

Figure 3A. The group 1 was: women with cervical length ≥ 20 mm and non-prolapsed membranes (n=24). The group 2 was: women with cervical length < 20 mm and non-prolapsed membranes (n=26). The group 3 was: women with cervical length ≥ 20 mm and prolapsed membranes (n=6). Group 4 was: women with cervical length < 20 mm and prolapsed membranes (n=17).



The log-rank test pairwise comparisons were:

		1	2	3	4
Log-Rank	**1**		0.004	< 0.001	< 0.001
	2	0.004		0.087	0.083
	3	< 0.001	0.087		0.539
	4	< 0.001	0.083	0.539	

Patients with non-prolapsed membranes and cervical length ≥ 20 mm (group 1) are the group with the significantly latest gestational age at delivery compared to groups 2, 3 and 4. Patients with non-prolapsed membranes and cervical length < 20 mm (group 2) have not significantly better outcomes compared to groups 3 and 4.
Patients with prolapsed membranes and cervical length ≥ 20 mm (group 3) have not significantly better outcomes compared to group 4. Patients with prolapsed membranes have a poor prognosis regardless of cervical length. In conclusion, the cervical length is not useful for patients with prolapsed membranes beyond the external os.

2. MULTIPLE PREGNANCIES

Our retrospective cohort study included unpublished data with multiple pregnancies: 18 twins and 2 triplet pregnancies. Only data with singleton pregnancies have been published. All multiple pregnancies were dichorionic or trichorionic. Multiple pregnancies were divided into two groups follow the absence (n=13) or presence (n=7) of prolapsed membranes beyond the external os. Maternal characteristics were similar for each group. Mean gestational age at cerclage was the same: 22.89±2.31 vs 22.26±1.22 weeks (P=0.394), respectively (Table 1A). The mean gestational age at delivery was 32.51±4.51 vs 26.69±5.16 weeks (P=0.003). The mean interval from cerclage to delivery was 67.38±32.56 vs 31.14±29.25 days (P=0.032) (Table 2A). The mean birth weight was 2014.2±580.1 vs 1224.5±796.5 grams (P=0.002) (Table 3A). Preterm deliveries were assessed with the use

of Kaplan-Meier analysis, in which gestational age was time scale and delivery the event. When comparing the non-prolapsed membranes group vs the prolapsed membranes group, the risk of preterm delivery showed a later decrease in multiple pregnancies with non-prolapsed membranes ($P=0.055$). For the same comparison between the non-prolapsed membranes group and the prolapsed membranes group, the risk was significantly decreased in singleton pregnancies ($P<0.001$) (Figure 4A). These results suggest that physical exam indicated cerclage in multiple pregnancies could be as successful as in singleton pregnancies.

Table 1A. Baseline characteristics.[a]

	Patients with non-prolapsed membranes (N=13)	Patients with prolapsed membranes (N=7)	*P* value[b]
Age (years)	31.1 ± 4.0	31.4 ± 5.8	0.128
BMI	26.5 ± 4.7	27.1 ± 5.2	0.384
Past surgical procedure in the cervix (conization, operative hysteroscopy, curettage)	2 (15.4%)	0 (0%)	0.521[d]
Gestity	2 (1-5)	1 (1-3)	0.891
Parity	1 (0-3)	0 (0-1)	0.141
Nuliparity/Primiparity	2 (15.4%)	4 (57.1%)	0.121[d]
Multiparity	11 (84.6%)	3 (42.9%)	0.121[d]
Gestational age at cerclage (weeks)	22.89±2.31	22.26±1.22	0.394
Cervical length (mm)	20.20±11.68	10.17±7.98	0.128

Abbreviation : BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

[a]Values are given as mean±SD, number (percentage), or median (range), unless indicated otherwise.

[b]Mann-Whitney *U* test, unless indicated otherwise.

[c]Student *t* test.

[d]Fisher exact test.

Table 2A. Obstetric outcomes.[a]

Outcome	Patients with non-prolapsed membranes (n=13)	Patients with prolapsed membranes (n=7)	Odds ratio (95% CI)	Mean difference (95% CI)	*P* value[b]
Late spontaneous abortion (< 24 wk)	1 (7)	2 (28)	0.27 (0.02-3.51)	NA	0.270
Chorioamnionitis	1 (100)	1 (50)			0.711
Length of pregnancy at delivery, wk	32.51±4.51	26.69±5.16	NA	5.82 (1.15-10.48)	0.003[c]
Latency between cerclage and delivery, d	67.38±32.56	31.14±29.25	NA	36.24 (5.21-67.26)	0.032[c]

Abbreviation : CI, confidence interval ; NA, not applicable

[a]Values are given as mean±SD, number (percentage), unless indicated otherwise.

[b]Fisher exact test unless indicated otherwise.

[c]Mann-Whitney *U* test.

Table 3A. Perinatal outcomes.[a]

Outcome	Neonates of patients with non-prolapsed membranes (n=25)	Neonates of patients with prolapsed membranes (n=11)	Odds ratio (95% CI)	Mean difference (95% CI)	*P* value[b]
Neonatal mortality	0 (0)	2 (18)	0.09 (0.01-2.03)	NA	0.130
Chorioamnionitis	0 (0)	0 (0)			
Neonatal survival	25 (100)	9 (82)	11.08 (0.49-229.88)	NA	0.130
Histological chorioamnionitis among live neonates[c]	1 (4)	4 (44)	0.09 (0.01-0.91)	NA	0.041
Birth weight, g	2014.2±580.1	1224.5±796.5	NA	792.7 (313.8.2-1271.5)	0.002[d]
Preterm delivery					
< 28 weeks	2 (8)	6 (55)	0.14 (0.02-0.84)	NA	0.004
< 32 weeks	9 (36)	9 (82)	0.44 (0.13-1.41)	NA	0.194
< 36 weeks	19 (76)	9 (82)	0.92 (0.32-2.69)	NA	1.0
≥ 36 w	6 (24)	2 (18)	1.32 (0.22-7.59)	NA	1.0
Neonatal morbidity[c]					
Severe	12 (48)	7 (78)	0.61 (0.18-2.05)	NA	0.432
Minimal	7 (28)	0 (0)	5.58 (0.29-107.63)	NA	0.254
None	6 (24)	2 (22)	1.08 (0.18-6.35)	NA	0.932

Neonates lost to follow-up	0 (0)	0 (0)
Neonatal intensive care unit admission[e]	25 (100)	11 (100)

Abbreviation: CI confidence interval, NA not applicable

[a]Values are given as number (percentage) or mean±SD, unless indicated otherwise.

[b]Fisher exact test, unless indicated otherwise.

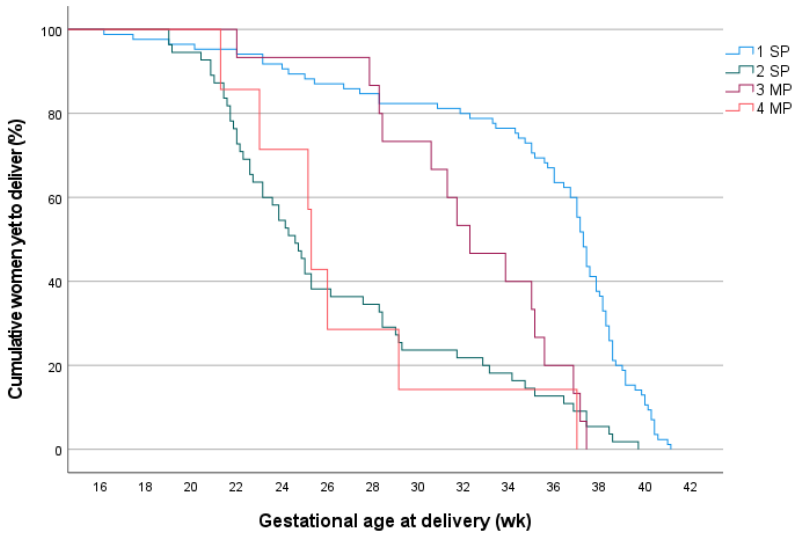
[c]Percentages calculated based on 25 neonates of patients with non-prolapsed membranes and 9 neonates of patients with prolapsed membranes.

[d]Mann-Whithney *U* test.

[e]Among neonates.

Figure 4A. Survival curves of singleton (SP) and multiple (MP) pregnancies that remained undelivered across gestation. The group 1 was singleton pregnancies with non-prolapsed membranes. The group 2 was singleton pregnancies with prolapsed membranes. The group 3 was multiple pregnancies with non-prolapsed membranes. Group 4 was multiple pregnancies with prolapsed membranes. The log-rank test pairwise comparisons were:

		1	2	3	4
Log-Rank	**1**		< 0.001	< 0.001	< 0.001
	2	< 0.001		0.072	0.977
	3	< 0.001	0.072		0.055
	4	< 0.001	0.977	0.055	



Our results showed that transvaginal emergency cerclage could be used in multiple pregnancies with similar outcomes than in singleton

pregnancies. Another retrospective cohort study confirmed that emergency cervical cerclage, even with 0-mm cervical length or prolapsed membranes is as effective in twin pregnancies as in singletons (211). After physical exam indicated cerclage in twin pregnancies, a recent RCT showed overall a significant decrease of the incidence of preterm birth and longer latency interval from cerclage to delivery (151). As the results of transvaginal cerclage are promising for twin pregnancies, we have also published a series of seven cases of TAC in twins. Perinatal outcomes were considerably improved with TAC (153).

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ORIGINAL ARTICLE

Transabdominal cerclage for cervical insufficiency in twins: series of seven cases and literature review

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ABSTRACT

Background: The diagnosis of cervical insufficiency is based on the previous history of recurrent second or early third trimester losses. Its incidence among pregnant women is 0.5–1% but can be as high as 75% among women with preterm birth. Transvaginal cerclage (TVC) is the common therapy of cervical insufficiency. However, this technique has several limits, especially in twin pregnancies. As some selected conditions, a transabdominal cerclage (TAC) is indicated, it has been offered to patients with multiple pregnancies.

Aim: To evaluate the outcomes of twin pregnancies with transabdominal cerclage in terms of preterm birth rate and neonatal morbidity and mortality.

Materials and methods: We conducted a retrospective study of seven patients with twin pregnancies managed with transabdominal cerclage at the end of the first trimester (12–15 weeks). We selected patients with a history of fetal loss who met the indications criteria of TAC (history of TVC failure or short cervix unable to have TVC). The antenatal and delivery data were collected and compared to those of their previous pregnancy.

Outcomes: All patients carried their pregnancy throughout the second trimester and delivered during the third trimester. Mean gestational age was 34 4/7 week. All newborns were alive and neonatal morbidity rate was 50%, mostly related to preterm birth. Mean duration of neonatal intensive care stay was 32 days. There were no operative complications following TAC.

Conclusions: Perinatal outcomes are considerably improved in twin pregnancies with transabdominal cerclage. Our findings corroborate those of previous case reports and support the efficacy of TAC for managing cervical insufficiency in twin pregnancies.

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Abdominal cerclage; prematurity; twins

Introduction

The diagnosis of cervical insufficiency is based on the previous history of recurrent second or early third trimester losses, but its pathophysiology is unclear, probably involving a mechanical disorder of the cervix prior to 37 weeks of gestation, in the absence of uterine contractions or labor [1].

Cervical insufficiency may be congenital or result from acquired cervical abnormalities such as cervical trauma from delivery or gynecologic surgery (conization), collagen disorders such as Ehlers–Danlos, uterine anomalies, and *in utero* diethylstilbestrol exposure [2,3].

There is no consensus regarding the diagnostic criteria of cervical insufficiency. Therefore, the diagnosis of cervical insufficiency should be based on the obstetrical history of recurrent pregnancy losses [4].

The usual management of cervical insufficiency is transvaginal cerclage (TVC). According to randomized controlled trial data, it is indicated in three populations: women with history of at least three or more deliveries before 37 weeks (prophylactic cerclage between 12 and 14 weeks) [5]; women with history of 1 or 2 seconds/early third trimester fetal loss and a US cervical length <25 mm (therapeutic cerclage) [6]; women with a cervical dilatation >2 cm discovered during physical examination, after a reasonable delay or an amniocentesis to preclude a subclinical infection (emergency cerclage) [7].

In the specific condition of multiple pregnancies, TVC is not recommended, according to a 2014 systematic review of randomized trials reporting no benefits of transvaginal cerclage in multiple pregnancies [8].

In 1965, Beson and Durfee reported the first transabdominal cerclage (TAC) technique in women with cervical insufficiency. These patients had previous unsuccessful pregnancies despite a TVC [9].

Due to the increased procedure-related morbidity and the need for two laparotomies (one for the

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cerclage and one for elective caesarean section), most experts restrict the indication of the transabdominal approach to women with second-early third-trimester pregnancy loss and either failed previous transvaginal cerclage or with a too short cervix to allow the placement of a vaginal cerclage [10].

Although experiences of TAC in singleton pregnancies have been published, there are only a few case reports on TAC in twin pregnancies. The objective of this study is to evaluate the outcomes of TAC in twin pregnancies.

Materials and methods

This retrospective unicenter study includes data on twin pregnancies in women with TAC between 2011 and 2016 at the Cliniques Universitaires Saint-Luc.

We selected women with twin pregnancies presenting indications for TAC: history of TVC failure or a cervix too short to allow TVC with a history of fetal loss [10]. The antenatal and delivery details were reviewed. We compared the outcomes of TAC with those of the patients' prior pregnancies. Obstetric outcomes included gestational age at delivery, incidence of delivery <32 weeks of gestation, birth weight, neonatal morbidity, and neonatal mortality. Operative outcomes included blood loss, intra and extraoperative complications such as fetal death, intrauterine growth restriction, suture migration, rupture of uterine vessels, infection, premature labor, premature rupture of membranes, uterine rupture, recto-vaginal fistula, and bladder injuries.

All TAC were performed by the same obstetrician (FD). Under general anesthesia, through a Pfannenstiel incision, the abdominal wall was opened until exposition of the uterus. The bladder was repressed and the cervicoisthmic region was exposed through sharp and blunt dissection of the vesicouterine peritoneum. The uterine vessels were displaced laterally to confirm avascular space. The avascular space was perforated with a steel clamp. A 5 mm mersilene tape was passed through the tunnel from the anterior to posterior, in-between uterine arteries, and was tied anteriorly. The correct position of the cerclage around the cervix was checked with ultrasound. After assuring hemostasis, the abdominal layers were closed, as done routinely.

All neonates were delivered by cesarean section and the Mersilene band was left in place as suggested by Cammarano et al. for the benefit in any future pregnancy [11].

Table 1. Maternal characteristics.

Patient	Age	GxPy	Indication	Gestational week at placement
1	31	G2P1	3 Conizations 1 PTB (29 5/7 w)	12
2	29	G4P0	1 Fetal loss (12 w) 1 PTB (24 w) despite TVC	12 3/7
3	35	G4P1	1 Fetal loss [20 w] despite TVC	Former pregnancy
4	38	G3P1	1 Conization 1 PTB (24 w) 1 Fetal loss (20 6/7 w) despite TVC	11 5/7
5	24	G4P0	3 Fetal losses (>12 w) TVC failure	10 6/7
6	28	G5P1	3 Fetal loss (>15 w) TVC and pessary failure	Former pregnancy
7	26	G6P0	4 Fetal loss (>15 w) despite TVC	12

PTB: preterm birth; TVC: transvaginal cerdage.

Results

Our study included seven women with a mean maternal age of 31 years old, all with dichorionic/diamniotic twin pregnancies. They underwent prophylactic TAC between 11 and 14 weeks of gestation (Table 1).

Six patients out of seven had previous TVC (or pessary) failures and pregnancy losses; the seventh patient had an extremely short cervix from three conizations enabling the placement of a vaginal cerclage and history of preterm birth before 30 weeks gestation.

The obstetrical outcomes are presented in Table 2. Gestational age at delivery ranged from 28 to 37 weeks: 1/7 < 29 weeks, 5/7 between 32 and 36 weeks, 1/7 at 37 weeks. The mean gestational age was 34 + 4/7 weeks and the incidence of delivery <34 weeks was 29%.

Three women went into spontaneous preterm labor; one patient had a spontaneous preterm premature rupture of membranes, one woman underwent elective cesarean delivery at term. We performed one emergency cesarean delivery for nonreassuring fetal status associated with growth retardation at 28 + 6/7 weeks.

All newborns were delivered alive. Birth weight ranged from 680 to 3030 grams (mean birth weight of 2124 g): two extremely low-birth weights (<1500 g), five low-birth weights (<2500 g), and three normal birth weights.

Major neonatal complications occurred in two pairs of twins born before 34 weeks and presented as hyaline membrane disease, chronic lung disease, and intestinal obstruction as a result of meconium ileus. The other complications occurred in the late preterm newborns and included transient tachypnea of the

Table 2. Obstetrical outcomes.

Patient	Gestational week of delivery	Indication	Birth weight	Apgar scores	Neonatal complications	Length of hospital stay
1	28 6/7	SC (fetal distress)	680 g	2-9-9	HMD CLD MI&IO	3 months
			980 g	4-9-9	HMD CLD	3 months
2	37 1/7	SC	2350 g	8-9-10	/	/
			2460 g	9-10-10	/	/
3	36 4/7	PTL	2820 g	8-9-10	TTN	10 days
			2500 g	8-9-9	/	/
4	35 5/7	PPROM	3030 g	7-8-9	/	/
			2440 g	8-9-10	RDS	24 hours
5	32 5/7	PTL	2040 g	9-10-10	/	4 weeks
			1940 g	9-10-10	HMD	4 weeks
6	36 4/7	SC	2620 g	9-10-10	/	/
			3050 g	9-10-10	/	/
7	34 4/7	PTL	1445 g	7-8-8	/	6 weeks
			1860 g	3-7-8	/	6 weeks

PTL: preterm labor; SC: scheduled caesarean section; PPROM: premature preterm rupture of membranes; HMD: hyaline membrane disease; CLD: chronic lung disease; MI&IO: meconium ileus and intestinal obstruction; TTN: transient tachypnea of the newborn; RDS: respiratory distress syndrome.

newborn and respiratory distress syndrome. Mean duration of neonatology stay was 32 days.

The median estimated blood loss during cerclage placement was 200 ml, no blood transfusion was required. No operative complications were reported.

Discussion

In 2006, Lotgering et al. published a study including 101 women with a history of cervical insufficiency and a cervix too short to allow for effective transvaginal cerclage. The conclusions showed successful outcomes of TAC in terms of delivery >32 weeks with improved neonatal survival [10].

The advantages of transabdominal cerclage over transvaginal cerclage are the following: placement of the stitch near internal cervical os, decreased risk of suture migration, absence of a foreign body in the vagina that could promote infection and the ability to leave the suture in place for future pregnancies [12]. Those potential benefits are challenged by two disadvantages: a higher procedure-related morbidity and the need for two laparotomies during pregnancy (one to place the cerclage and one for the elective cesarean section).

A systematic review compared the outcomes of either TVC or TAC after a prior failed TVC. TAC was associated with a lower perinatal death rate and a lower incidence of delivery <24 weeks [13]. According to these results and given the potential disadvantages of TAC, most experts recommend to offer the transabdominal approach only to women who have either failed previous transvaginal cerclage or to women with a short cervix (cervical trauma, collagen disorders, *in utero* diethylstilbestrol) and history of second-early third-trimester pregnancy loss [10].

In multiple pregnancies, a 2014 systematic review of randomized trials comparing cervical cerclage with no cervical cerclage in multiple gestations did not find current evidence of benefit for transvaginal cerclage in multiple pregnancies, regardless of the indications [8].

Furthermore, a review of 14 studies showed an increased risk of preterm birth, very low-birth weight and respiratory distress [14]. Therefore, transvaginal cerclage is not recommended in multiple pregnancies for preventing preterm birth.

Only 16 cases of patients with twin pregnancies who underwent TAC were reported in the literature with overall successful outcomes (Table 3).

These cases resulted in 28/32 healthy infants (87.5%) and 4/32 perinatal deaths (12.5%). In one woman, both twins died from extreme prematurity after spontaneous labor at 20 weeks. TAC was history indicated in this case because of failed vaginal cerclage in the previous pregnancy.

Two other women delivered a stillborn and a viable infant, one after a preterm premature rupture of membranes at 25 weeks and the other at 37 weeks.

Fifteen women out of 16 underwent TAC by laparotomy, one by laparoscopic transabdominal cervicoisthmic cerclage. Three of the 16 interventions took place before the pregnancy, either before conception or in a former pregnancy. The remaining interventions took place during the pregnancy between 12 and 14 weeks of gestation. Gestational age at delivery ranged from 20 to 38 weeks: 1/16 at 20 weeks, 3/16 < 32 weeks, 5/16 between 32 and 37 weeks, and 7/16 after 37 weeks.

Table 3. Review of the literature of transabdominal cerclage (TAC) in twin pregnancies.

Authors (number of twin pairs)	Technique	Gestational week of placement	Pregnancy	Gestational week of delivery	Outcome
Cammarano et al. (2) [11]	TAC	14	3rd	38	2 alive
		13	3rd	33 4/7	2 alive
Isci et al. (1) [15]	TAC	Former pregnancy	2nd	33	2 alive
Lee et al. (1) [16]	TAC	12 5/7	1st	30 5/7	2 alive
Olatunbosun et al. (2) [17]	TAC	12-14	?	20	2 perinatal deaths
				37	1 stillborn, 1 alive
Olawaiye et al. (1) [18]	TAC	Preconceptional	1st	36	2 alive
Pereira et al. (1) [19]	LTCC	Preconceptional	2nd	38	2 alive
Lotgering et al. (7) [10]	TAC	End of 1st trimester		25(1)	1 alive; 1 perinatal death
				>32 (6)	12 alive
Kyvernitakis et al. (1) [20]	TAC	12 + 4/7	1st	34 3/7	2 alive

TAC: transabdominal cervicoisthmic cerclage (laparotomy); LTCC: laparoscopic transabdominal cervicoisthmic cerclage.

In our series of seven twin pregnancies, one was delivered at 28+6/7 weeks for fetal distress but all the others at more than 32 weeks and five over 36 weeks gestation.

Combining results from the literature review and our own series of seven twin pregnancies, we observe an overall perinatal survival of 91%. Neonatal complications are related to prematurity stage, with 80% of pregnancies delivered beyond 32 weeks and 35% after 37 weeks gestation. Out of the morbidity related to Pfannenstiel incisions, no adverse events were observed on the maternal side. These results are comparable with "normal" twin pregnancies. TAC efficiently suppresses the risk of cervical incompetency but obviously not the other risks associated with twin pregnancies.

TAC can be performed either before or after conception using laparotomy or laparoscopic approach. The benefit of TAC during pregnancy is that the cervix has reached its full thickness, which allows for maximal tight of the knot and, thereby, decreases the potential risk of loss if the knot is too loose. The risk of hemorrhage, however, is theoretically higher than pre-conceptional TAC because of the enlargement of the uterine arteries.

Laparoscopic TAC is associated with better recovery and is a good option, in particular, when performed in nonpregnant women. Nevertheless, if there is no pregnancy, the preconceptional TAC exposes the woman to unnecessary risks.

A meta-analysis of 16 studies showed no difference in live birth rate and third-trimester delivery between pre and postconceptional TAC, neither when performed by laparoscopy or laparotomy [21]. Nevertheless, in series with laparoscopic cerclage, the indication does not necessarily include previous fetal losses.

So far, the following question remains: why is transvaginal cerclage efficient in singleton but not in twins? Transvaginal cerclage is the classic treatment of cervical insufficiency [4]. The standard definition of cervical insufficiency is a painless cervical dilatation leading to recurrent second-early third-trimester pregnancy losses [22]. In 2014, the American College of Obstetricians and Gynecologists updated this definition to the inability of the uterine cervix to retain a pregnancy in the second trimester in the absence of clinical contractions, labor, or both [1].

The diagnosis of cervical insufficiency is quite challenging. Indeed, although cervical insufficiency is the source of some second-trimester losses, most are caused by threatened preterm labor, such as decidual inflammation, infection, hemorrhage or uterine overdistension. Those events can initiate biochemical changes in the cervix that will secondly lead to cervical shortening and pregnancy loss [23]. The key to tell those entities apart is history of recurrent pregnancy losses [4].

Thus, a short cervix without a history of pregnancy loss is more likely to be due to threatened preterm labor than a cervical insufficiency, as it is statistically more common. Thereby, if we perform a TAC regardless of the history of fetal loss, the likelihood to do so in threatened preterm labor rather than a cervical insufficiency is very high. The TAC is then totally useless as the patient will go into labour and deliver anyway.

This hypothesis might be the reason why Berghella did not find any benefit of TVC in multiple pregnancies, whereas TAC showed successful outcomes so far [8]. Indeed, indications for TVC are less strict regarding history of loss pregnancy and thereby allow the possibility to perform a TVC in a threatened preterm labour. This is especially true in twin pregnancies, since uterine overdistension very often leads to cervical modifications and preterm labour [23].

In conclusion, in our series of seven patients, we find that TAC improves perinatal outcomes compared to those in their previous pregnancy without TAC when following strict indications. Our data

show a decrease of second trimester pregnancy loss of 100%. This finding corroborates those of previous case reports.

Unfortunately, our study population is too small to allow a firm conclusion. The decision to perform or not a TAC in woman pregnant with twins with history of preterm birth remains controversial.

Nevertheless, even if data are limited, the neonatal outcomes are improved, which suggests that the results of TAC in twin pregnancies are similar to those in singleton pregnancies and indications for TAC in singletons may be valid in twins.

Further studies are needed to make more accurate recommendations, preferably RCTs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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4. PART 2 : TRANSABDOMINAL CERCLAGE – EXPERIMENTAL STUDY

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ABSTRACT

OBJECTIVE: To determine whether term fetal membranes from transabdominal cerclage (TAC) patients have favorable characteristics compared with membranes from patients without TAC.

METHODS: A prospective study of consecutive pregnant women who had undergone TAC and were delivered by elective cesarean after 37 weeks before the onset of labor at Cliniques universitaires Saint-Luc, Brussels, between January 2015 and June 2016. Membranes were collected from two areas: overlying the cervix and located far from the cervix. Membrane thickness, 15-hydroxyprostaglandin dehydrogenase (PGDH), toll-like receptor-2 (TLR2) expression, and senescence were measured and compared between the TAC group and a control group without TAC enrolled using the same study criteria.

RESULTS: In the cervical area of the TAC group, the chorion was significantly thicker (P=0.003). PGDH and TLR2 expression were also significantly increased in the cervical area of the TAC group (P=0.021 and P=0.043, respectively). Senescence was significantly decreased in the TAC group (P=0.001).

CONCLUSION: A significant relationship between chorion thickening and increase in PGDH and TLR2 expression and decrease in

senescence was reported in the cervical area of membranes in the TAC group. These membrane changes could prevent triggering of parturition and may account for favorable outcomes and clinical success in pregnancies with TAC.
KEYWORDS : Cellular senescence; Cervical insufficiency; Chorioamniotic membranes; Fetal membranes; PGDH; TLRs; Transabdominal cerclage; Zone of altered morphology.

1. INTRODUCTION

Transabdominal cerclage (TAC) is a surgical procedure that can be performed in women who have experienced previously failed prophylactic transvaginal cerclage and fetal loss. TAC is also indicated when transvaginal cerclage is technically infeasible if the cervix is absent or lacerated as a result of developmental abnormality or previous surgery. TAC poses the last opportunity to surgically close the cervix to carry a pregnancy to term and requires laparotomy and cesarean delivery. Fetal survival rate is high when TAC is performed by laparotomy during pregnancy. Adverse outcomes such as preterm premature rupture of membranes, chorioamnionitis, and preterm delivery are rare. Most women who undergo TAC are delivered by cesarean at more than 36 weeks of pregnancy.[1]

Fetal membranes show unique morphological characteristics in a restricted area known as the “zone of altered morphology” (ZAM). The characteristics of this zone were first described at the rupture site during labor. This zone was characterized by significant changes in the thickness of membrane layers.[2] Similar changes in an area of the membranes overlying the cervix were also detected during late pregnancy.[3] The main morphological feature of membranes in the

ZAM was a decrease in the thickness of the chorion layer; accordingly, the ZAM represents an area of weakness in membranes predisposed to rupture.[4] In the cervical area, the significant morphological changes have functional implications for the process of labor.[5]

Fetal membranes also exhibit functional characteristics for maintenance of birth. Several pathways are involved in the pathophysiological process of labor, including the contribution of membranes to synthesis and release of mediators. Fetal membranes constitute a main site of prostaglandin synthesis and metabolism. The key enzyme for metabolism of prostaglandins is 15-hydroxyprostaglandin dehydrogenase (PGDH). The level of PGDH expression in the chorion is significantly lower at term, allowing more active prostaglandins to cross the myometrium and activate labor.[6]

In addition, the membrane immune pathway also participates in the onset of labor.[7,8] Innate immune cells mediate the activation of labor by releasing pro-inflammatory cytokines.[7] Innate immune cells and nonimmune cells in fetal membranes express receptors known as toll-like receptors (TLRs) that bind to sequences known as pathogen-associated molecular patterns (PAMPs).[9] In addition, TLRs interact with endogenous molecules called danger-associated molecular patterns (DAMPs) or alarmins in response to danger.

Furthermore, cellular senescence in fetal membranes also contributes to initiation of birth. Oxidative stress-induced damage in cells promotes cellular senescence and secretion of senescence-associated secretory phenotype (SASP) markers. Therefore, SASP factors secreted from senescent cells promote birth at term.[10,11] DAMPs are

molecules that activate the innate immune system and increase the inflammatory load to induce labor.

It is apparent therefore that multiple pathways contribute and interact in the pathophysiological process of labor and subsequent preterm or term delivery: prostaglandins, inflammation, local immune response, and cellular senescence. However, a recent study showed that cellular senescence was not always present in chorioamniotic membranes after spontaneous labor at term.[12]

The aim of the present study was to examine fetal membrane histology by comparing membrane thickness in pregnant women who had undergone TAC with membrane thickness in pregnant women without TAC, at term, before labor, and without premature rupture of membranes.

We hypothesized that TAC determines morphological changes in fetal membranes that can lead to functional changes, thus maintaining the homeostatic balance at term without complications.

2. MATERIALS AND METHODS

The present prospective observational study enrolled all pregnant women who had undergone TAC and delivered at the Cliniques universitaires Saint-Luc, Brussels, Belgium, between January 2015 and June 2016. Inclusion criteria were presence of prophylactic TAC performed in the first trimester of pregnancy, gestational age greater than or equal to 37 weeks at time of delivery, and elective cesarean delivery before onset of labor. Exclusion criteria were premature rupture of membranes, multiple pregnancy, maternal disease, or fetal anomaly. To act as a control group, we enrolled an equal number of

pregnant women who had not undergone TAC, with the same study criteria during the same period. The research protocol was approved by the hospital's ethics committee (2013/07NOV/509). All women provided informed consent.

Fetal membrane samples were collected during cesarean delivery in two separate areas: membranes overlying the cervix (the cervical area) and membranes located far from the cervix and the placental bed (the distal area). Samples were cut into strips and fixed in buffered formalin. The strips were embedded in paraffin and sectioned at 5 μm. Histology was evaluated on hematoxylin and eosin-stained sections. Amnion and chorion thickness were measured, as well as the full thickness of the membrane.

To determine whether functional changes are associated with morphological changes in chorioamniotic membranes we measured, using quantitative real-time polymerase chain reaction (PCR), the expression of genes coding for the key enzyme of prostaglandin metabolism, PDGH, and toll-like receptor-2 (TLR2). Membrane samples were immediately stored in RNAlater stabilization solution (Ambion, Life Technologies, Carlsbad, CA, USA) and kept at −80 °C. Total RNA was extracted and purified. After quantitation, 0.5 μg of cellular RNA was used as a template for the reverse transcription using qScript cDNA superMix (Quanta Biosciences, Gaithersburg, MD, USA). cDNA was diluted 10 times with RNase-free water. We used 5 ng of cDNA for quantitative real-time PCR using specific primers for PGDH (forward: 5′-CTG-CAC-CAT-GCA-CGT-GAA-CG-3′; reverse: 5′-AAG-TGT-CTC-TCA-GTT-GTT-GCT-G-3′); TLR2 (forward: 5′-GAG-ACC-TAT-AGT-GAC-TCC-CAG-3′; reverse: 5 ′CTG-CCC-TTG-

CAG-ATA-CCA-TTG-3′); and internal control gene TATA-binding protein (TBP) (forward: 5′-GAA-CAT-CAT-GGA-TCA-GAA-CAA-CA-3′; reverse: 5′-ATA-GGG-ATT-CCG-GGA-GTC-AT-3′). To prevent bacterial infection, 16S RNA PCR was also examined (forward: 5′-CCA-TGA-AGT-CGG-AAT-CGC-TAG-3′; reverse: 5′-ACT-CCC-ATG-GTG-TGA-CGG-3′). PCR was performed in a StepOne Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The specificity of the reaction was verified using melting curve analysis, which generated a single peak product for each transcript. The reactions were performed in duplicate for each cDNA, averaged and normalized to TBP. The comparative 2−ΔΔCt method was used to calculate the relative quantification of gene expression, and data were represented as fold change between groups. A sample from term membrane without TAC located far from the cervix (distal area) was used as the reference.

Membranes obtained from each sample were prepared for protein localization by immunofluorescence. After deparaffinization, 5 µm sections of chorioamniotic membranes were first subjected to antigen retrieval in 10 mM citrate buffer pH 5.7 followed by incubation for 60 minutes in Tris-buffered saline (TBS) blocking solution containing 5% bovine serum albumin (BSA), 2% milk, 1% human immunoglobulins and 0.1% Tween 20 (Sigma-Aldrich, Steinheim, Germany). Sections were then co-incubated with rabbit anti-PGDH (Abcam #ab187160, Cambridge, UK, clone EPR14332, 1/6000 dilution) and mouse anti-TLR2 (Millipore #MABF84, Burlington, MA, USA, clone 19B6.2, 1/100 dilution) primary antibodies overnight at 4 °C diluted in TBS solution containing 1% BSA and 0.05% Triton X-100 (Sigma-Aldrich). The

slides were washed three times with TBS solution containing 0.05% Triton followed by incubation with anti-rabbit AlexaFluor555 conjugated secondary antibody (Cell Signaling #4413, Danvers, MA, USA) and anti-mouse AlexaFluor488 conjugated secondary antibody (Cell Signaling #4408) at 1/500 dilution for 1 hour at room temperature. Nuclei were then stained with 4′-6-diamidino-2- phenylindole (DAPI). Slides were mounted with Dako Agilent (Santa Clara, CA, USA) fluorescence mounting medium. Labeled sections were imaged by epifluorescence with a 10× objective EC Plan-Neofluar using a Zeiss Axio Imager microscope (Zeiss, Oberkochen, Germany).

The presence of senescence-associated β-galactosidase (SA β-gal) enzyme, a senescent cell marker, was also evaluated by immunohistochemical staining. After deparaffinization, 5 µm sections of chorioamniotic membranes were subjected to: (1) endogenous peroxidase inhibition (3% H2O2 in methanol); (2) antigen retrieval in 10 mM citrate buffer pH 5.7; and (3) blocking of specific antigen binding sites with TBS solution containing 5% BSA, 2% milk, 1% human immunoglobulins, and 0.1% Tween. Sections were then incubated with mouse anti-beta-galactosidase primary antibody (Santa Cruz #sc-37B-127257, Dallas, TX, USA, clone, 1/100 dilution) overnight at 4 °C in TBS solution containing 1% BSA and 0.05% Triton. This was followed by incubation with peroxidase-conjugated Envision+ anti-mouse secondary antibodies (Dako #K4001) for 30 minutes at room temperature. The reaction detection was done using 3,3 ′-diaminobenzidine (DAB) (Dako #K3468). The slides were counter-stained with hematoxylin (Dako #S3301), dehydrated and cover slipped. They were finally digitalized at a 20× magnification with

a SCN400 slide scanner (Leica, Wetzlar, Germany) and visualized on the Digital Image Hub (Leica Biosystems, Dublin, Ireland). An immunohistochemical score was calculated by multiplying the staining intensity by the percentage of the β-gal positive membrane area (ImageJ 1.52a, National Institutes of Health, Bethesda, MD, USA).[13] Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong).

SPSS software version 25.0 (IBM, Armonk, NY, USA) was used for statistical evaluation. Samples were analyzed using nonparametric Mann-Whitney U tests for non-normally distributed continuous variables. $P<0.05$ was considered statistically significant.

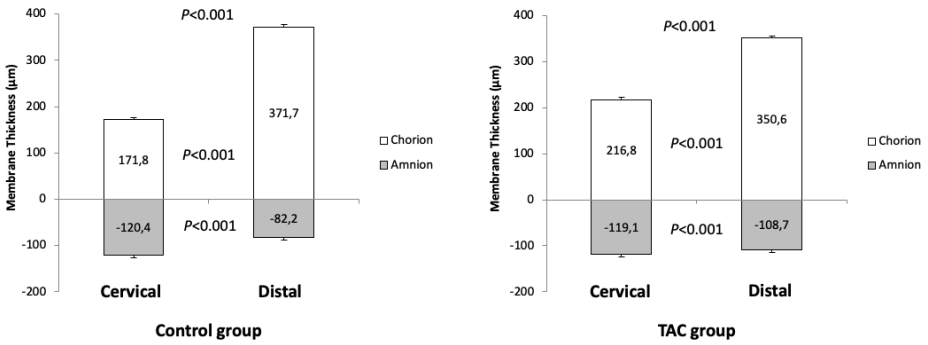
3. RESULTS

During the study period, five women who had undergone TAC met the study criteria and were enrolled. For the control group, five women were recruited during the same study period.

We observed a thinning of the full-membrane thickness in the cervical area compared with the distal area (Fig. 1A). In the distal area, there was no significant difference in mean membrane thickness between the TAC and control groups (Fig. 1B). In the cervical area, mean membrane thickness was significantly higher in the TAC group compared with controls ($P=0.003$). The chorion layer was significantly thicker in the cervical area of the TAC group than in controls ($P=0.010$). There was no significant difference in the mean amnion thickness between the TAC and control groups ($P=0.521$) (Fig. 1B).

Figure 1. Chorioamniotic membrane thickness. Thinning of full-membrane thickness in the cervical area in the control and TAC

groups. Amnion thickness is significantly increased whereas chorion thickness is significantly decreased in the cervical area compared with the distal area in the control and TAC groups (A). In the cervical area, chorion thickness is significantly increased in the TAC group compared with the control group; there is no difference in amnion layer thickness between the TAC and control groups. No difference in full-membrane thickness is seen in the distal area (B). Membrane thickness measurements (amnion and chorion) (C). Representative figures (scale bar 20 μm) with hematoxylin and eosin staining in cervical (1,2) and distal (3,4) areas in control (1,3) and TAC (2,4) groups (D). Values are mean ± SEM.



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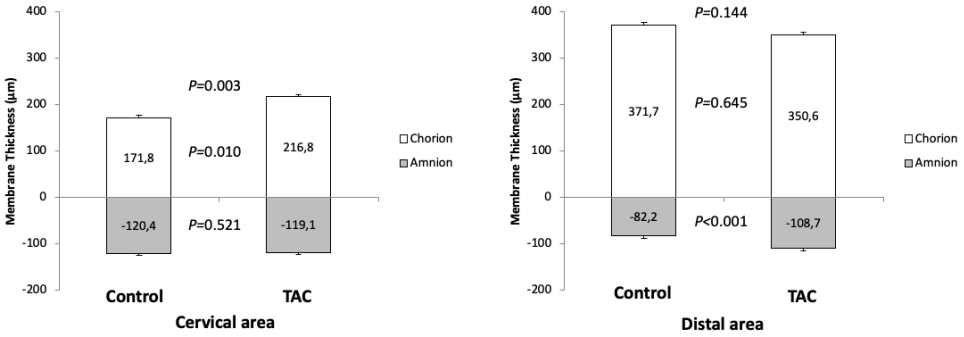

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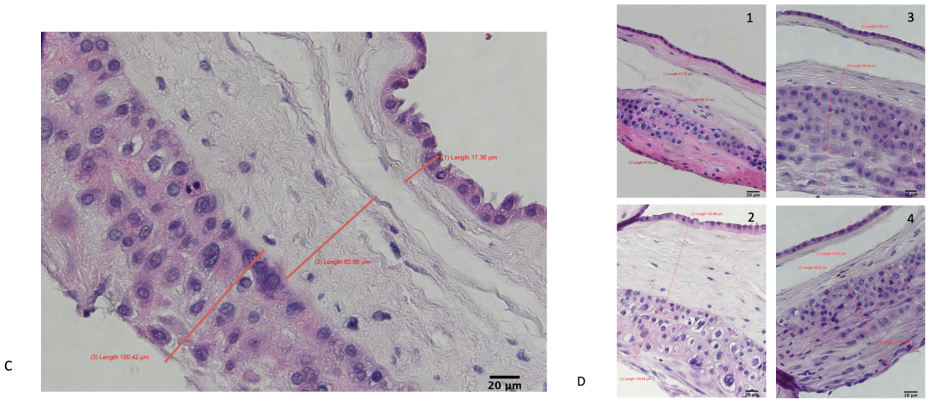
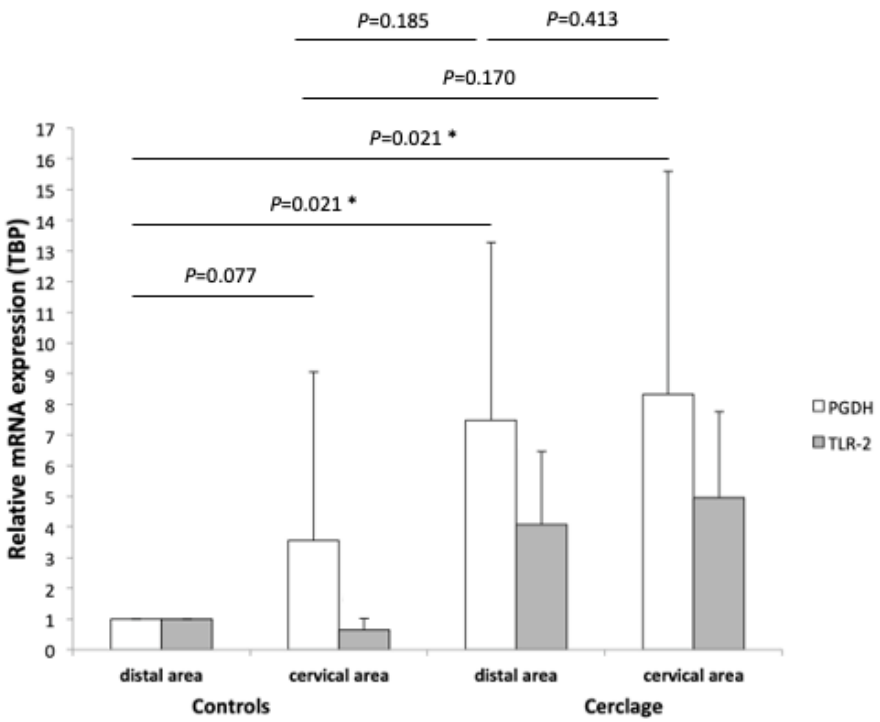
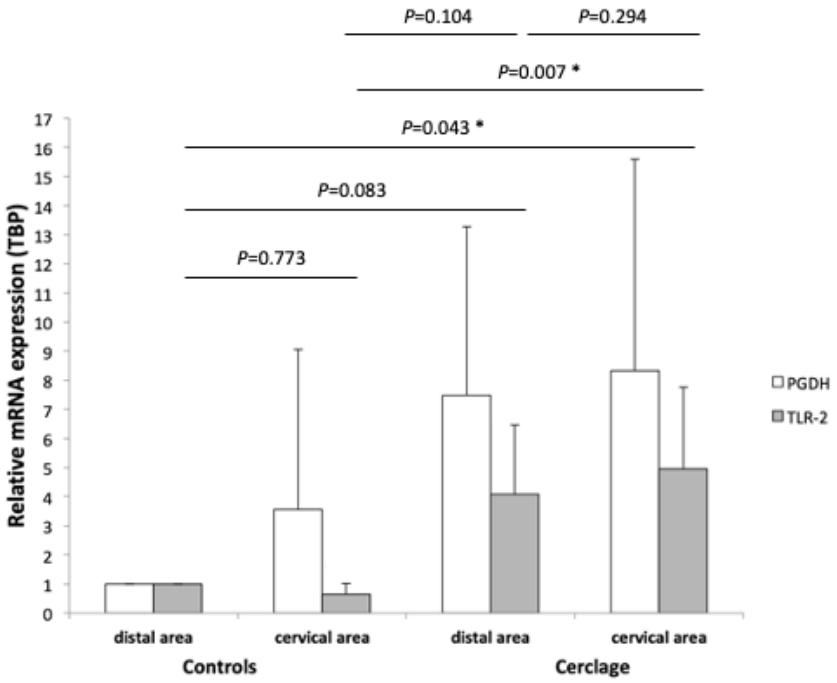


We then determined whether thickened chorion had an effect on the expression of genes potentially involved in preterm and term labor. We first evaluated PGDH mRNA expression by real-time PCR. As presented in Figure 2, PGDH mRNA expression was found to increase in the cervical area and to increase significantly in fetal membranes in the TAC group. This was confirmed by immunofluorescence membrane staining. Immunofluorescence also indicated that PGDH was expressed in the chorion layer of fetal membranes (Fig. 3).

Figure 2. RT-qPCR data of PGDH and TLR-2 expression. PGDH mRNA expression is significantly increased in the TAC group, both in cervical and distal areas. PGDH expression is increased in cervical area of controls. TLR-2 expression is increased in the TAC group with significantly increased expression in the cervical area. PGDH and TLR-2 mRNA expression is significantly increased in the cervical area in the TAC group. The results were normalized to the TBP gene. Expression in the distal area in the control group was used as a reference. All comparisons are mentioned. Values are mean ± SD of eight independent experiments done in duplicate. n=5 patients in the TAC group and n=5 patients in the control group.





Similarly, we evaluated TLR2 mRNA expression by real-time PCR. TLR2 mRNA expression was higher in fetal membranes from the TAC group than in the control group. In addition, TLR2 mRNA expression was significantly higher in the cervical area of the TAC group compared to the distal area in the control group (Fig. 2). This was also observed by immunofluorescence. Immunofluorescence showed that TLR2 protein was expressed in both the chorion layer and in the amniotic epithelium (Fig. 4).

Figure 3. Representative immunofluorescence staining of PGDH (red) in the chorion layer in the cervical area (A) and in the distal area (B) of membranes from the control group. Immunofluorescence staining of PGDH in the chorion layer in the cervical area (C) and in the distal area (D) of membranes from TAC group. Staining intensity is greater in the chorion cells of membranes in the TAC group, especially in the cervical area (C) compared with the distal area (D). Nuclei were counterstained with DAPI (blue).

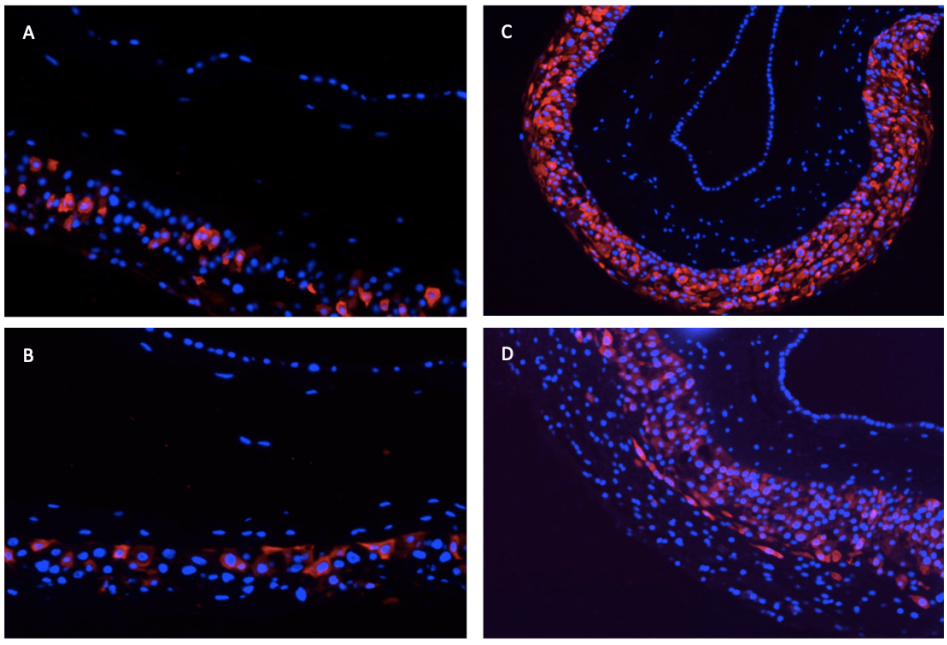
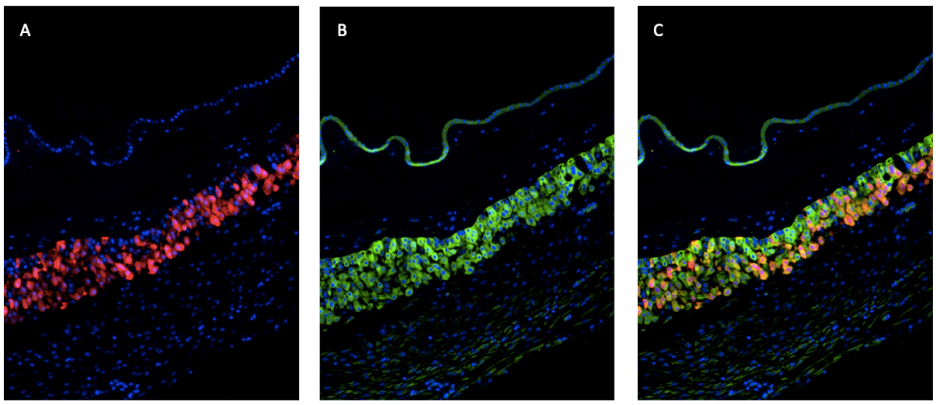
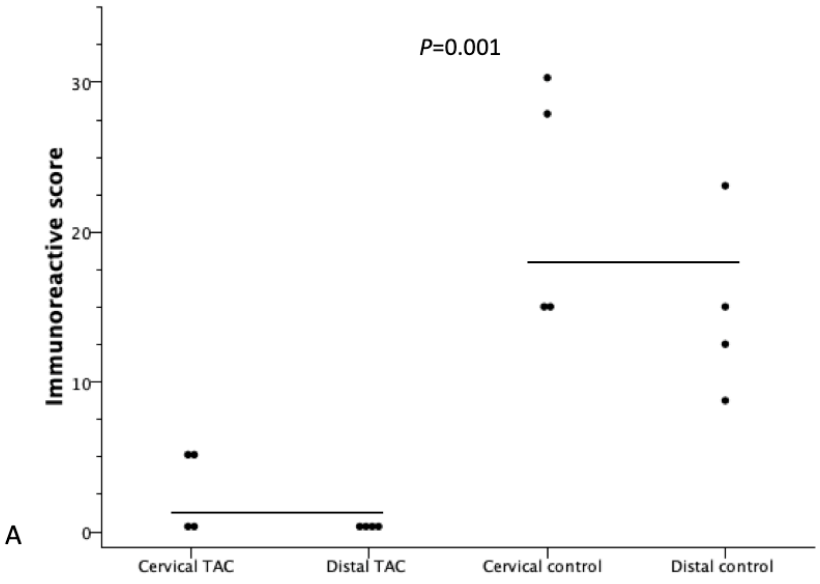


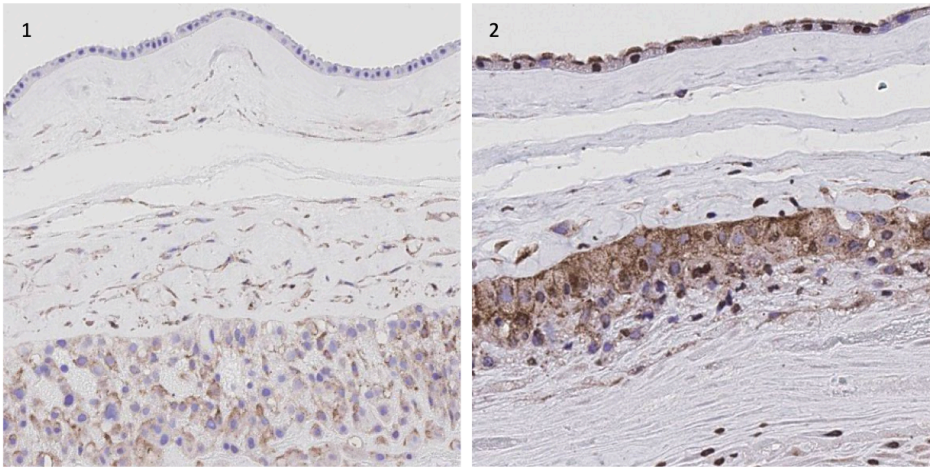
Figure 4. Fetal membranes in the cervical area in the TAC group. Representative immunofluorescence staining of PGDH (red) in the chorion layer (A), of TLR-2 (green) both in the chorion and in the amniotic epithelium (B), and merged figure (C).



Finally, we evaluated the level of senescence in fetal membranes by detecting and quantifying the SA β-gal enzyme by immunohistochemistry. According to the calculated immunoreactive score, fetal membrane senescence was significantly lower in the TAC group compared with the control group (P=0.001) (Fig. 5A). Representative immunostaining of SA ß-gal presence is shown in membranes in cerclage and in control group (Fig. 5B).

Figure 5. Senescence-associated β-galactosidase (SA β-gal) presence calculated by the immunoreactive score. Regardless of the area, senescence is significantly decreased in membranes in the TAC group compared with controls (A). Representative immunohistochemical staining of SA β-gal presence in membranes from the TAC group (1) and control group (2) (B). Bar represents the mean in the TAC and control group.





4. DISCUSSION

Our results showed a thinning of the full-membrane thickness in the cervical area. The fetal membranes were thinner in the cervical area compared with the distal area. As previously described in a ZAM overlying the cervix, we observed amnion swelling and chorion thinning.[2–5] In the distal area, there was no significant difference in the mean membrane thickness between the TAC group and controls. In the cervical area, mean membrane thickness was significantly higher in the TAC group compared with controls. The chorion was significantly thicker in the cervical area of the TAC group than in controls. There was no significant difference in the mean amnion thickness between the TAC group and controls. Therefore, thickening of the fetal membrane in the cervical area is related to increased chorion thickness.

Could we associate the thickened chorion to functional changes in the chorion layer in the cervical area and to the clinical success of TAC?

The chorion is known to act as a coordinating center for metabolites exchange across the fetal-maternal interface, it provides immune protection, and is a site for prostaglandins production.[11] In human parturition, the fetal membranes are the main source of prostaglandins. The level of active prostaglandins is controlled by the metabolism and the initial oxidative step of metabolism uses the enzyme PGDH. The main site of PGDH expression is in the chorion. This constitutes a metabolic barrier to control intrauterine concentrations of prostaglandins.[14–17] Some studies have reported that PGDH expression was lower in the chorion at term in spontaneous labor, compared with term elective cesarean delivery.[18,19] This suggests that the decrease in PGDH expression allows synthesized prostaglandins to escape metabolism in the chorion and allows labor. In the present study, all of the cesarean deliveries were performed before labor, and we observed a difference in the chorion thickness between the TAC group and controls. To understand whether thickened chorion had an effect on the gene expression, PGDH mRNA expression was evaluated by real-time PCR. In the cervical area, PGDH mRNA expression was significantly higher in the chorion of the TAC group than its expression in the distal area of controls. These results suggest that the thicker chorion only in the cervical area of the TAC group can partially explain the increased PGDH mRNA expression. Considering that PGDH mRNA expression is significantly increased in the TAC group (cervical and distal areas), having undergone TAC can also partially explain the increased PGDH mRNA expression. This suggests that the level of prostaglandins is decreased at the fetal-maternal interface with a thickened chorion with

TAC and that the uterine quiescence status is protected, thus avoiding triggering parturition.

At the fetal-maternal interface, TLRs are widely expressed not only by immune cells but also by nonimmune cells.[20] TLRs form the major family of pattern recognition receptors (PRRs) that are involved in innate immunity. TLR2 is a transmembrane protein with the widest specificity able to bind to sequences known as PAMPs expressed by bacterial lipoproteins, gram-positive bacterial peptidoglycan (PDG), and fungal zymosan. In addition to detecting pathogen-derived ligands, TLRs interact with other endogenous molecules, typically in response to danger. These molecules have sequences known as DAMPs. Senescent cells release DAMP mediators that will activate the innate immune system and enhance the inflammatory load.[11] As TLR2 mRNA expression was significantly higher in cervical area membranes in the TAC group, it can be seen that TAC had an effect on the innate immune response in chorioamniotic membranes. This suggests that membranes at term with TAC may be more reactive to pathogen stimuli compared with term membranes without TAC. As seen by immunofluorescence, TLR2 was expressed in both chorion cells and in the amniotic epithelium. Immunostained cells for TLR2 were previously described both in the chorion and in the amniotic epithelium. Moreover, intra-amniotic infection by Ureaplasma pathogens triggers innate immune response via TLR2. Activation via TLR2 results in a subdued inflammatory response.[21] Any bacterial infection in our fetal membranes was initially precluded by 16S RNA PCR. Our fetal membranes from women in the TAC group at term expressed more TLR2 without any bacterial infection.

Recent studies provide evidence that, at the fetal-maternal interface, programed membrane senescence generates sterile inflammation that triggers parturition.[10,22–24] In utero, cellular senescence is recognized as a contributor to labor by inducing signals. Aging in utero coincides with fetal growth and organ maturation and is an inevitable process in the life of all cells, especially in intrauterine tissues during pregnancy. Membranes undergo cumulative oxidative stress during late gestation. The senescent fetal cells with their SASP release signals like DAMP markers. DAMPs are sterile inflammatory mediators that can be propagated to maternal tissues (myometrium and cervix) resulting in myometrial contractions and cervical remodeling.[11,25] The increase in inflammatory load in maternal tissues will trigger parturition.[25] In the present study, SA β-gal presence was significantly decreased in membranes in the TAC group compared with controls.

The strength of the present study is its prospective design. Some limitations must also be considered. The principal limitation is the relatively small number of women included in the TAC and control groups. Some potential biases cannot be excluded in selecting a small number of controls. To limit individual variations, membrane samples were taken and compared at different locations (cervical and distal areas) for each group.

In conclusion, our results suggest that, compared with controls, fetal membranes from women who underwent TAC exhibit changes at term before the onset of labor. In the cervical area of membranes from the TAC group, significant chorion thickening was found to be associated with increased PGDH and TLR2 mRNA expression and decreased senescence. These membrane changes could prevent triggering of

parturition. Our results confirm our hypothesis that membrane changes account for favorable outcomes and clinical success for pregnancies among women who have undergone TAC.

AUTHOR CONTRIBUTIONS

PS was involved in the study design, data acquisition, data analysis, and wrote the draft manuscript. CD, CH, and FD were involved in the study design, data analysis, and co-writing of the manuscript.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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5. DISCUSSION AND PERSPECTIVES

This PhD thesis aims to study some aspects of cervical insufficiency and its management strategies and outcomes.

The first part of this thesis is a retrospective clinical study comparing perinatal outcomes in emergency cerclage between patients with prolapsed and non-prolapsed membranes. We confirmed as others that emergency cerclage can be a safe and effective procedure in attempt to prolong the pregnancy (74, 79, 212-220). We showed that perinatal outcomes were more favorable in patients with non-prolapsed membranes. Patients with prolapsed membranes had a significant higher risk to have a shorter time interval between cerclage and delivery, to deliver at early gestational age, thus increasing neonatal morbidity and mortality. The main interest of our paper is to provide more information about the risks and benefits of emergency cerclage to clinicians for patients counseling. It also showed that prolapsed membranes are a predictive factor of adverse perinatal outcomes. We published perinatal outcomes only for singleton pregnancies. The clinical study had also included unpublished data about multiple pregnancies. These data suggested that physical exam indicated cerclage could be as successful as in singleton pregnancies. As we found that cervical cerclage is less effective when there are prolapsed membranes both in singleton and multiple pregnancies, we postulate that fetal membranes may play an essential role in the success rate of the surgical procedure.

This hypothesis is validated in **the second part of this thesis.** We studied in vitro the structural and functional changes of fetal

membranes throughout pregnancy. We searched to evaluate fetal membranes in attempt to better understand the mechanisms that maintain pregnancy and pathways leading to parturition. We know that transabdominal cerclage has a high success rate (54-57).Then we studied and compared fetal membranes at term in two groups of patients, with and without transabdominal cerclage. All fetal membranes specimens were collected before labor during cesarean sections, as we know, that fetal membranes have a zone of altered morphology overlying the cervix (154-161).

Fetal Membrane Structure and Function

Firstly, we evaluated morphological changes in fetal membranes in cervical and distal area in each group. As previously described in a ZAM overlying the cervix, we observed a thinning of fetal membranes (155-157, 161). We showed that fetal membrane thickness was significantly higher in cervical area of patients with TAC compared to controls. The chorion layer was significantly thicker in the TAC group. We then determined whether thickened chorion had an effect on the expression of genes potentially involved in preterm and term labor.

In transvaginal cerclages, structural and functional changes are more difficult to evaluate before labor at term. Data about the fetal membrane thickness with transvaginal cerclage are lacking. Most patients have a vaginal delivery after rupture of membranes. Further investigations could be evaluated in patients with transvaginal cerclage requiring a cesarean section at term before labor.

The 15-Hydroxyprostaglandin Dehydrogenase

Then, we evaluated some functional changes in fetal membranes. As the chorion layer acts as a barrier at the fetal-maternal interface for prostaglandins transfer (169, 170, 172), we evaluated in fetal membranes the expression of a key 15-HPGD enzyme in prostaglandins metabolism. This expression was significantly increased in fetal membranes with TAC where the chorion layer was thicker. We hypothesized that fetal membrane thickness in patients with TAC may reflect an increased barrier effect for reducing prostaglandins role on myometrium. This suggests that the level of prostaglandins is decreased at the fetal-maternal interface with a thickened chorion with TAC and the uterine quiescence status is protected. TAC could also have a beneficial effect in reducing uterine contractions. A recent study evaluated the localization of prostaglandin synthase (PGES) in fetal membranes and the PGE_2 concentrations in both maternal (decidual) and fetal (amniotic fluid) compartments. They suggested that both amnion and chorion are able to produce and release PGE_2. Because the amnion directly contacts amniotic fluid, it may contribute to PGE_2 accumulation in amniotic fluid (221). In order to validate the increased 15-HPGD expression, we could also investigate the PGE_2 concentrations in amniotic fluid in patients with TAC and controls. Like another recent study (222), we could also investigate the effect of PGE_2 on the output of pro-inflammatory cytokines (PGE_2 increase the concentrations of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα)) in cultured human uterine smooth

muscle cells from term pregnant women between the TAC and control group.

Toll-Like Receptors

Then, we evaluated innate immune response in fetal membranes via toll-like receptors, present at the fetal-maternal interface in a spatio-temporal specific manner (183, 184, 187, 189, 190). We demonstrated that TLR2 mRNA expression was significantly higher in cervical area of membranes with TAC, in absence of infection. This suggests that immune response is increased in presence of TAC which could be beneficial to prevent an ascending infection. This benefit could explain a low rate of adverse outcomes with TAC (chorioamnionitis, preterm premature rupture of membranes).

Senescence

Lastly, we investigated fetal membrane senescence. Cellular senescence in membranes can trigger initiation of labor. Senescence in fetal membranes is likely reflection of fetal growth and organs maturation (194-197). Aging, an inflammatory condition, generates inflammatory mediators, including DAMPs and well-characterized uterotonins and propagates them to various fetal-maternal tissues through exosomes. We reported that senescence (evaluated by the presence of SA β-gal) is significantly decreased in fetal membranes in patients with TAC compared to controls. Therefore, we can speculate on the consequences of the decreased senescence in fetal membranes which could explain the very low rate of adverse

outcomes with TAC (preterm birth, preterm premature rupture of membranes).

In conclusion of this part, we observed that fetal membranes overlying the cervix with TAC change their phenotype with a thicker chorion. We reported that the 15-PGDH and TLR2 mRNA were significantly higher expressed between TAC and controls. Senescence was also reported decreased in fetal membranes with TAC. Therefore, our results infer that the uterine contractions and the membranes rupture are unlikely to be manifested prior to labor in patients with TAC. We suggest that the success rate of TAC is maybe not only dependent on the mechanical effect of the cerclage but also on the observed changes in membranes.

TAC and high-risk pregnancies

Patients with multiple pregnancies, collagen disorders, “short cervical length” without history of preterm birth or fetal loss are at high-risk of preterm delivery. The challenge is to identify among these patients those who have genuinely a cervical insufficiency. The currently available literature provides little evidence on the benefits of cerclage in these high-risk pregnancies (148-150). Our data and those of retrospective studies report successful outcomes after emergency cerclage in twin pregnancies, which indirectly support the potential benefit of emergency cerclage in twins (211, 223-227). Our series of seven cases of TAC in twins also support the benefit of the abdominal procedure in these high-risk pregnancies (153). The perinatal outcomes are considerably improved after TAC in women with cervical insufficiency and twins. A retrospective cohort study in triplet

pregnancies had already concluded that prophylactic placement of TAC seems to decrease the incidence of delivery before 28 weeks (152). We advocate that the indications of these procedures could be enlarged to high-risk pregnancies. Recently, a first RCT showed the decreased incidence of spontaneous preterm birth after emergency cerclage in twin pregnancies (32). Further research and RCT are needed to confirm the value of TAC in suitable high-risk pregnancies. TAC could become the choice procedure with the least risk of adverse outcomes. Further research should define the optimal timing (interval or during pregnancy) and the surgical technique (laparoscopy or laparotomy) of TAC. Long-term maternal morbidity (pelvic pain, repeat surgery) should be included in the outcomes.

FUTURE PERSPECTIVES

1. As explained above, in clinical practice, further research and RCTs are needed to confirm the value of TAC in high-risk pregnancies. Further research should define the optimal timing and the surgical technique of TAC. Long-term maternal morbidity should be included in the outcomes.
2. In the field of the research, there is still unknown knowledge in PTB. PTB therapeutic options such as tocolytic agents briefly delay PTB but lack of efficacy (228). Mechanical therapies such as transvaginal and transabdominal cerclages were discussed in this thesis. It is known that the success of a pregnancy after TAC is not related to the cervical length. Women with a very short cervix of after cervical amputation can experience a

normal pregnancy until term with TAC. We do not know if the mechanical support of the TAC is sufficient to explain the successful outcomes after TAC. A better understanding of the pathophysiology of fetal membranes is needed to open new preventive and therapeutic options which aim to reduce PTB.

3. We showed a significantly increased mRNA expression of PGDH and TLR2 in the cervical area after TAC but the corresponding quantification of proteins level has not been reported. Low levels of prostaglandins are associated with low myometrial contractility. As the chorionic expression of the 15-HPGD is significantly down regulated with term and preterm labor, we could consider therapeutic approach to target the 15-HPGD in prolonging the pregnancy. The pharmacologic targeting of the 15-HPGD was already studied in cancers (229) and pulmonary fibrosis (230, 231). The 15-HPGD targeted therapy may be beneficial for patients suffering from advanced and metastatic disease (232). The down regulation of the 15-HPGD has been shown in several cancers (including lung, colon, bladder, endometrial and gastric cancer) and has been shown to have a tumor suppressor role (233). In the same way, we could consider potential therapeutic approach targeted to fetal membranes (such as 15-HPGD activators) in preventing PTB. Activation of 15-HPGD expression could be a potential target for therapeutics to prevent PTB. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the secretion of prostaglandins and are used as tocolytics but they have potential neonatal complications of early closure of the ductus

arteriosus, intracranial hemorrhage, renal dysfunction, periventricular leukomalacia, and necrotizing enterocolitis (234). Further investigation is needed to know if a potential target in fetal membranes such as 15-HPGD activators could be effective for the tocolysis with less adverse neonatal outcomes compared to NSAIDs.

4. The high incidence of infection and chorioamnionitis associated with PPROM is well established in preterm birth (235). The chorioamnionitis after PPROM is associated with a higher risk of early-onset neonatal sepsis (EONS) and necrotizing enterocolitis (NEC) in neonates (236). The early diagnosis of chorioamnionitis using amniocentesis and the management of pregnancies with PPROM are essential to improve neonatal outcomes (237). The role of TLRs in fetal membranes with chorioamnionitis has been described (238-241). Our data suggest that the fetal membranes vary their TLR expression levels in presence of TAC. This information could be used in further research to determine the role of these receptors in the prevention of infection at different gestational age as well as the defense against bacterial infection.

5. CONCLUSIONS

Prevention of PTB remains the principal concern of obstetricians and neonatologists. Despite preventive and therapeutic management measures, the incidence of PTB is not significantly reduced nowadays. Many reasons are responsible for that: new risk factors such as stress, advanced maternal age and increased of multiple pregnancies.

However, there is still unknown knowledge specifically in the area of fetal membranes (https://www.fetalmembranesociety.org).

The present thesis highlights the contribution of the fetal-maternal interface for maintaining pregnancy to term. A synergy between each compartment is essential for the success of pregnancy. Interventions (transvaginal and transabdominal cerclage) and management strategies are mostly targeted to cervix (maternal tissue). We suggest the crucial role of fetal membranes in PTB both in transvaginal and transabdominal cerclages. This focus into the fetal membranes could increase the potential for new therapeutics to be developed (228).

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