To My Family.

LASER COAGULATION IN COMPLICATED MONOCHORIONIC PREGNANCIES

Submitted by

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LASER COAGULATION IN COMPLICATED MONOCHORIONIC PREGNANCIES. SUMMARY

Monochorionic pregnancies are found in 1 in 200 births, but the incidence is increasing because of the widespread use of assisted reproductive technology. Monochorionic twins, compared to dichorionic twins, have a considerably higher risk of pregnancy complications that are dues to the process of splitting of the single embryonic mass [congenital abnormalities, conjoint twins, selective fetal growth restriction (sFGR)] and the communicating placental vessels between the two circulations [twin-to-twin transfusion syndrome (TTTS), twin reversed arterial perfusion (TRAP) sequence, twin anemia polycythemia sequence (TAPS)].

This thesis reports on the outcome of pregnancies with sFGR (with and without superimposed TTTS) treated by endoscopic laser separation of the inter-twin communicating placental vessels.

Triplet pregnancies in which two or all three fetuses are monochorionic, are at very high risk of perinatal death. The incidence of dichorionic triamniotic pregnancies has dramatically increased following the practice of IVF with delayed transfer of two embryos.

This thesis reports on the outcome of monochorionic and dichorionic triplet pregnancies complicated by TTTS (with and without sFGR) treated by endoscopic laser separation of the communicating placental vessels. The thesis also reports on the development of a first-trimester technique for embryo reduction of one of the two monochorionic twins by ultrasound-guided interstitial laser ablation of the pelvic vessels.

Professor Kypros Herodotou Nicolaides

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I confirm that Dr Gergana Peeva has carried out under my supervision the studies presented in the Thesis: Laser coagulation in complicated monochorionic pregnancies.

I have read the Thesis and I agree for the Thesis to be presented to the Tribunal for the degree of Doctor of Philosophy in Medicine.

Professor Kypros Herodotou Nicolaides
London, March 2018

Professor María de la Calle Fernández-Miranda

Universidad Autónoma de Madrid,
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Madrid, March 2018

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CONTENTS

AA	arterioarterial
AEDF	absent end-diastolic flow
AFDV	absent flow ductus venosus
AREDF	absent or reversed end diastolic flow
ART	artificial reproductive technologies
AV	arteriovenous
BCTA	bicoriales triamniónicas
CIRs	crecimiento intrauterino restrictivo selectivo
CIRs-II	crecimiento intrauterino restrictivo selectivo tipo II
cm	centimetre
cm/s	centimetre per second
DC	dichorionic
DCDA	dichorionic-diamniotic
DCT	dichorionic triplet
DVP	deepest vertical pool
ER	embryo reduction
FFTS	feto-fetal transfusion syndrome
FGR	fetal growth restriction
KCL	potassium chloride, cloruro de potasio
MC	monochorionic
MCA	middle cerebral artery
MCBA	monocoriales biamniónicas

ABBREVIATIONS

MCDA	monochorionic-diamniotic
MCMA	monochorionic-monoamniotic
MoM	multiple of median
NDI	neurodevelopmental impairment
PSV	peak systolic velocity
PTB	preterm birth
PUVF	pulsatile umbilical venous flow
RE	reducción embrionaria
REDV	reverse end-diastolic flow in the umbilical artery
RFDV	reversed flow in ductus venosus
sFGR	selective fetal growth restriction
sFGR-II	selective fetal growth restriction type II
STFF	síndrome de transfusión feto-fetal
TA	triamniotic
TAPS	twin anemia polycythemia sequence
TCT	trichorionic triplets
TRA	reproducción asistida
TRAP	twin reversed arterial perfusion
TTTS	twin-to-twin transfusion syndrome
UA	umbilical artery
VV	venovenous

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1. INTRODUCTION

1.1 PRESENTACIÓN EN ESPAÑOL

La prevalencia de la gestación monocorial es de 1 en 200 nacimientos, pero su incidencia está en aumento consecuencia del rápido aumento en el uso de las técnicas de reproducción asistida (TRA). Los gemelos monocoriales, en contraste con los bicoriales, tienen un riesgo considerablemente mayor de complicaciones del embarazo debidas al proceso de separación de una masa embrionaria única (anomalías congénitas, gemelos siameses, crecimiento intrauterino restrictivo selectivo (CIRs)) y a la presencia de vasos placentarios comunicantes entre las dos circulaciones fetales [síndrome de transfusión feto-fetal (STFF), la secuencia TRAP (twin reversed arterial perfusion) y la secuencia TAPS (twin anemia polycythemia sequence).

Esta tesis trata el resultado perinatal de gestaciones con CIRs (con y sin STFF sobreañadido) tratadas con láser endoscópico para la separación de los vasos placentarios comunicantes.

Las gestaciones triples en las que dos o los tres fetos son monocoriales presentan un riesgo muy alto de muerte perinatal. La incidencia de gestaciones bicoriales triamniónicas (BCTA) ha aumentado considerablemente consecuencia del aumento de las TRA con la transferencia diferida de dos embriones.

Esta tesis trata el resultado perinatal de gestaciones triples monocoriales y dicoriales complicadas con STFF (con y sin CIRs) tratadas con láser endoscópico para la separación de los vasos comunicantes. Esta tesis también analiza el desarrollo de una técnica para embrioreducción de uno de los dos gemelos monocoriales en el primer trimestre mediante ablación con láser intersticial de los vasos pélvicos fetales.

1.2 CHORIONICITY AND ZYGOSITY

1.2.1 Overview

The incidence of multiple pregnancies is rising, mainly due to delayed childbirth and advanced maternal age at conception and the resultant widespread use of assisted reproduction techniques (National Collaborating Centre for Women's and Children's Health 2011). About 1 in 90 spontaneous conceptions results in the birth of twins, however due to artificial reproductive technologies (ART) the rate of twining has increased by 75% over the last 3 decades and overall twins constitute 2-4% of all births (Ananth et al., 2012).

Twinning can be categorised according to zygosity and chorionicity. Zygosity refers to the type of conception. Chorionicity refers to the type of placentation and is closely related to zygosity. Although all monochorionic (MC) twins are monozygotic, and all unlike-sex twins are dizygotic, zygosity remains unknown by clinical means in all like-sex dichorionic twins.

Dizygotic twins (non-identical, fraternal) twins (70% of all twins) result from multiple ovulation with synchronous fertilization of two ova by two sperm cells. The causes of dizygotic twinning are multifactorial. Maternal family history, ethnicity, age, gonadotropin levels, use of artificial conception technology and dietary factors are all likely to play a role (Weber et al., 2010; Shur et al., 2009). Dizygotic twins always have separate placentas and are therefore per definition dichorionic.

Monozygotic twins (identical, 30% of all twins) result from division of a zygote originating from the fertilization of one ovum by one sperm cell (Hall et al., 2003). The exact origin of monozygotic twinning remains unknown. The single fertilised egg or zygote may divide into two embryos at any stage between 1 and 16 days after fertilisation. The time at which the embryo divides is a critical factor in subsequent placental development (Hall et al., 2003; Figure 1). The process of division is called cleavage. If cleavage occurs early (0-3 days) after fertilisation (25-30% of cases), the pregnancy develops into dichorionic-diamniotic twins (DCDA). Division at 4-7 days after fertilisation (70-75% of cases) when the chorionic sac has already developed will result in monochorionic-diamniotic twins (MCDA). Division at 8-13 days after fertilization (1-2% of monozygotic twins), the amnion has developed and therefore the cleavage at this stage results in monochorionic-monoamniotic twins (MCMA). Later zygotic splitting (14-16 days), when the development of the embryo has already commenced, results in conjoined monochorionic-monoamniotic twinning.

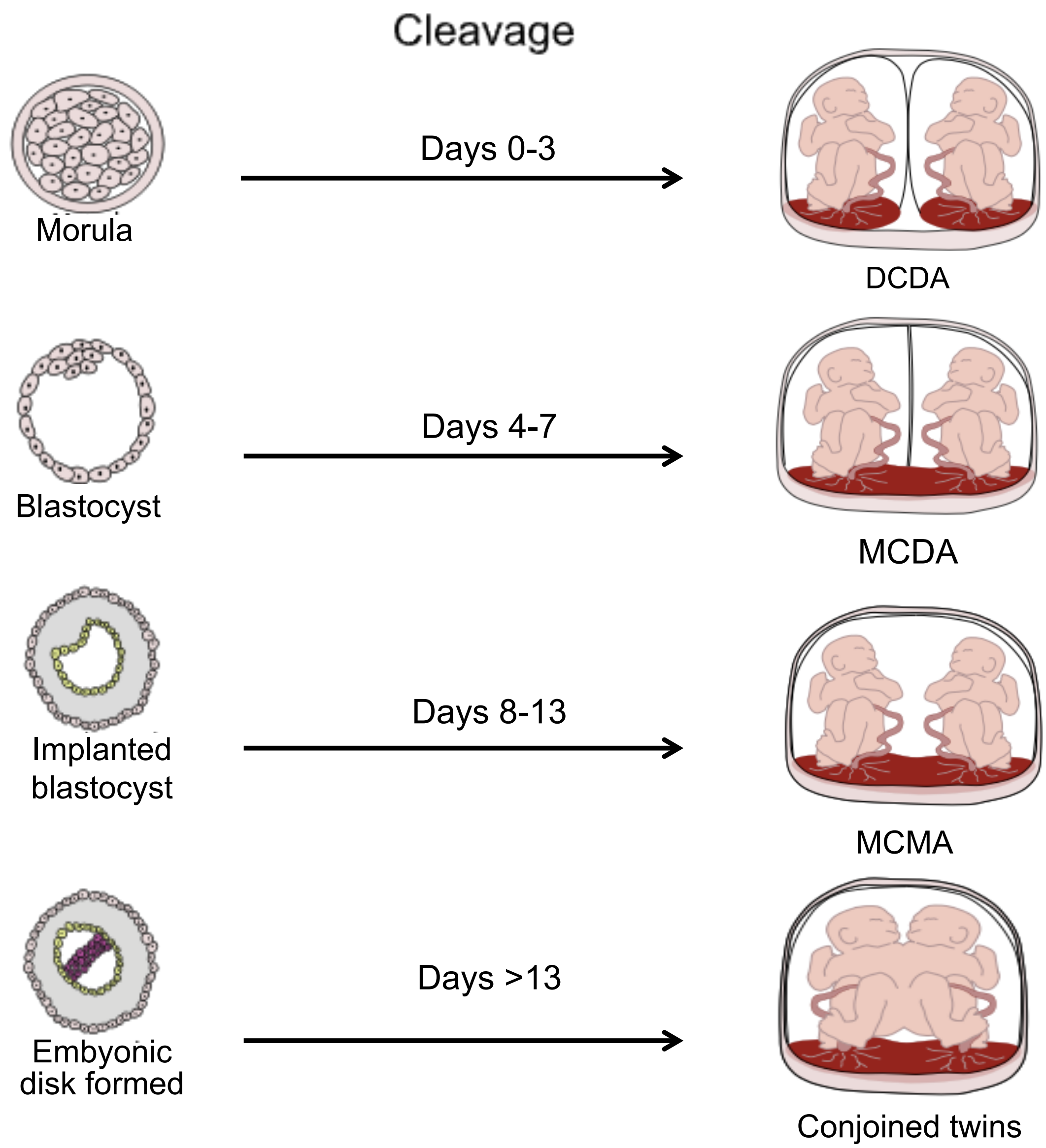

Figure 1. Possible relations of chronicity in monozygotic twins based on the cleavage timing (Dufendach, 2008).

It is neither zygosity nor mode of conception, but chronicity that is the main determinant for pregnancy outcome (Benirschke et al, 1973; Neilson et al., 1989; Bejar et al., 1990).

1.2.2 Prenatal assessment of chorionicity

Accurate determination of chronicity is of paramount importance for the optimal management of twin pregnancies. Therefore the distinction between a high-risk monochorionic and lower risk dichorionic twin pair should be performed as early as possible in the gestation, preferably in the first trimester. A number of different markers have been proposed for ultrasound determination of chorionicity (Scardo et al., 1995). Accuracy of antenatal chorionicity determination has been reported to be between 75% and 100%. This variation can be attributed not only to the use of different ultrasound markers, but also to the timing of scans and the lack of placental histology to confirm chorionicity (Scardo et al., 1995; Sepulveda et al., 1996). The most accurate markers of chorionicity (>99%) appear to be the combination of placental number with the use of the membrane T or λ-signs for a single placental mass at 11-13 weeks gestation (Sepulveda et al., 1996; Dias et al., 2011).

In dichorionic (DC) twins the inter-twin membrane is formed by one thick layer in the middle consisting of the two adjacent chorionic membranes with thin layers of amniotic membrane on each side (Figures 2 and 3). Sonographically this appears as the so-called λ-sign (Sepulveda et al., 1996). Also the yolk sacs are in separate exocoelomic cavities.

In monochorionic-diamniotic twins the dividing membrane consists only with the two fused amniotic membranes and has been described as the T-sign (Sepulveda et al., 1996). With advancing gestation there is regression of the chorion leave and the 'lambda' sign becomes progressively more difficult to identify. Thus by 20 weeks only 85% of DC pregnancies demonstrate the λ-sign.

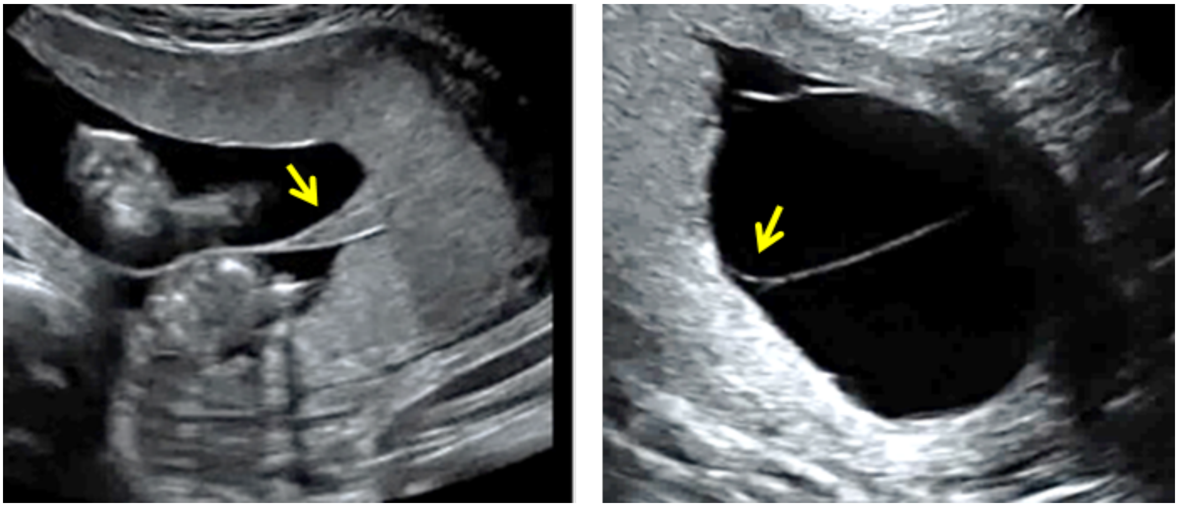

Figure 2. Ultrasound images in the first trimester of a DCDA pregnancy with λ-sign (left) and MCDA pregnancy with T-sign (right).

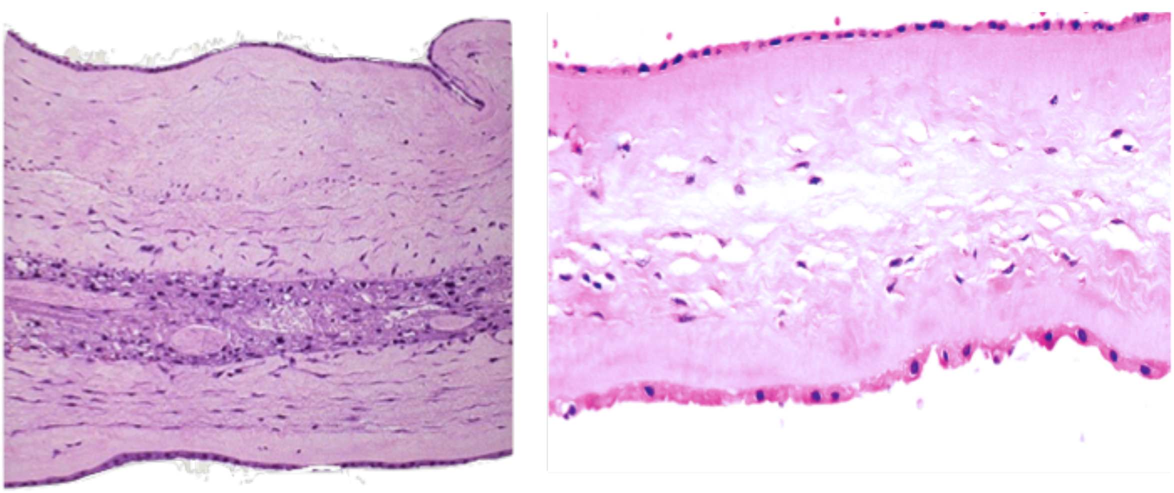

Figure 3. Histological section of DCDA placenta: thin cuboidal layer of amnion on each side and the two chorions plastered together in the centre (left; Pathology Outlines 2011). Histological section of MCDA placenta: thin cuboidal layer of amnion on each surface without visible chorion in-between (right; Baergen et al., 2015)

It is important to emphasize line that amnionicity cannot be predicted accurately by the number of the yolk sacs. Indeed the majority (85%) of diamniotic twins have two yolk sacs, whereas monoamniotic twins have a single yolk sac (Shen et al., 2006); however, rare exceptions have been described (Corbett et al., 2012).

1.2.3 Impact of ART of monozygotic twinning

In spontaneous conceptions the rate of monozygotic twinning is 0.4 - 0.45% (MacGillivray 1986; Derom et al. 1987), whereas the rate after assisted reproduction is increased to 2.1 - 2.2%. Factors, which have significant impact on the monozygotic twinning in assisted reproduction, are younger oocyte age (female age at time of retrieval <35 years), fresh embryo transfer and extended culture (defined as noncleavage embryos transferred on / after day 4). In fact, the risk for monozygotic twinning is increased by 50% after day 5 transfer (blastocyst stage) than after day 3 transfers (Knoppmann et al., 2014; Mateizel et al., 2016).

1.2.4 Consequences of monochorionicity

The placenta in monochorionic twins is unique. Both fetuses share a single placenta and vascular anastomoses on the fetal surface connect the blood circulations of both twins. Through these anastomoses, there is a continuous exchange of blood between the twins (Couck et al., 2016). Therefore the monochorionic placenta can be divided into three parts, one part, which belongs to one twin, second which belongs to the co-twin and third which both monochorionic twins are sharing via anastomoses.

Anastomoses can be of three types: arterioarterial, arteriovenous, and venovenous (Figure 4). Arterioarterial (AA) and venovenous (VV) anastomoses are superficial and bidirectional anastomoses. “Superficial” refers to the fact that they are visible on the surface of the chorionic plate, forming direct communications between the arteries and veins. “Bidirectional” means that they allow flow in both directions depending on the relative intertwin vascular pressure gradients. Most monochorionic placentas typically have only one AA anastomosis. VV anastomoses are more rare and seen in only about 25% of monochorionic placentas. Arteriovenous (AV) anastomoses are very common in a monochorionic placenta and present as deep and unidirectional anastomoses. “Deep” refers to the fact that the anastomosis itself occurs at the capillary level within a shared placental lobule. “Unidirectional” means that they allow flow in one direction only. It receives its arterial supply from one twin and gives its venous (well-oxygenated) drainage to the other. Because of their unidirectional nature, AV anastomoses can create a transfusion imbalance, unless an oppositely directed transfusion by other superficial or deep anastomoses provides adequate compensation (Lewi et al., 2013).

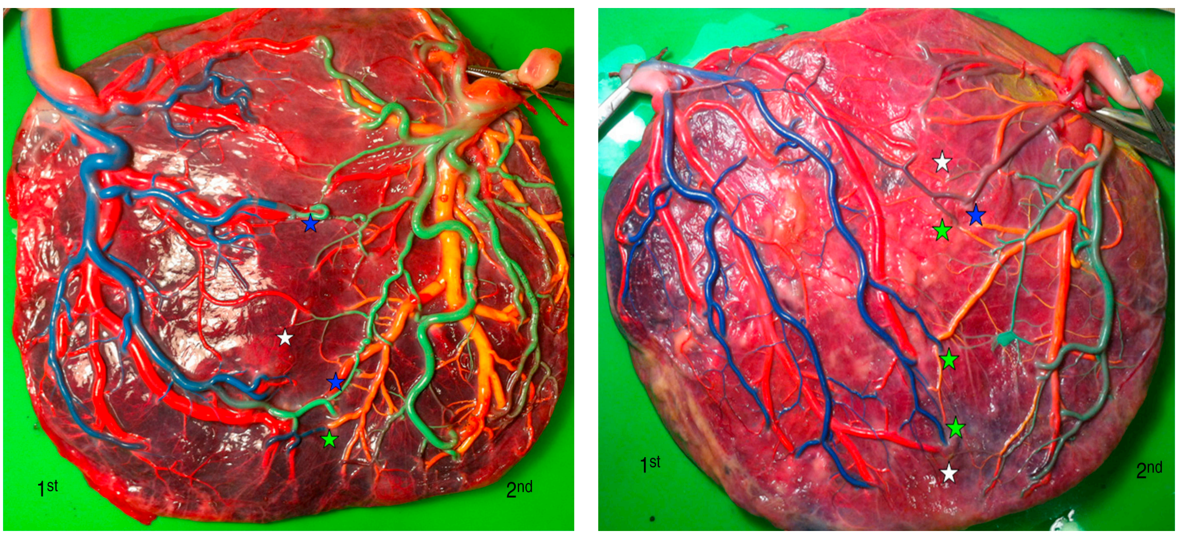

Figure 4. Normal MC placenta showing several AV and VA anastomoses (green and white stars, respectively) and 2 AA anastomoses (blue stars; left). TTTS placenta treated with amnioreduction showing several AV anastomoses (green stars) and VA anastomoses (white stars) and 1 AA anastomosis (blue star; right; Reprinted from Zhao et al., 2014).

Imbalances in this intertwin exchange are often the cause of severe and specific for monochorionic twins’ complications, such as twin-to-twin transfusion syndrome (TTTS), selective fetal growth restriction (sFGR), twin reversed arterial perfusion (TRAP) and twin anemia polycythemia sequence (TAPS) (Table 1).

Major congenital anomalies are more common in monochorionic twin pregnancies and occur in about 6% of pairs (Sperling et al., 2007). Concordance (both twins similarly affected) for a structural anomaly is rare. Several hypotheses exist to explain this increased incidence. Possibly the zygotic splitting itself is teratogenic, resulting in abnormalities, such as midline defects (Table 1).

Table 1. Complications in monochorionic twins due to unique placentation presented in percentage of overall incidence of monochorionic twin pregnancies.

Complications in MC twins	%	Study
Twin-to-twin transfusion syndrome	15	Sebire et al., 2000
Selective fetal growth restriction	12	Sebire et al., 1997
Twin reversed arterial perfusion	1	Moore et al., 1990
Twin anemia polycythemia sequence	5	Lewi et al., 2008 (spontaneous)
	13	Robyr et al., 2006 (post laser)
Discordant anomalies	6	Sperling et al., 2007

Furthermore, in monochorionic twin gestations, transfusion imbalances through the anastomoses during embryogenesis or during later fetal life may account for at least part of the cardiac or brain anomalies observed in these pregnancies. As such, the prevalence of congenital cardiac anomalies in monochorionic twins has been reported to be 2.3% in those without TTTS and 7% in those with TTTS, compared to 0.6% in the general population (Lewi et al., 2013).

Due to the shared circulation within the common placenta, the well being of one twin critically depends on that of the other. As consequence of this, a single death may lead to double demise in about 15% or antenatal brain damage with neurodevelopmental impairment in another 25%, because of acute exsanguination of the surviving co-twin into its demised twin's circulation, in addition to a 68% risk of preterm birth (Hillman et al., 2011).

Twin Anemia and Polycythemia Syndrome

TAPS occurs due to a slow transfusion of blood from the donor to the recipient via few minuscule arteriovenous vascular anastomoses leading gradually to highly discordant hemoglobin levels. The spontaneous form complicates approximately 5% of all monochorionic twin pregnancies (Lewi et al., 2008), whereas the iatrogenic form following incomplete laser coagulation of the communicating vessels occurs in approximately 13% of the cases (Robyr et al., 2006). TAPS can be diagnosed antenatal based on discordant fetal middle cerebral artery peak systolic velocity (MCA-PSV) measurements (Figure 5). The normal values of MCA PSV are given in Table 2. The fetal MCA PSV in the anemic fetus is increased (>1.5 multiples of median), whereas that in the polycythemic fetus is decreased (<1 multiples of the median; Mari et al., 2000, Slaghekke et al., 2010). The placenta of the anemic fetus looks thick and hyperechogenic, whereas that of the polycythemic fetus looks thin and translucent (Figure 5).

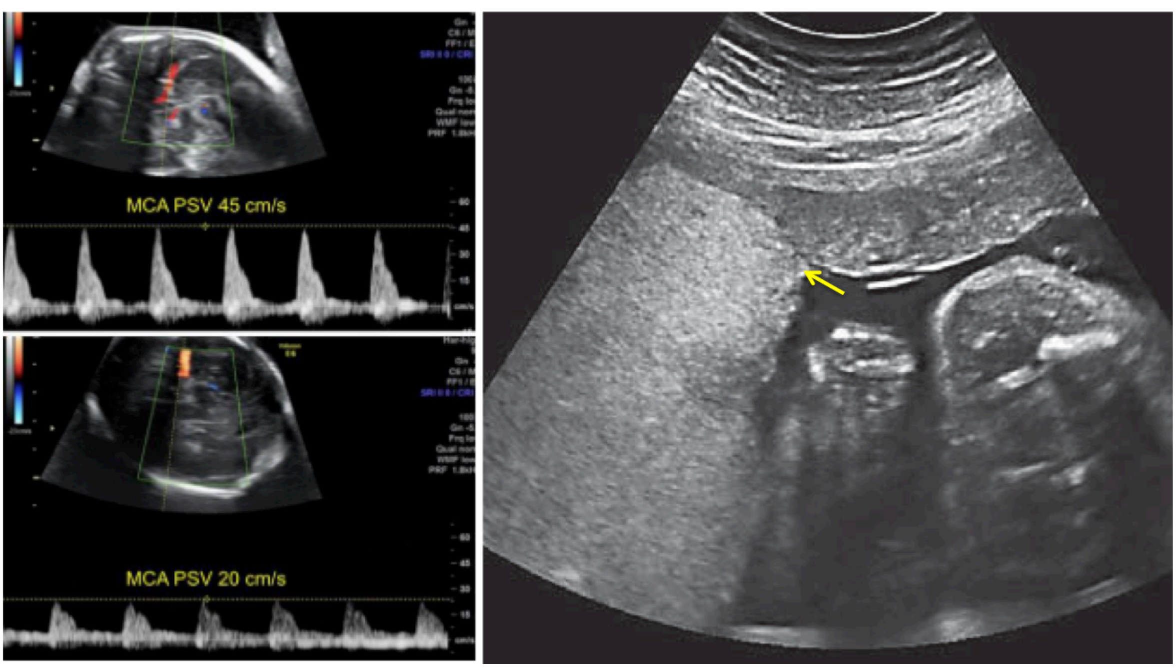

Figure 5. Doppler assessment of flow in the fetal middle cerebral artery (left; FMF) and typical placenta appearance in TAPS (right; Reprinted from Slaghekke et al., 2010)

Table 2. Reference values of peak systolic velocity (cm/s) of the middle cerebral artery (Mari et al., 2000).

Gestational age weeks	Peak Systolic Velocity of the Middle Cerebral Artery (cm/s)		
	Multiple of Median (MoM)		
	1	1.5	2
17	22	33	44
18	23	35	46
19	24	36	48
20	25	38	50
21	26	39	52
22	28	42	56
23	29	44	58
24	30	45	60
25	32	48	64
26	33	50	66
27	35	53	70
28	37	56	74
29	38	57	76
30	40	60	80
31	42	63	84
32	44	66	88
33	46	69	92
34	48	72	96
35	50	75	100
36	53	80	106
37	55	83	110
38	58	87	116

The anemic fetus may have a dilated heart, tricuspid regurgitation and ascites. The liver of the polycythemic fetus has a starry sky pattern due to diminished echogenicity of the liver parenchyma and an increased brightness of the portal venule walls (FMF website 2017). Postnatal, TAPS is defined by hemoglobin discordance of at least 8 g/dl.

TAPS usually presents in the viable period, so the mortality and morbidity is much lower compared to TTTS. The management will mainly depend on and accessibility of the equator. Cordocentesis and intrauterine transfusion is an option by gestational age prior to 30 weeks and persistent discordance in MCA-PSV or a hydrops. Further options depending on the gestational age are an elective delivery, a selective reduction, or a re-laser of the vascular anastomoses (Lewi et al., 2013).

Twin Reversed Arterial Perfusion Sequence

TRAP sequence is a rare condition and complicates about 1% of monochorionic twin pregnancies, which results in an incidence of 1: 35,000 pregnancies. In this unique for monochorionic placentation condition there is a discordant development or an intrauterine demise of one of the monochorionic twins (true parasite) allowing for reversal of blood flow from an umbilical artery of the pump twin in a reversed direction into the umbilical artery of the perfused twin, via an arterioarterial anastomosis and usually returns via a venovenous anastomosis back to the pump twin. The perfused twin's blood supply is by definition deoxygenated and results in variable degrees of deficient development of the head, heart, and upper limb structures. Usually, the perfused twin does not have any functional cardiac activity, hence also the name "acardiac twin." TRAP is diagnosed by the detection of color Doppler ultrasound showing the reversed blood flow in the umbilical artery (Figure 6; Zhao et al., 2014). The acardiac twin is hemodynamically dependent on the pump twin, and its continuous growth threatens the survival of the co-twin, either by increasing the risk for the development of congestive cardiac failure with subsequent intrauterine death, or due to the development of polyhydramnios, preterm premature rupture of membranes, preterm labour and premature delivery. The size of the acardiac mass seems to be of prognostic value for the survival of the pump twin. The perinatal mortality rate for the healthy co- twin has been reported to be in the range 35% to 55% (Hecher et al., 2007). The incidence of chromosomal abnormalities, genetic syndromes or fetal defects is not increased.

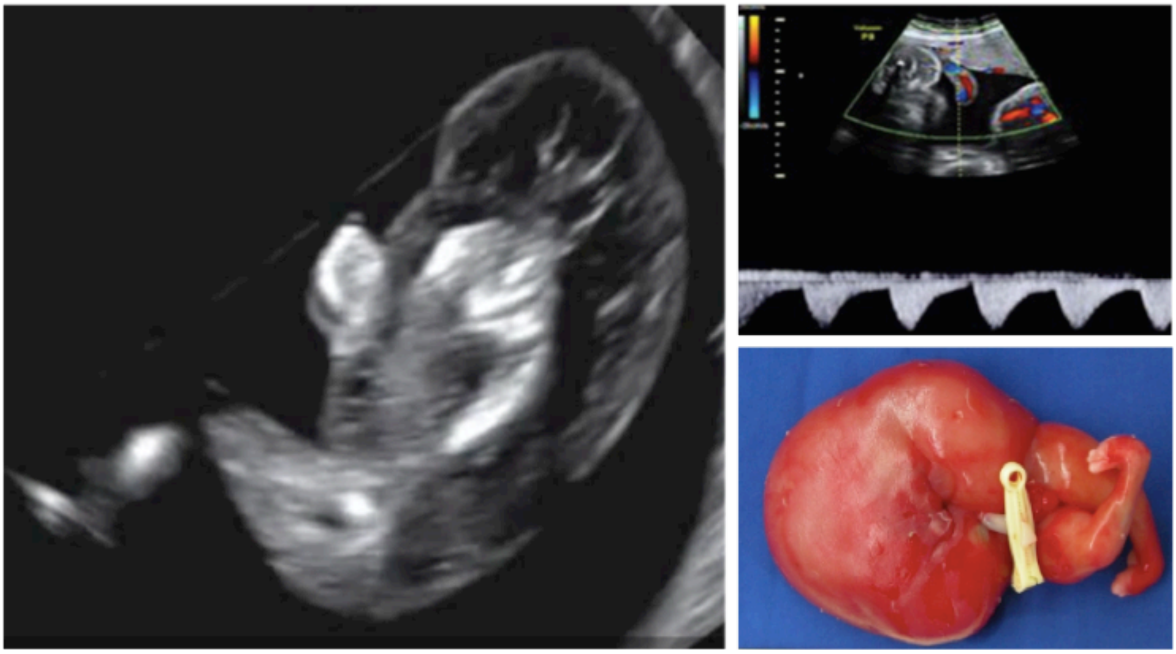

Figure 6. TRAP sequence (left; FMF), pulsed Doppler of the perfused twin (TRAP) demonstrating reversal of flow in the umbilical artery (right; Reprinted from Khanduri et al., 2015) and TRAP appearance post delivery (right; anomalias congenitas/es.atlaseclamc.org)

Several methods for intrauterine intervention have been developed aiming to improve the outcome for the pump twin. In combined data, the survival rate of the pump twin was about 80% for most techniques, including ablation of umbilical cord vessels by laser or diathermy, coagulation of placental anastomoses by laser, or ablation of intrafetal vessels by monopolar diathermy, laser, or radiofrequency. However, these survival rates relate to pregnancies with TRAP sequence diagnosed at or after 16 weeks' gestation. The delay in intervention between the diagnosis of TRAP sequence at 11-13 weeks until 16-18 weeks is associated with spontaneous cessation of flow in the acardiac twin in 60% of cases and in about 60% of these there is also death or brain damage in the pump twin. Therefore survival may be improved by elective intervention at 12-14 weeks by avoiding the hidden mortality of the pump twin until a second trimester intervention and is described to be 77.4% (Chaveeva et al., 2014).

1.3 TWIN-TO-TWIN TRANSFUSION SYNDROME

1.3.1 Classification

One of the major complications that may occur in monochorionic pregnancies before fetal viability is reached is the onset of severe TTTS, complicating about 10-15% of mid-trimester monochorionic twin pregnancies (Sebire et al., 2000; Quintero et al., 1999). Its onset can be attributed to a net blood flow from one fetus (the donor twin) to the other (the recipient twin) via unidirectional arteriovenous placental anastomoses (Lewi et al., 2013).

The syndrome is defined sonographically by the presence of a polyhydramnios in the sac of one twin (recipient twin) and oligohydramnios in the sac of the other twin (donor twin) (Quintero et al., 1999). Whereas the definition of oligohydramnios is world wide accepted as deepest vertical pool (DVP) of ≤ 2 cm, there are differences in the description of polyhydramnios. Because amniotic fluid increases with gestation, most Europeans adhere to the gestational age-dependent cut-off of a DVP ≥ 10 cm after 20 weeks to define the degree of polyhydramnios instead of the primary proposed cut-off of vertical deepest pool ≥ 8 cm (Senat et al., 2004).

The severity of the disease is classified as stage I if the Doppler findings in the umbilical artery and ductus venosus of both fetuses are normal and the bladder of the donor is visible, stage II with normal Doppler findings but not visible bladder, stage III with abnormal Doppler findings in either twin defined as absent (AEDF) or reverse end-diastolic flow in the umbilical artery (REDV), absent (AFDV) or reversed flow in ductus venosus (RFDV) or pulsatile umbilical venous flow (PUVF) and stage IV with presence of ascites or hydrops in either twin. Stage V is characterised with intrauterine demise of one or both twins. (Table 3; Quintero et al., 1999)

Table 3. Staging of TTTS based on ultrasound findings.

Stage	Polyhydramnios* / Oligohydramnios	Absent bladder in donor	Impaired Dopplers*	Hydrops	Intrauterine demise
I	+	-	-	-	-
II	+	+	-	-	-
III	+	+	+	-	-
IV	+	+	+	+	-
V	+	+	+	+	+

* Polyhydramnios: DVP ≥8 cm before 20 weeks and ≥10 cm after 20 weeks
* Impaired Dopplers include AEDF, REDF, AFDV, RFDV and PUVF

Although the vascular anastomoses are an anatomical prerequisite, the pathogenesis of TTTS must be more complex than a simple net transfer of red blood cells, because both twins usually have similar hemoglobin values (Saunders et al., 1999). As mentioned above, TTTS is a problem of amniotic fluid discordance with a volume-overloaded recipient and a volume-depleted donor and not a problem of hemoglobin discordance with a polycythemic recipient and anemic donor. Therefore, endocrine factors related to fluid and pressure homeostasis are likely to be involved as well. Due to the intertwin blood exchange, one twin is exposed to the endocrine environment of the other. As such, transfer of renin-angiotensin-aldosterone effectors from the donor may partly explain the recipient's hypertensive cardiomyopathy and volume overload (Mahieu-Caputo et al., 2005).

1.3.2 Management

The natural history of TTTS is variable. Although not all cases of TTTS may show progressive deterioration, time-related changes may occur if the disease is managed expectantly or symptomatically. The natural history of stage I TTTS is that > 75% of cases remain stable or regress without invasive intervention and with good perinatal outcome. In contrast, the natural history of stages III and IV TTTS is bleak, with perinatal loss rate of 70-100%, particularly when it presents before 26 weeks (SMFM clinical guideline 2013; Hecher et al., 2000).

Several studies have shown that in severe TTTS presenting before 26 weeks, endoscopic laser coagulation of the chorionic-plate-communicating vessels results in higher survival rates and lower rates of neurologic complications at 6 months of age than serial amnioreduction (Table 4; Ville et al., 1992; Ville et al., 1995; Senat et al., 2004; Huber et al., 2006; Rossi et al., 2008). Serial amnioreductions are associated with mortality rates up to 60%, a median gestational age at delivery around 28 weeks and up to 50% severe neurodevelopmental impairment in survivors (Roberts et al., 2008)

Table 4. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome (Rossi et al., 2008).

Outcome	Laser %	Amnioreduction %
Overall survivors	57-77	38-81
At least 1 survivor	75-87	9-49
Neonatal death	4-12	14-39
Cerebral injury	2-33	18-83

Endoscopic laser coagulation involves photocoagulating the vascular anastomoses crossing from one side of the placenta to the other. The goal of laser ablation is to functionally separate the placenta into 2 regions, each supplying one of the twins. Disease recurrence after fetoscopic laser is attributable to residual anastomoses. The reported rate of residual anastomoses in several studies varies from 4% to 33%, depending on the used technique (Zhao et al., 2014). Residual anastomoses lead to chronic inter-twin transfusion and insufficient compensation. Large unidirectional residual channels are more likely to cause TTTS, whereas small vessels less than 1 mm located within 2 cm of the placental edge have been specifically implicated in post-laser TAPS (Baschat et al., 2013).

The recommended technique is a selective coagulation of the anastomoses at the vascular equator combined with additional coagulation of the area between the selectively coagulated anastomoses, known as ‘Solomon technique’ or equatorial laser dichorionisation of the placenta (Figure 7).

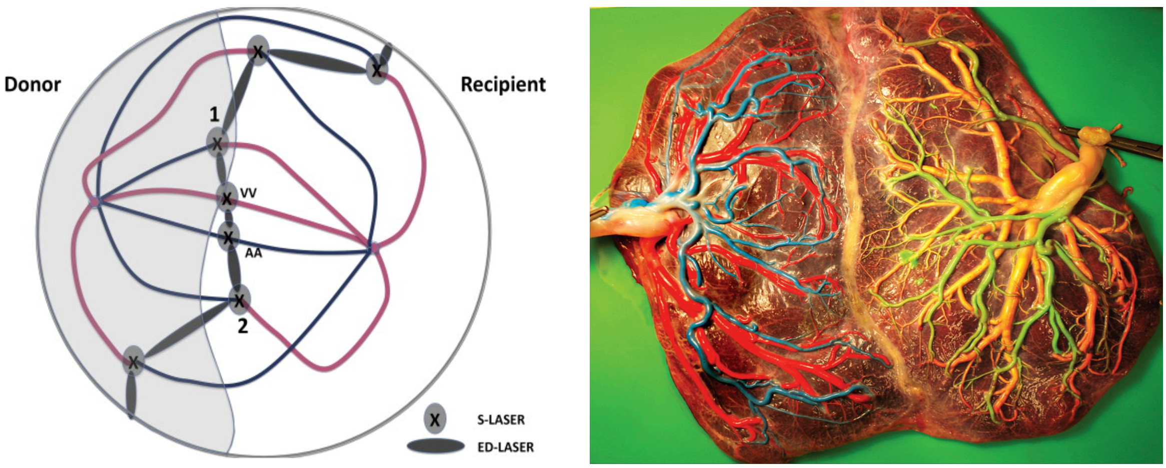

Figure 7. Schematic representation of laser techniques (left; Reprinted from Baschat et al., 2013) and TTTS placenta treated with laser using the Solomon technique (right; Reprinted from Slaghekke et al., 2014).

The Solomon technique significantly reduces the incidence of TAPS and recurrent TTTS and may improve survival and neonatal outcome, without identifiable adverse outcome or complications (Table 5; Slaghekke et al., 2016; Ruano et al., 2013; Baschat et al., 2013).

Table 5. Comparison between the two techniques for laser coagulation of the communicating intertwin vascular anastomosis (Slaghekke et al., 2016).

Outcome	Solomon technique (%)	Selective technique (%)
TAPS	0 - 2.9	4.2 - 15.6
Recurrent TTTS	0 - 3.9	5.3 - 8.5
Dual survival	64 – 85	46 – 76

1.3.3 Outcome after laser surgery

In a combined data in 34 studies on a total of 3,868 pregnancies complicated by TTTS treated with fetoscopic laser coagulation the mean perinatal survival of both twins was 52% (±14.8), one twin was 29% (±10.5) and at least one twin was 81% (±8.3). The overall survival of fetuses was 5,348/7,736 (69%). However, the meta-analysis did not show detailed data about stage related survival (Akkermans et al., 2015).

I performed a literature search to identify all articles in English, which reported Quintero-stage related outcomes and the results are summarised in Tables 6-8. In total, there were 1641 cases of TTTS with or without coexistent IUGR treated with endoscopic laser coagulation of the chorionic-plate-communicating vessels. There were 244 cases with stage I TTTS, 480 cases with stage II TTTS, 784 cases stage III TTTS and 153 cases stage IV TTTS.

- In stage I, overall survival rate was 85%, at least one survival was 92%, and two survivors were present in 78%.

- In stage II, overall survival rate was 77%, at least one survival was 87%, and two survivors were present in 66%.

- In stage III, overall survival rate was 69%, at least one survival was in 83%, and two survivors were present in 49%.

- In stage IV, overall survival rate was 69%, at least one survival was in 83%, and two survivors were present in 55%.

In cases of severe TTTS, combining stage III and IV, overall survival rate was 69%, at least one survival was in 83%, and two survivors were present in 50%.

Table 6. Survival in stage I TTTS treated by endoscopic laser coagulation.

Study	n	Laser weeks	Survival of babies			Delivery weeks
			Overall (%)	≥ 1 (%)	Both (%)	
Quintero et al., 2003	21	20 (16-25)*	32 (76.2)	18 (85.7)	14 (66.7)	32 (16-40)*
Huber et al., 2006	29	22 (17-25)	49 (84.5)	27 (93.1)	22 (75.9)	32 (25-38)
Middeldorp et al., 2007	10	20 (16-26)*	19 (95.0)	10 (100)	9 (90.0)	33 (18-40)*
Chmait et al., 2011	112	21 (16-26)	191 (85.3)	103 (91.9)	88 (78.6)	33 (20-40)
Müllers et al., 2015	9	20 (16-25)*	14 (77.8)	8 (88.9)	6 (66.7)	32 (24-40)*
Emery et al., 2015	45	21.1 ± 2.1	78 (86.7)	42 (93.3)	36 (80.0)	31.9 ± 4.4
Chang et al., 2016	18	21.3 ± 2.4	31 (86.1)	17 (94.4)	14 (77.8)	33.7 ± 4.7
Total	**244**		**414 (84.8)**	**225 (92.2)**	**189 (77.5)**	

Table 7. Survival in stage II TTTS treated by endoscopic laser coagulation.

Study	n	Laser weeks	Survival of babies			Delivery weeks
			Overall (%)	≥ 1 (%)	Both (%)	
Quintero et al., 2003	35	20 (16-25)*	47(67.1)	29 (82.9)	16 (45.7)	32 (16-40)*
Gray et al., 2006	5	21 (19-23)*	3 (30.0)	2 (40.0)	1 (20.0)	34 (29-36)*
Huber et al., 2006	81	21 (16-24)	116 (71.6)	67 (82.7)	49 (60.5)	34 (23-40)
Middeldorp et al., 2007	42	20 (16-26)*	64 (76.2)	35 (83.3)	29 (69.0)	33 (18-40)*
Sepulveda et al., 2007	9	22 (20-25)	11 (61.1)	7 (77.8)	4 (44.4)	31 (25-36)
Morris et al., 2010	8	19 (19-20)	11 (68.8)	8 (100)	3 (37.5)	34 (31-35)
Chmait et al., 2011	177	20 (16-27)	300 (84.7)	165 (93.2)	135 (76.3)	33 (19-38)
Ruano et al., 2013	50	20 (15-26)*	77 (77.0)	45 (90.0)	32 (64.0)	31 (16-39)*
Müllers et al., 2015	42	20 (16-25)*	55 (65.5)	32 (76.2)	23 (54.8)	32 (24-40)*
Chang et al., 2016	29	20.6 ± 2.7	48 (82.8)	25 (86.2)	23 (79.3)	32.4 ± 5.8
Thia et al., 2016	2	19 (19-20)	3 (75.0)	2 (100)	1 (50.0)	31 (28-34)
Total	**480**		**735 (76.6)**	**417 (86.9)**	**316 (65.8)**	

*Reported median gestation combined for all TTTS stages

Table 8. Survival in stage III and stage IV TTTS treated by endoscopic laser coagulation.

Study	n	Laser weeks	Survival of babies			Delivery weeks
			Overall (%)	≥ 1 (%)	Both (%)	
Stage III						
Quintero et al., 2003	28	20 (16-25)*	29 (51.7)	22 (78.6)	7 (25.0)	32 (16-40)*
Gray et al., 2006	17	21 (19-23)*	28 (82.4)	16 (94.1)	12 (70.6)	34 (29-36)*
Huber et al., 2006	80	19 (15-25)	109 (68.1)	66 (82.5)	43 (53.6)	34 (27-40)
Middeldorp et al., 2007	41	20 (16-26)*	57 (69.5)	33 (80.5)	24 (58.3)	33 (18-40)*
Sepulveda et al., 2007	21	20 (17-24)	20 (47.6)	16 (76.2)	4 (19.0)	31 (23-38)
Morris et al., 2010	129	20 (18-22)	165 (64.0)	113 (87.6)	52 (40.3)	33 (30-34)
Chmait et al., 2011	328	19 (15-27)	482 (73.4)	290 (88.4)	192 (58.5)	33 (17-39)
Ruano et al., 2013	47	20 (15-26)*	53 (56.4)	32 (68.1)	21 (44.7)	31 (16-39)*
Müllers et al., 2015	50	20 (16-25)*	88 (88.0)	34 (68.0)	18 (36.0)	32 (24-40)*
Chang et al., 2016	40	20.2 ± 2.7	53 (66.3)	30 (75.0)	13 (32.5)	29.7 ± 5.6
Thia et al., 2016	3	21 (19-21)	3 (50.0)	2 (66.7)	1 (33.3)	29 (27-30)
Total	**784**		**1087 (69.3)**	**654 (83.4)**	**387 (49.4)**	
Stage IV						
Quintero et al., 2003	11	20 (16-25)*	14 (63.6)	9 (81.8)	5 (45.5)	32 (16-40)*
Gray et al., 2006	9	21 (19-23)*	17 (94.4)	9 (100)	8 (88.9)	34 (29-36)*
Huber et al., 2006	10	20 (18-22)	12 (60.0)	7 (70.0)	5 (50.0)	32 (26-36)
Middeldorp et al., 2007	7	20 (16-26)*	7 (50.0)	4 (57.1)	3 (42.9)	33 (18-40)*
Sepulveda et al., 2007	3	22 (19-25)	3 (50.0)	2 (66.7)	1 (33.3)	32 (31-33)
Morriset al., 2010	27	20 (17-21)	26 (48.1)	18 (66.7)	8 (29.6)	31 (27-35)
Chmait et al., 2011	63	21 (16-26)	101 (80.2)	58 (92.1)	43 (68.3)	33 (20-39)
Ruano et al., 2013	5	20 (15-26)*	9 (90.0)	5 (50.0)	4 (80.0)	31 (16-39)*
Müllers et al., 2015	5	20 (16-25)*	7 (70.0)	5 (100)	2 (40.0)	32 (24-40)*
Changet al., 2016	13	21.3 ± 3.3	15 (57.7)	10 (76.9)	5 (38.5)	30.2 ± 6.6
Total	**153**		**211 (69.0)**	**127 (83.0)**	**84 (54.9)**	

*Reported median gestation combined for all TTTS stages

1.3.4. Long-term neurodevelopmental outcome for TTTS

TTTS is associated with an increased risk of neonatal mortality and morbidity, including severe cerebral injury. Twins treated with amnioreduction are less likely to survive, compared to twins treated with laser surgery.

The incidence of long-term neurodevelopmental impairment (NDI) in TTTS treated with amnioreduction is high, on average 20%. The long-term outcome in TTTS treated with laser is more favorable; NDI is reported in 10% of cases. This significant difference in outcome is partly due to the higher rate of severe prematurity in TTTS treated with amnioreduction.

The association between low gestational age at birth and NDI is not surprising, as prematurity is a well-recognised risk factor for adverse neurodevelopmental outcome. Other important risk factors are advanced gestational age at intervention, higher Quintero stage, severe cerebral injury, low birth weight, and low parental educational level (Van Klink et al., 2016).

1.4 SELECTIVE FETAL GROWTH RESTRICTION

1.4.1 Classification

The definition of sFGR in monochorionic twins is not universally established. Previous studies have used variable criteria including estimated fetal weight of the small twin < 5th or < 10th centile, (Sebire et al., 1997; Quintero et al., 2001; Gratacos et al., 2004; Chauhan et al., 2004), abdominal circumference (Vanderheyden et al., 2005) and/or the degree of fetal weight discordance of 25% (Sebire et al., 1997; Gratacos et al., 2004; Chauhan et al., 2004; Victoria et al., 2001). Selective FGR, defined by the estimated fetal weight of the small twin < 5th and the presence of a > 25% discordance in estimated weight between the fetuses, is observed in 12% of MCDA twins (Sebire et al., 1997).

One parameter, which best reflects the differences in sFGR in MC pregnancies with respect to singletons or dichorionic twins is umbilical artery (UA) Doppler flow. The characteristics of UA Doppler flow may be strongly influenced by the existence of intertwin vascular connections. The condition is subdivided into types I, II and III according to the Dopp ler finding of the end diastolic flow in the umbilical artery of the small fetus, which is positive in type I, absent or reversed (AREDF) in type II or intermittent AREDF (iAREDF) in type III (Figure 8; Gratacos et al., 2007).

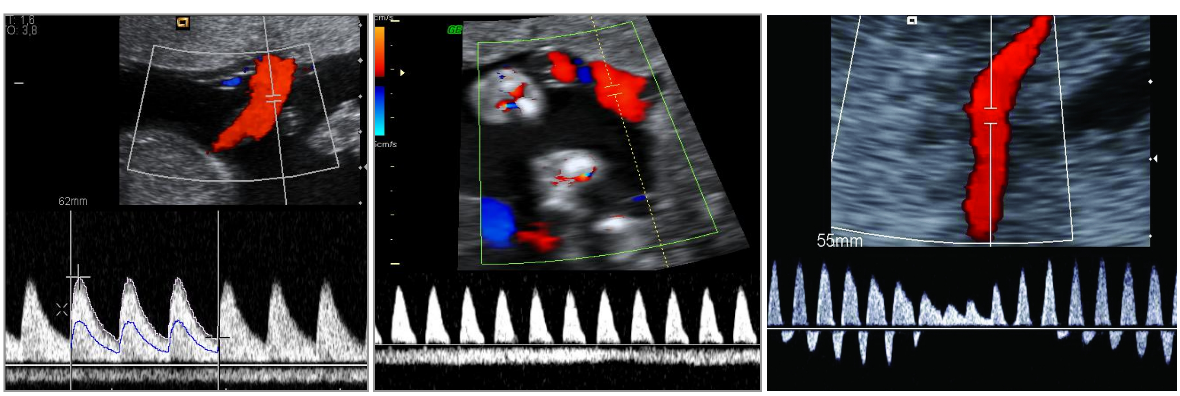

Figure 8. Doppler examination of umbilical artery in sFGR with positive EDF (left), absent EDF (middle) and intermittent AREDF (right) (Reprinted from FMF and Gratacos et al., 2009).

1.4.2 Management

In type I sFGR expectant management with close monitoring to define the best time of delivery is associated with good perinatal outcome for both twins (Table 9). In the combined data from four studies on a total of 115 such pregnancies 97% of small and big babies survived.

Table 9. Reports on outcome of MC twin pregnancies with type I sFGR managed expectantly.

Study	n	Definition sFGR*	Survival of babies (%)		Delivery weeks
			Small	Large	
Gratacos et al., 2004	22	Small <5th, 25%discordance	22 (100)	22 (100)	29 (24-35)
Gratacos et al., 2007	39	Small <10th	38 (97.4)	38 (97.4)	35 (16-38)
Ishii et al., 2009	23	Small <10th	22 (95.7)	22 (95.7)	36 (26-38)
Ishii et al., 2011	31	Small <10th, Large >10th	30 (96.8)	30 (96.8)	29 (26-38)
Total	**115**		**112 (97.4)**	**112 (97.4)**	

* No associated TTTS

In type II sFGR without TTTS management options include close monitoring and early delivery if there is evidence of deterioration of the small fetus, endoscopic laser ablation of placental vascular communications between the two fetuses and cord occlusion of the FGR twin either in all cases or in those with evidence of deterioration at an early gestational age. In type II sFGR with superimposed TTTS the preferred management is endoscopic laser.

Table 10 summarises the data of studies on different management strategies of sFGR with or without coexistent TTTS.

Table 10. Reports on management of MC twin pregnancies with type II sFGR.

Study	n	Definition of sFGR	Survival of babies (%)		Delivery weeks
			Small	Large	
Expectant					
Quintero et al., 2001	12	Small <10th, 25% discordance	6 (50.0)	9 (75.0)	30 (26-39)
Huber et al., 2006	19	Small <5th	10 (52.6)	13 (68.4)	32 (26-36)
Ishii et al., 2009	27	Small <10th	10 (37.0)	18 (66.7)	28 (18-40)
Ishii et al., 2011	55	Small <10th	27 (49.1)	41 (74.5)	32 (18-40)
Visentin et al., 2013	14	Small <10th	14 (100)	14 (100)	30 (28-34)
Total	**127**		**67 (52.8)**	**95 (74.8)**	
Laser surgery					
- No TTTS					
Quintero et al., 2001	8	Small <10th	3 (37.5)	4 (50.0)	31 (25-34)
Chalouhi et al., 2013	23	Small <5th, 25% discordance	7 (30.4)	17 (73.9)	33 (31-34)
Total	**31**		**10 (32.3)**	**21 (67.7)**	
- TTTS					
Chalouhi et al., 2013	166	Small <5th, 25% discordance	69 (41.6)	92 (55.4)	33 (32-34)
Cord occlusion					
- In all cases					
Bebbington et al., 2012	24	Small <10th, 25% discordance	0	21 (87.5)	-
Chalouhi et al., 2013	22	Small <5th, 25% discordance	0	20 (90.9)	31 (29-34)
Total	**46**		**0**	**41 (89.1)**	
- In 9 / 30 cases					
Gratacos et al., 2007	30	Small <10th	21 (70.0)	30 (100)	31 (27-40)

In a combined data in five studies in type II sFGR without TTTS managed expectantly on a total of 127 pregnancies, 53% of small and 75% of big babies survived.

Two studies reported on endoscopic laser ablation of placental vascular communications in type II sFGR without TTTS; in the combined total of 31 pregnancies, 32% of small and 68% of big babies survived (Quintero et al., 2001; Chalouhi et al., 2013). One study reported on endoscopic laser ablation of placental vascular communications in type II sFGR with TTTS; in the total of 166 pregnancies, 42% of small and 55% of big babies survived (Chalouhi et al., 2013).

Elective cord occlusion of the small fetus in type II sFGR without TTTS was reported in two studies; in the combined total of 46 pregnancies, 89% of the big babies survived (Bebbington et al., 2012; Chalouhi et al., 2013). Another study reported on the expectant management of 30 cases of type II sFGR without TTTS, but in 9 cases there was early deterioration in the condition of the small fetus and cord occlusion was then undertaken; in the 30 cases 70% of small and 100% of big babies survived (Gratacos et al., 2007).

In type III sFGR there is a high risk of perinatal death and handicap for both twins. In this condition the close proximity of the umbilical cords precludes the option of endoscopic laser surgery. In a combined series of 142 pregnancies that were managed expectantly the small baby survived in 79% of cases and the big baby survived in 89% of cases (Table 11).

Table 11. Outcome of MC twin pregnancies with type III sFGR without superimposed TTTS managed expectantly.

Study	n	Definition of sFGR	Survival of babies (%)		Delivery weeks
			Small	Large	
Gratacos et al., 2004	18	Small <10th, 25% discordance	13 (72.2)	16 (88.9)	
Gratacos et al., 2007	65	Small <10th	51 (78.4)	61 (93.8)	32 (23-39)
Gratacos et al., 2008	31	Small <10th	25 (80.6)	28 (90.3)	33 (23-38)
Ishii et al., 2010	13	Small <10th	11 (84.6)	10 (76.9)	31(25-37)
Ishii et al., 2011	15	Small <10th	12 (80.0)	11 (73.3)	31(25-37)
Total	**142**		**112 (78.9)**	**126 (88.7)**	

1.5 MANAGEMENT OF TRIPLET PREGNANCIES DIAGNOSED IN THE FIRST TRIMESTER

1.5.1 Incidence of triplet pregnancies

The prevalence of spontaneous triplet pregnancy is about one in 7000 deliveries, but with the increasing availability of assisted reproductive technologies, particularly in vitro fertilisation and controlled ovarian hyperstimulation, the rate of triplet and other higher-order multiple pregnancies has risen dramatically in the last 2 decades.

Although most triplet pregnancies resulting from assisted reproductive techniques are trizygotic and therefore trichorionic, the rate of monozygosity is two to three times higher in assisted conceptions compared with spontaneous pregnancies (Sepulveda et al., 1996; Edwards et al., 1986). The rate of monozygotic triplet pregnancies in a triplet population is estimated as 4.5%, but the exact fraction of monochorionic triplets remains unknown (De Catte et al., 2000). Although multiple births have increased and most of the reported monochorionic triplet pregnancies have been conceived by in-vitro-fertilisation, the monochorionic triplet pregnancy is rare, and is estimated to be approximately 1 in 100,000 births (Ghulmiyyah et al., 2003; Ulug et al., 2004)

1.5.2 Consequences of expectant management and embryo reduction

In triplet pregnancies diagnosed during the first trimester management options include continuing with the whole pregnancy or embryo reduction (ER) to twins or singletons.

In trichorionic triplets (TCT), ER is achieved by fetal intracardiac injection of potassium chloride (KCl). The beneficial consequence of ER is the decrease in the rate of early preterm birth (PTB), but at the expense of increased rate of miscarriage. Table 12 summarises the results of studies reporting on the management of TCT pregnancies with three live fetuses at 8-14 weeks' gestation. In the combined data from these studies the rates of miscarriage at <24 weeks' gestation were 4.2% for expectant management, 7.4% for ER to twins and

10.3% for ER to singleton. The respective rates for preterm birth at <33 weeks were 35.1%, 13.1% and 8.0%.

Table 12. Studies reporting on the management of TCT pregnancies.

Study	n	Miscarriage (%)	Preterm birth (%)		
			<32w	<33w	<34w
Expectant management					
Antsaklis et al., 2004	70	2 (2.9)		25/68 (36.8)	
Skiadas et al., 2011	59	0 (0.0)			36/59 (61.0)
Chaveeva et al., 2013	229	9 (3.9)		76/220 (34.5)	109/220 (49.5)
Drugan et al., 2013	36	2 (5.6)	13/34 (38.2)		
Shiva et al., 2014	58	6 (12.1)	16/52 (30.8)		
Total	**452**	**19 (4.2)**	**29/86 (33.7)**	**101/288 (35.1)**	**145/279 (52.0)**
Embryo reduction to 2					
Antsaklis et al., 2004	185	15 (8.1)		19/170 (11.2)	
Athanasiadis et al., 2005	160	12 (7.5)			
Skiadas et al., 2011	87	6 (6.9)			16/81 (19.8)
Kuhn-Beck et al., 2012	136	7 (5.1)		16/128 (12.4)	
Chaveeva et al., 2013	265	21 (7.9)		36/244 (14.8)	51/244 (20.9)
Drugan et al., 2013	46	3 (6.5)	3/34 (8.8)		
Haas et al., 2014	55	2 (3.6)	0/53 (0.0)		4/53 (7.5)
Shiva et al., 2014	57	7 (12.3)	6/50 (12.0)		
Total	**991**	**73/991 (7.4)**	**9/137 (6.6)**	**71/542 (13.1)**	**71/378 (18.8)**
Embryo reduction to 1					
Kuhn-Beck et al., 2012	44	4 (9.1)		4/40 (10.0)	
Chaveeva et al., 2013	34	5 (14.7)		2/29 (6.9)	3/29 (10.3)
Haas et al., 2014	19	1 (5.3)	1/18 (5.6)	1/18 (5.6)	1/18 (5.6)
Total	**97**	**10 (10.3)**	**1/18 (5.6)**	**7/87 (8.0)**	**4/47 (8.5)**

In dichorionic triplet (DCT) pregnancies, ER by intracardiac injection of KCl involves either the dichorionic fetus or both monochorionic twins; the injected KCl to only one of the monochorionic twins could be transferred to the co-twin through the inter-twin placental vascular anastomoses or death of one fetus could lead to hemorrhage from the co-twin into the dead feto-placental unit with consequent death or neurodevelopmental impairment in the survivor. Table 13 summarises the results of studies reporting on the management of DCT pregnancies with three live fetuses at 8-14 weeks' gestation. In DCT pregnancies, compared to TCTs, there is a higher rate of miscarriage both with expectant management and after ER to twins, which could, at least in part, be attributed to complications arising from inter-twin placental vascular communications and / or unequal sharing of the placenta in the monochorionic pair.

Table 13. Studies reporting on the management of DCT pregnancies.

Study	n	Miscarriage (%)	Preterm birth (%)		
			<32w	<33w	<34w
Expectant management					
Skiadas et al., 2010	13	2 (15.4)			5/11 (45.5)
Chaveeva et al., 2013	123	10 (8.1)		52/113 (46.0)	78/113 (69.0)
Morlando et al., 2015	77	7 (9.1)		17/70 (24.3)	70/70 (100)
Abel et al., 2016	16	1 (6.3)			14/15 (93.3)
Van de Mheen et al., 2016	42	4 (9.5)	17/38 (44.7)		
Total	**271**	**24 (8.9)**		**69/183 (37.7)**	**167/209 (79.9)**
Embryo reduction to 2					
Chaveeva et al., 2013	15	2 (13.3)		3/13 (23.1)	4/13 (30.8)
Embryo reduction to 1					
Skiadas et al., 2010	13	3 (23.1)			1/10 (10.0)
Chaveeva et al., 2013	29	4 (13.8)		2/25 (8.0)	2/25 (8.0)
Morlando et al., 2015	10	2 (20.0)		1/8 (12.5)	8/8 (100)
Abel et al., 2016	30	6 (20.0)			1/24 (4.2)
Total	**82**	**15 (18.3)**		**3/33 (9.1)**	**12/67 (17.9)**

1.5.3 Intrafetal laser for embryo reduction of dichorionic triplets to dichorionic twins

A recent study has proposed a new approach for ER of DCT pregnancies to dichorionic twins by ultrasound-guided laser ablation of the pelvic vessels of one of the monochorionic twins (Chaveeva et al., 2014). In a series of 22 DCT pregnancies undergoing intrafetal laser ultrasound examination within two weeks of the procedure demonstrated that the co-twin had died in 11 cases and was alive in the other 11; in the dichorionic group there was one miscarriage at 23 weeks and in the other 10 cases there were two live births at a median gestational age of 35 weeks, whereas in the 11 cases where both monochorionic fetuses died the separate triplet was live born at a median gestation of 38 weeks.

One other study has reported on the outcome of a series of DCT pregnancies managed by occlusion of the blood supply to one of the monochorionic pair (Morlando et al., 2015). In this multicenter study ultrasound guided laser or radiofrequency ablation of pelvic vessels was carried out in 12 DCT pregnancies at 10-14 weeks' gestation. Two (17%) pregnancies miscarried and in the remaining 10 there were two survivors in seven and one survivor in three; PTB at <33 weeks occurred in 40% (4 of 10) of cases.

1.6 OBJECTIVES

The aims of this thesis are:

- To examine the outcome of monochorionic diamniotic twins with selective fetal growth restriction type II, with or without coexistent twin-to-twin transfusion syndrome, treated by endoscopic placental laser coagulation and to determine predictors of survival of the growth restricted and appropriately grown for gestational age twins.

- To report outcome of monochorionic and dichorionic triamniotic triplet pregnancies treated with endoscopic laser coagulation of communicating placental vessels for severe feto-fetal transfusion syndrome and selective fetal growth restriction.

- To report the outcome of dichorionic triplet pregnancies reduced to dichorionic twins by laser ablation of the pelvic vessels of one of the monochorionic twins.

2. PUBLISHED STUDIES

STUDY 1

Endoscopic placental laser coagulation in monochorionic diamniotic twins with type II selective fetal growth restriction.

STUDY 2

Endoscopic placental laser coagulation in dichorionic and monochorionic triplet pregnancies.

STUDY 3

Intrafetal laser for embryo reduction from dichorionic triplets to dichorionic twins.

STUDY 1

Peeva G, Bower S, Orosz L, Chaveeva P, Akolekar R, Nicolaides KH: Endoscopic placental laser coagulation in monochorionic diamniotic twins with type II selective fetal growth restriction. Fetal Diagn Ther 2015; 38: 86-93.

Selective fetal growth restriction, defined by the presence of a ≥ 25% discordance in estimated weight between the fetuses, is observed in 12% of monochorionic diamniotic twin pregnancies. The condition is subdivided into types I, II and III according to the Doppler finding of the end diastolic flow in the umbilical artery of the small fetus, which is normal in type I, absent or reversed (AREDF) in type II or intermittent AREDF in type III. Some cases of sFGR are complicated by twin-to-twin transfusion syndrome, defined by marked discordance in amniotic fluid volume with deepest vertical pool of ≤2 cm in one sac and ≥ 8 cm before 20 weeks and > 10 cm after 20 weeks in the other sac. In TTTS the severity of the disease is classified as stage I if the Doppler findings in the umbilical artery and ductus venosus of both fetuses are normal and the bladder of the donor is visible, stage II with normal Doppler findings but not visible bladder, stage III with abnormal Doppler findings in either twin and stage IV with presence of ascites or hydrops in either twin.

This study reports our experience with endoscopic laser surgery in MCDA twins with sFGR type II, with or without coexistent TTTS, and investigates predictors of survival of the growth restricted and appropriately grown for gestational age.

STUDY 2

Peeva G, Chaveeva P, Gil Guevara E, Akolekar R, Nicolaides KH: Endoscopic placental laser coagulation in dichorionic and monochorionic triplet pregnancies. Fetal Diagn Ther 2016; 40: 174-180.

Monochorionic pregnancies are often complicated by severe feto-fetal transfusion syndrome (FFTS) and / or selective fetal growth restriction. The established treatment of choice in the management of severe FFTS or some cases of sFGR in MC twin pregnancies is endoscopic laser coagulation of the inter-twin communicating placental vessels. Few studies in a small number of patients have reported on endoscopic laser coagulation of communicating placental vessels for the management of FFTS in MC or dichorionic triamniotic (TA) triplet pregnancies complicated by FFTS.

This study reports our experience with endoscopic laser surgery in the management of severe FFTS or sFGR in MC and DC triplet pregnancies and compares the results with those of previous studies.

STUDY 3

Chaveeva P, **Peeva G**, Gil Pugliese S, Shterev A, Nicolaides KH: Intrafetal laser for embryo reduction from dichorionic triplets to dichorionic twins. Ultrasound Obstet Gynecol 2017; Aug 9. doi: 10.1002/uog.18834.

In dichorionic triplet pregnancies diagnosed during the first trimester there is a high risk of miscarriage and early preterm birth at <33 weeks' gestation. Management options include continuing with the whole pregnancy or embryo reduction by intracardiac injection of KCl into the dichorionic fetus or both monochorionic twins. Previous studies reported that with expectant management the rates of miscarriage and PTB are 9% and 38%, respectively. The rates after ER to twins are 13% and 23% and after ER to singleton are 18% and 9%.

This study examines the outcome of 60 DCT pregnancies undergoing ER to dichorionic twins using a new method of ultrasound-guided laser ablation of the pelvic vessels of one of the monochorionic twins.

STUDY 1

Peeva G, Bower S, Orosz L, Chaveeva P, Akolekar R, Nicolaides KH: Endoscopic placental laser coagulation in monochorionic diamniotic twins with type II selective fetal growth restriction. Fetal Diagn Ther 2015; 38: 86-93.

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Endoscopic Placental Laser Coagulation in Monochorionic Diamniotic Twins with Type II Selective Fetal Growth Restriction

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Key Words
Monochorionic twins · Selective fetal growth restriction · Twin-to-twin transfusion syndrome · Endoscopic laser coagulation

Abstract
Objective: To determine predictors of survival in monochorionic diamniotic twins with selective fetal growth restriction type II (sFGR-II), with or without twin-to-twin transfusion syndrome (TTTS), treated by endoscopic placental laser coagulation. ***Methods:*** Laser surgery was performed at 20 (15–27) weeks' gestation in 405 cases of sFGR-II with a without coexisting TTTS. Multivariable logistic regression analysis was performed to determine significant predictors of survival to discharge from hospital. ***Results:*** There was survival of the small twin in 216 (39.5%) and of the large twin in 379 (69.3%) cases. Significant predictors of survival of both the small and larger twin were ductus venosus Doppler findings in the small twin, gestational age at laser and cervical length, but not the presence of TTTS or Doppler findings in the large twin. ***Conclusions:*** In sFGR-II, survival after laser surgery is primarily dependent on the condition of the small twin.

Introduction

Selective fetal growth restriction (sFGR), defined by the presence of a ≥25% discordance in estimated weight between the fetuses, is observed in 12% of monochorionic diamniotic (MCDA) twin pregnancies [1]. The condition is subdivided into types I, II and III according to the Doppler finding of the end diastolic flow (EDF) in the umbilical artery of the small fetus, which is normal in type I, absent or reversed (AREDF) in type II or intermittent AREDF in type III [2]. Some cases of sFGR are complicated by twin-to-twin transfusion syndrome (TTTS), defined by marked discordance in amniotic fluid volume with the deepest vertical pool of ≤2 cm in one sac and ≥8 cm before 20 weeks and >10 cm after 20 weeks in the other sac [3, 4]. In TTTS, the severity of the disease is classified as stage I if the Doppler findings in the umbilical artery and ductus venosus of both fetuses are normal and the bladder of the donor is visible, stage II with normal Doppler findings but not visible bladder, stage III with abnormal Doppler findings in either twin and stage IV with presence of ascites or hydrops in either twin [3].

In the management of TTTS, endoscopic laser coagulation of the inter-twin communicating placental vessels

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Table 1. Pregnancy characteristics in monochorionic twin pregnancies with sFGR-II following treatment with endoscopic laser surgery in fetuses that survived and those that did not survive

Characteristic	Smaller fetus		Larger fetus	
	alive (n = 216)	dead (n = 331)	alive (n = 379)	dead (n = 168)
Discordance	32.1 (28.1–38.3)*	35.4 (29.9–42.4)	33.8 (28.7–41.6)	34.5 (30.0–39.7)
Coexisting twin-to-twin transfusion				
Yes	161 (74.5)	244 (73.7)	283 (74.7)	122 (72.6)
No	55 (25.5)	87 (26.3)	96 (25.3)	46 (27.4)
Umbilical artery end diastolic flow				
Positive	–	–	340 (89.7)	146 (86.9)
Negative	202 (93.5)	282 (85.2)	37 (9.8)	21 (12.5)
Reversed	14 (6.5)*	49 (14.8)	2 (0.5)	1 (0.6)
Ductus venosus a-wave (n = 516)				
Positive	165 (80.5%)	170 (54.7%)	277 (76.9%)	116 (74.4%)
Negative/reversed	40 (19.5%)*	141 (45.3%)	83 (23.1%)	40 (25.6%)
Year of laser – 1992	15 (10–19)*	14 (8–18)	15 (10–18)*	13 (7–18)
Placental position				
Anterior	108 (50.0)	165 (49.8)	190 (50.1)	83 (49.4)
Posterior	108 (50.0)	166 (50.2)	189 (49.9)	85 (50.6)
Gestation at laser, weeks	20.4 (18.3–23.1)*	19.7 (17.6–22.0)	20.1 (18.0–22.7)	19.7 (17.6–21.9)
Cervical length, mm	31 (27–36)*	30 (25–34)	31 (27–35)*	29 (22–35)
Gestation at outcome, weeks	32.0 (29.8–34.2)*	21.7 (19.1–25.0)	32.1 (29.9–34.6)*	22.2 (19.8–25.4)
Birth weight, g	1,200 (860–1,710)	–	1,674 (1,287–2,100)	–

Values are presented as median (interquartile range) or n (%). * $p < 0.01$.

is the established treatment of choice [4–6]. In type I sFGR without TTTS, expectant management with close monitoring to define the best time of delivery is associated with good perinatal outcome for both twins [2, 7]. In types II and III sFGR without TTTS, there is a high risk of perinatal death and handicap for both twins; the best management, which includes early delivery, cord occlusion of the FGR twin or endoscopic laser surgery, is uncertain because of the small number of reported cases [2, 7–11].

The objectives of this study are to, firstly, examine the outcome of MCDA twins with sFGR type II, with or without coexistent TTTS, treated by endoscopic placental laser coagulation and, secondly, determine predictors of survival of the growth-restricted and appropriately grown for gestational age twins.

Patients and Methods

This study comprised all cases of MCDA twins complicated by sFGR-II, with and without coexisting TTTS, which were treated by endoscopic laser coagulation of the inter-twin communicating placental vessels in our fetal medicine centre between 1992, when we first introduced endoscopic laser surgery [4], and April 2014. Umbilical cord occlusion was not a treatment option for sFGR or TTTS in our centre. In total, there were 556 cases that fulfilled the entry criteria, but 9 were excluded from further analysis because they were lost to follow-up. The study population comprised 547 cases of sFGR-II, including 405 (74%) with coexisting TTTS and 142 (26%) without TTTS. The TTTS was stage III in 385 (95%) cases and stage IV in 20 (5%).

Gestational age was determined by ultrasound measurements of the larger twin, including crown-rump length at <14 weeks [12] and head circumference at ≥14 weeks [13]. Diagnosis of sFGR was based on the demonstration that, firstly, the abdominal circumference at <22 weeks or estimated fetal weight at ≥22 weeks was below the 5th percentile of the appropriate reference range [13–15] and, secondly, the inter-twin discordance in estimated fetal weight (weight difference divided by the weight of the large twin) was ≥25%. The estimated fetal weight at <20 weeks was derived by the formula by Warsof et al. [16], and at ≥20 weeks it was derived by the formula of Hadlock et al. [14].

Doppler assessment of the umbilical arteries of both twins and classification of the EDF as normal, absent or reversed was carried out in all cases. Doppler assessment of the fetal ductus venosus and classification of the a-wave as positive, negative or reversed was systematically carried out only after 1995 [17].

Endoscopic laser surgery was carried out using a semi-rigid 2.0-mm diameter fetoscope (Karl Storz GmbH, Tuttlingen, Germany) through a 3.3-mm diameter cannula (Cook Medical, Blooming-

Table 2. Logistic regression analysis for predicting survival of the smaller fetus in monochorionic twin pregnancies with sFGR-II treated with endoscopic laser surgery

Variable	Univariable analysis			Multivariable analysis		
	OR	95% CI	p value	OR	95% CI	p value
Degree of discordance – 25, %	0.965	0.946–0.985	0.001	0.973	0.949–0.998	0.032
Coexisting twin-to-twin transfusion						
No (reference)	1.000					
Yes	1.044	0.705–1.545	0.830			
Donor: umbilical artery EDF						
Negative (reference)	1.000			1.000		
Reversed	0.399	0.214–0.742	0.004	0.393	0.196–0.788	0.009
Donor: ductus venosus a-wave						
Positive (reference)	1.000			1.000		
Negative/reversed	0.292	0.194–0.441	<0.0001	0.416	0.265–0.653	<0.0001
Recipient: umbilical artery EDF						
Positive (reference)	1.000					
Negative/reversed	0.993	0.576–1.713	0.981			
Recipient: ductus venosus a-wave						
Positive (reference)	1.000					
Negative/reversed	1.369	0.909–2.061	0.133			
Date of endoscopic laser – 1992	1.042	1.010–1.074	0.008			
Placental location						
Posterior (reference)	1.000					
Anterior	1.006	0.714–1.418	0.972			
Gestational age at laser (weeks)	1.087	1.024–1.155	0.006	1.189	1.103–1.282	<0.0001
Cervical length (mm)	1.031	1.008–1.055	0.007	1.037	1.012–1.063	0.003

OR = Odds ratio; CI = confidence interval.

ton, Ind., USA), which was introduced transabdominally into the sac of the larger twin after the administration of prophylactic antibiotics and local anaesthesia. In the cases of sFGR without TTTS, we did not undertake amnioinfusion, as previously described [10]. A 400-μm diameter Nd:YAG laser fibre (Dornier Med Tech, Wessling, Germany) with a power output of 40 W was used to coagulate the inter-twin communicating placental vessels. In the early 1990s, all vessels crossing the inter-twin membrane were coagulated [4, 5], but from 1999 the technique of selective coagulation was adopted [18] with additional coagulation of the placenta between the coagulated vessels. Subsequently, in the cases with coexisting TTTS, amnioreduction of the polyhydramnios was undertaken through the cannula over a period of 10–15 min to obtain subjective normalisation of the amniotic fluid volume on ultrasonographic examination. After a period of rest for 1–3 h, the patients were discharged home, and follow-up was usually undertaken in the referral hospitals.

Maternal demographic characteristics, ultrasound findings and details of intrauterine intervention were recorded in a database. Pregnancy outcomes were collected into the same database when they became available from the referring hospitals, general practitioners or from the patients themselves.

Statistical Analysis

Data from categorical variables were presented as n (%) and from continuous variables as median and interquartile ranges. The MCDA pregnancies with sFGR were divided into smaller and larger fetuses, and comparisons between the fetuses alive and dead in each group were carried out using the χ^2 test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. The tests were considered significant at a p value of <0.05 using two-tailed tests.

Logistic regression analysis was used to determine the variables providing a significant contribution in predicting survival of the smaller and larger fetuses. In the analysis, discordance in estimated inter-twin weight was centred at 25%, and experience in laser surgery was centred at 1992, when the first procedure was carried out. Univariable analysis was carried out to examine the individual variables contributing significantly to the survival of sFGR and larger fetuses by assessing their odds ratios and 95% confidence intervals. Subsequently, multivariable logistic regression analysis with backward stepwise elimination was performed to determine which of these variables provided a significant independent contribution in the logistic model. Kaplan-Meier survival analysis was used to examine the effect of ductus venosus Doppler of the FGR-II fetus in determining the proportion of fetuses surviving following endoscopic laser surgery.

The statistical software package IBM SPSS 21.0 (IBM Corp., Armonk, N.Y., USA) was used for data analyses.

Table 3. Logistic regression analysis for predicting survival of the larger fetus in monochorionic twin pregnancies with sFGR-II treated with endoscopic laser surgery

Variable	Univariable analysis			Multivariable analysis		
	OR	95% CI	p value	OR	95% CI	p value
Degree of discordance – 25, %	0.999	0.979–1.019	0.930			
Coexisting twin-to-twin transfusion						
No (reference)	1.000					
Yes	1.112	0.737–1.676	0.614			
Donor: umbilical artery EDF						
Negative (reference)	1.000					
Reversed	1.123	0.629–2.004	0.695			
Donor: ductus venosus a-wave						
Positive (reference)	1.000			1.000		
Negative/reversed	0.487	0.331–0.718	<0.0001	0.576	0.377–0.880	0.011
Recipient: umbilical artery EDF						
Positive (reference)	1.000					
Negative/reversed	0.761	0.436–1.329	0.337			
Recipient: ductus venosus a-wave						
Positive (reference)	1.000					
Negative/reversed	0.869	0.562–1.343	0.527			
Date of endoscopic laser – 1992	1.049	1.017–1.082	0.003			
Placental location						
Posterior (reference)	1.000					
Anterior	1.030	0.716–1.481	0.875			
Gestational age at laser (weeks)	1.056	0.990–1.127	0.095	1.132	1.050–1.221	0.001
Cervical length (mm)	1.043	1.019–1.068	<0.0001	1.050	1.025–1.077	<0.0001

Results

The median gestational age at laser surgery was 20 (range 15–27) weeks for both groups of sFGR-II. In the pregnancies with no TTTS (n = 142), there was survival of the smaller twin in 55 (38.7%) cases, the larger twin in 96 (67.6%), at least one twin in 102 (71.8%) and both twins in 49 (34.5%) cases; in total, 151 (53.2%) of 284 babies survived. In the sFGR-II pregnancies with coexisting TTTS (n = 405), there was survival of the smaller twin in 161 (39.7%) cases, the larger twin in 283 (69.8%), at least one twin in 291 (71.9%) and both twins in 153 (37.7%) cases; in total, 444 (54.8%) of 810 babies survived. The median gestational age at delivery for the survivors was 32 (range 24–41) weeks, and for the intrauterine or postnatal deaths it was 22 (range 16–37) weeks. Of the 595 survivors, 63 (10.6%), 211 (35.5%), 281 (47.2%) and 40 (6.7%) were born at 24–27, 28–31, 32–36 and at ≥37 weeks' gestation, respectively.

The pregnancy characteristics of the survivors are compared with those with intrauterine or neonatal death in table 1. In the smaller twins that survived, compared to those who did not survive, there was significantly lower estimated inter-twin weight discordance, prevalence of reversed EDF in the umbilical artery and negative or reversed a-wave in the ductus venosus, and higher year of laser surgery, gestational age at laser, cervical length before laser and gestational age at delivery. In the larger twins that survived, compared to those who did not survive, there was significantly higher year of laser surgery, cervical length before laser and gestational age at delivery.

Logistic regression analysis demonstrated that significant predictors of the survival of the smaller twin were estimated inter-twin weight discordance, Doppler findings in the umbilical artery and ductus venosus of the smaller twin, year of endoscopic laser surgery, gestational age at laser and cervical length before laser, but not the presence or absence of coexisting TTTS, Doppler findings in the umbilical artery or ductus venosus of the larger twin or placental location (table 2). In the multivariable analysis, the significant predictors of survival were estimated inter-twin weight discordance, Doppler findings in the umbilical artery and ductus venosus of the smaller twin, gestational age at laser and cervical length before laser, but not year of endoscopic laser surgery. Multivariable logistic regression analysis demonstrated that signif-

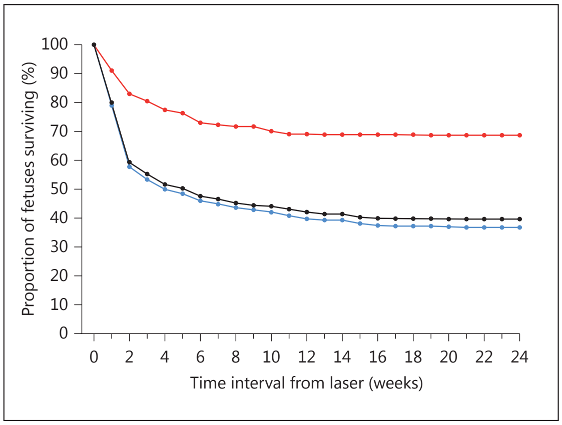

Fig. 1. Kaplan-Meier survival curve illustrating the proportion of surviving twins in relation to time interval from endoscopic laser surgery. The red line is for the larger twin, the black line is for the smaller twin and the blue line for both twins.

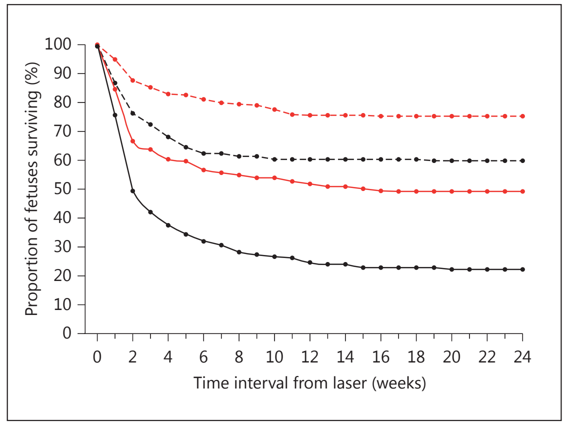

Fig. 2. Kaplan-Meier survival curve demonstrating the effect of negative or reversed a-wave in the ductus venosus of the growth-restricted twin on the proportion of smaller and larger twins surviving after treatment with endoscopic laser surgery. Black lines indicate negative or reversed a-wave and red lines indicate positive a-wave in ductus venosus. Interrupted lines are for the larger twins and solid lines are for the smaller twins.

icant predictors of the survival of the larger twin were Doppler findings in the ductus venosus of the smaller twin, gestational age at laser and cervical length before laser (table 3).

The Kaplan-Meier survival curve illustrating the proportion of surviving twins, irrespective of presence or absence of coexisting TTTS, in relation to time interval from endoscopic laser surgery is shown in figure 1. There was survival of the smaller twin in 216 (39.5%) cases, the larger twin in 379 (69.3%) and both twins in 201 (36.7%) cases. Most deaths occurred within 2 weeks of laser surgery, including 244 (73.7%) of the 331 deaths of the smaller twin and 109 (64.9%) of the 168 deaths of the larger twin.

The survival curves in figure 2 illustrate the relation between the Doppler findings in the ductus venosus of the FGR-II twin and the survival of the smaller and larger twin. Data on ductus venosus Doppler were available for 516 pregnancies. The smaller twin died in 170 (51.2%) of the 335 cases with positive a-wave and in 141 (77.9%) of the 181 cases with negative or reversed a-wave. The larger twin died in 83 (24.7%) of the 335 cases with positive a-wave in the ductus venosus of the FGR-II twin a 73 (40.3%) of the 181 with negative or reversed a-wave. Death of the smaller twin within 2 weeks of endoscopic laser surgery occurred in 33.4% of cases with positive a-wave in the ductus venosus of the FGR-II twin and in 50.3% of cases with negative or reversed a-wave ($p < 0.001$). The respective values for the larger twin were 12.2 and 23.8% ($p = 0.005$).

Discussion

Principal Findings of This Study

In about 75% of our cases with sFGR-II, there was coexisting severe TTTS. This high percentage of coexisting TTTS may represent selection bias by referring obstetricians because isolated sFGR has only recently been recognised as a condition for referral to specialist fetal medicine units. In the management of sFGR-II by endoscopic laser coagulation of the inter-twin communicating placental vessels, survival is not related to the presence or absence of coexisting TTTS and is observed in about 40% of the smaller twins and 69% of the larger twins. There is survival of at least one twin in 72% of cases, and in total 54% of all babies survive.

Most deaths of both the small and the larger twin occur within 2 weeks of laser surgery, and the rate of such death is related to the Doppler findings in the ductus venosus of the FGR-II twin, gestational age at laser and pre-laser cervical length. The rate of death of the smaller and larger twins is 33 and 12% if the a-wave is positive, and the

Table 4. Reports on elective intrauterine interventions in monochorionic twin pregnancies presenting with sFGR-II with and without coexistent twin-to-twin transfusion syndrome at about 20 weeks' gestation

Management/first author	n	Survival			Delivery, weeks
		small twin	large twin	overall	
Cord occlusion					
Bebbington, 2012 [11]	24	0	21 (87.5%)	21 (43.8%)	–
Chalouhi, 2013 [10]	22	0	20 (90.9%)	20 (45.4%)	31.1
Total	46	0	41 (89.1%)	41 (44.6%)	
Endoscopic placental vessel laser					
No twin-to-twin transfusion					
Quintero, 2001 [9]	8	3 (37.5%)	4 (50.0%)	7 (43.7%)	31.3
Chalouhi, 2013 [10]	23	7 (30.4%)	17 (73.9%)	24 (52.2%)	32.5
Peeva, 2015	142	55 (38.7%)	96 (67.6%)	151 (53.2%)	32.0
Total	173	65 (37.8%)	117 (67.6%)	182 (52.6%)	32.0
Twin-to-twin transfusion					
Chalouhi, 2013 [10]	166	69 (41.6%)	92 (55.4%)	161 (48.5%)	32.5
Peeva, 2015	405	161 (39.7%)	283 (69.8%)	444 (54.8%)	32.3
Total	571	230 (40.3%)	375 (65.7%)	605 (53.0%)	32.4

respective rates increase to 53 and 24% if the a-wave is negative or reversed. The high mortality rate of the FGR-II twin within 2 weeks of laser surgery, which is particularly marked in those with abnormal flow in the ductus venosus, could at least in part be attributed to the interruption of the oxygenated blood supply from the larger to the smaller twin [2]. Death of the larger twin, which is also related to the degree of cardiovascular compromise of the FGR-II twin, could at least in part be the result of miscarriage triggered by the intrauterine presence of the dead fetoplacental unit of the smaller twin.

Limitations of the Study

The main limitation of the study is related to the lack of data on neonatal morbidity and long-term neurodevelopmental outcome. This is mainly because the patients were referred for surgery from other centres, often from other countries, where the subsequent management of the pregnancy and the neonates was undertaken. Nevertheless, comparisons can be made with other studies on the basis of the gestational age at delivery of the survivors after laser surgery, which is the main determinant of prevalence of neonatal cerebral lesions [19] and long-term neurological outcome [20]. Douglas et al. [19] performed neonatal imaging in survivors after endoscopic laser surgery and reported severe cerebral lesions in 18.4, 7.3 and 3.7% of those born at 24–27, 28–31 and ≥32 weeks' gestation, respectively. If these figures were applied to our population, the estimated rate of neonatal cerebral lesions would be 6.5%.

Comparison of the Findings with Previous Studies in the Literature

There is general agreement that the treatment of choice for severe TTTS is endoscopic laser coagulation of the inter-twin communicating placental vessels. However, the best management for sFGR-II without coexistent TTTS is uncertain. Gratacos et al. [2] reported that in about 90% of cases there is deterioration in the fetal condition with development of abnormal Doppler findings in the ductus venosus or abnormal fetal heart rate patterns necessitating early delivery or cord occlusion. Ishii et al. [7] described the natural history of the condition in a series of 27 pregnancies with FGR-II diagnosed at <26 weeks' gestation and managed expectantly. There was fetal or neonatal death of the small twin in 48% of cases and of the larger twin in 33%; at 6 months of age, there was evidence of neurological damage in 29% of the surviving smaller twins and in 17% of the larger twins [7]. In a study of 30 cases of sFGR-II diagnosed at a median gestational age of 20 weeks, the intended management was expectant but in 9 cases cord occlusion was carried out because of deterioration in the condition of the FGR twin; all remaining babies survived after delivery at a median gestation of 30 weeks, but in 14% of the small neonates there was evidence of brain damage [2].

The alternative options in the management of sFGR-II are elective cord occlusion of the FGR twin or endoscopic laser coagulation of the inter-twin communicating placental vessels (table 4). Two studies reported on elective occlusion of the umbilical cord of the FGR-II twin at about 20 weeks' gestation; in the combined data on a total of 46 pregnancies, there was survival of the larger twin in 89% of cases, and therefore the overall survival was 45% with delivery at a median gestational age of 31 weeks [10, 11]. Data on elective endoscopic laser coagulation of placental vessels in FGR-II without coexisting TTTS were provided by 2 previous studies, and the results are similar to those in our study. In the combined data on a total of 173 pregnancies, there was survival of the smaller twin in 38% of cases, of the larger twin in 68%, and the overall survival was 52% with delivery at a median gestational age of 32 weeks [9, 10]. Similar results were obtained in the combined data from a previous study [10] and our data on a total of 571 pregnancies with FGR-II and coexisting TTTS treated by elective endoscopic laser surgery.

Implications for Clinical Practice

In sFGR-II presenting at 28 weeks' gestation with abnormal Doppler findings in the ductus venosus, it would be reasonable to undertake early delivery if there is a good standard of neonatal care. If in such pregnancy the birth weight of the larger twin is on the 50th percentile, the weight of the smaller twin would be at least 25% less, which is equivalent to an average weight at 26 weeks [15].

In the last 20 years, sFGR-II has been identified at the ultrasound examination that is routinely performed at 20 weeks' gestation. Indeed, many cases are now detected earlier because of the widespread uptake of the 11–13 weeks' scan and the policy of following up MCDA twins at 1–2 weekly intervals thereafter [1, 21].

In sFGR-II presenting with abnormal Doppler findings in the ductus venosus at about 20 weeks' gestation, the likely outcome is fetal death of the smaller twin with consequent high risk of death or brain damage in the larger twin. In such cases, the management of choice would be endoscopic laser coagulation of the inter-twin communicating placental vessels or cord occlusion of the FGR twin. The best option would be determined by the wishes of the parents, technical issues in carrying out the procedure and local expertise. In terms of outcome, the two methods are associated with a similar gestational age at delivery and therefore likely risk of handicap. With cord occlusion, compared to laser surgery, it appears that the rate of survival of the larger twin may be higher but the overall survival is lower. However, these conclusions are drawn from the published data on all cases of FGR-II, rather than those with abnormal Doppler findings in the ductus venosus alone [9–11]. In our series of such cases, only 12% of the small twins and 60% of the larger ones survived. On the assumption that with cord occlusion the survival of the larger twin is not related to the severity of the disease, reflected in the abnormal ductus venosus flow in the small twin, such intervention that sacrifices the small twin could improve the survival of the larger twin to about 90% [10, 11]. If the rationale of this argument is accepted in cases of sFGR-II without TTTS, the same could be applied for cases with coexistent TTTS, and yet there is general agreement that the best option in the management of such cases is endoscopic laser surgery.

In sFGR-II and normal Doppler findings in the ductus venosus at the time of diagnosis at about 20 weeks, the best choice between elective laser surgery, elective cord occlusion or expectant management with subsequent options of early delivery or emergency intrauterine intervention remains uncertain. With expectant management, it is anticipated that in most cases there will be deterioration in the fetal condition leading to fetal death, extreme preterm delivery or the need for emergency intrauterine intervention [2, 7]. It could therefore be argued that elective cord occlusion or laser surgery at about 20 weeks would be preferable. However, in some cases such interventions may be unnecessary because the fetuses could survive to a gestational age at which early delivery can safely be undertaken. The exact proportion that will fall into the groups of early and late deterioration and the method of predicting the speed of such deterioration require further investigation.

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STUDY 2

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Endoscopic Placental Laser Coagulation in Dichorionic and Monochorionic Triplet Pregnancies

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Key Words
Monochorionic triplets · Dichorionic triplets · Feto-fetal transfusion syndrome · Selective fetal growth restriction · Endoscopic laser coagulation

Abstract
Objective: To report the outcome of monochorionic (MC) and dichorionic (DC) triamniotic (TA) triplet pregnancies treated with endoscopic laser coagulation of the communicating placental vessels for severe feto-fetal transfusion syndrome (FFTS) and selective fetal growth restriction (sFGR). ***Methods:*** Laser surgery was performed at 18 (15–24) weeks' gestation in 11 MCTA and 33 DCTA pregnancies complicated by FFTS and 14 DCTA pregnancies complicated by sFGR. Data from our study and previous reports were pooled using meta-analytic techniques. ***Results:*** Survival of at least one baby and survival among all fetuses was 97.0 and 72.7% in DCTA pregnancies with FFTS, 78.6 and 52.4% in DCTA pregnancies with sFGR and 81.8 and 39.4% in MCTA pregnancies with FFTS. In the combined data from our study and previous reports, the pooled survival rates in 132 DCTA pregnancies with FFTS were 94.4 and 76.1%, and in 29 MCTA pregnancies with FFTS, they were 80.6 and 57.5%. ***Conclusions:*** Survival after laser surgery is higher in DC triplets with FFTS than in those with sFGR and in DC than in MC triplets with FFTS.

Introduction

Monochorionic (MC) pregnancies are often complicated by severe feto-fetal transfusion syndrome (FFTS) and/or selective fetal growth restriction (sFGR). The established treatment of choice in the management of severe FFTS in MC twin pregnancies is endoscopic laser coagulation of the inter-twin communicating placental vessels [1–3]. In sFGR with abnormal Doppler findings in the umbilical artery of the affected fetus, there is a high risk of perinatal death and handicap for both twins; the management for affected pregnancies presenting at midgestation includes endoscopic laser coagulation of the inter-twin communicating placental vessels or cord occlusion of the FGR twin [4–8]. Few studies in a small number of patients have reported on endoscopic laser coagulation of communicating placental vessels for the management of FFTS in MC or dichorionic (DC) triamniotic (TA) triplet pregnancies complicated by FFTS [9–16].

The objectives of this study are to report our experience with endoscopic laser surgery in the management of severe FFTS or sFGR in MC and DC triplet pregnancies and to compare the results with those of previous studies.

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Methods

Study Design and Participants

This was a retrospective study of all cases of MCTA and DCTA triplet pregnancies treated by endoscopic laser coagulation of communicating placental vessels at 15–24 weeks' gestation in our fetal medicine centre between 1996 (4 years after we first introduced endoscopic laser surgery in twins [1]) and June 2015. Umbilical cord occlusion was not a treatment option for sFGR or FFTS in our centre. The cases included 11 MCTA and 33 DCTA pregnancies complicated by FFTS and 14 DCTA pregnancies complicated by sFGR without coexisting FFTS.

Chorionicity and amnionicity were established in the first trimester as previously reported [17]. Gestational age was calculated from the crown-rump length of the biggest fetus [18]. The diagnosis of FFTS was made if there was a marked discordance in amniotic fluid volume between the MC fetuses with a deepest vertical pool of ≤2 cm in at least one sac and of ≥8 cm in the other; the severity of the disease was classified as stage I if the Doppler findings in the umbilical artery and ductus venosus of both fetuses were normal and the bladder of the donor was visible, stage II with normal Doppler findings but no visible bladder, stage III with abnormal Doppler findings in either of the MC fetuses and stage IV with presence of ascites or hydrops in either MC fetus [19].

In the DCTA pregnancies, diagnosis of sFGR was based on the demonstration that, firstly, the abdominal circumference at <22 weeks or estimated fetal weight at ≥22 weeks was below the 5th percentile of the appropriate reference range [20–22] and, secondly, the inter-twin discordance in estimated fetal weight (weight difference divided by the weight of the larger twin) was ≥25%; the estimated fetal weight at <20 weeks was derived by the formula by Warsof et al. [23], and at ≥20 weeks it was derived by the formula of Hadlock et al. [21]. In all of our cases of sFGR, there was Doppler evidence of absent or reversed end-diastolic flow in the umbilical artery of the smaller fetus, and they were therefore classified as type II [24].

Endoscopic laser surgery was carried out transabdominally using a semi-rigid 2.0-mm diameter fetoscope (Karl Storz GmbH, Tuttlingen, Germany), through a 3.3-mm diameter cannula (Cook Medical, Bloomington, Ind., USA), after the administration of prophylactic antibiotics and local anaesthesia. In DC triplet pregnancies with FFTS or sFGR, the fetoscope was introduced into the sac of the larger fetus of the MC pair; a 400-μm diameter Nd:YAG laser fibre (Dornier MedTech, Wessling, Germany) and power output of 40 W was used to coagulate the inter-twin communicating placental vessels as previously described for MC twin pregnancies [7]. In MC triplet pregnancies with FFTS, the fetoscope was introduced into the sac of the recipient fetus, and laser was used to coagulate the vessels between this fetus and each of the other two fetuses; subsequently, the fetoscope was advanced through the inter-twin membrane into the sac of one of the other fetuses to coagulate the vascular connections between them. In cases of polyhydramnios, amnioreduction was undertaken through the cannula over a period of 10–15 min to obtain subjective normalization of the amniotic fluid volume. After a period of rest for 1–3 h, the patients were discharged home. Follow-up and management of the pregnancies was usually undertaken in the referral hospitals.

Maternal demographic characteristics, ultrasound findings and details of intrauterine intervention were recorded in a database. Pregnancy outcomes were collected into the same database when they became available from the referring hospitals, general practitioners or from the patients themselves.

Comparison and Synthesis with Results from Previous Studies

Searches of Medline and Embase (August 2015) were performed to identify all studies in the English language that reported on the use of endoscopic laser surgery for at least two triplet pregnancies. The inclusion criteria were DCTA or MCTA pregnancies complicated by FFTS and/or sFGR, treated by endoscopic laser and providing outcome data. In case of data duplication or overlap, only the largest or most recent study with available data was included.

Statistical Analysis

Data from our study and previous reports were pooled using meta-analytic techniques. Random effects models were used to estimate weighted neonatal survival rates, with 95% confidence interval (CI). Heterogeneity between studies was analysed using both Higgins' I^2 and Cochran's Q test [25, 26].

The statistical software package SPSS 20.0 (IBM SPSS Statistics for Windows, version 20.0, IBM Corp., Armonk, N.Y., USA) and StatsDirect version 2.0 (StatsDirect Statistical Software, UK, 2013) were used for data analysis.

Results

Study Population

During the study period of 1996–2015, we performed endoscopic laser coagulation of communicating placental vessels in 47 DCTA and 11 MCTA triplet pregnancies. The DCTA pregnancies included 14 with sFGR type II and 33 with FFTS; in the latter group, the Quintero stage was I in one, II in two, III in 29 and IV in one. In all MCTA pregnancies, the FFTS was stage III or IV; in 6 cases, there was one donor and one recipient, in 4 cases, there were two donors and one recipient, and in 1 case, there was one donor and two recipients.

DCTA Triplet Pregnancies with FFTS

In the group of 33 DCTA triplet pregnancies with FFTS, endoscopic laser surgery was performed successfully in all cases at a median gestational age of 18 (range 15–23) weeks (table 1). There was survival to discharge from hospital in 72.7% babies, and there was at least one survivor in 97.0% of the pregnancies. The median gestational age at delivery of live births was 29 weeks, and 68.8% were born at <32 weeks.

In 15 pregnancies, all three babies survived after delivery at 8–16 weeks following laser surgery. In 10 pregnancies, two babies survived. In 8 cases, the donor triplet died either in utero or in the neonatal period at 1–14 weeks after laser surgery; the recipient triplet and the separate one were live born and survived. In 1 case, selective feticide of the donor twin was carried out by intracardiac injection of potassium chloride immediately after the laser ablation at 17 weeks' gestation because, at presentation, this fetus was found to have ventriculomegaly and hyperechogenicity of the cerebral cortex; the other two babies were born 13 weeks later and survived. In another case, treated at 20 weeks' gestation, selective fetocide of the separate fetus was carried out because of the diagnosis of cerebellar atrophy after presentation with ruptured membranes at 29 weeks' gestation; the other two babies were live born and survived. In 7 pregnancies, both MC twins died, 6 twins within 2 weeks and one set of twins 4 weeks after laser surgery; in these cases, the separate triplet was live born and survived. In 1 pregnancy, all three babies died; the MC pair died 2 weeks after laser, and this was followed by spontaneous birth at 25 weeks' gestation of the separate triplet that died in the neonatal period.

DCTA Triplet Pregnancies with sFGR

In the group of 14 DCTA triplet pregnancies with sFGR, endoscopic laser surgery was performed successfully in all cases at a median gestational age of 18 (range 16–24) weeks (table 1). There was survival to discharge from hospital in 52.4% babies, and there was at least one survivor in 78.6% of the pregnancies. The median gestational age at delivery of live births was 32 weeks, and 45.5% were born at <32 weeks.

In 1 pregnancy, all three babies survived after delivery at 32 weeks' gestation following laser surgery at 18 weeks. In 9 pregnancies, the FGR triplet died within 2 weeks of laser surgery, but the other two babies were live born 6–20 weeks later and survived. In 1 pregnancy, the MC twins died 1 week after laser surgery at 17 weeks; the separate triplet was live born at 37 weeks and survived. In 3 pregnancies, all three babies died; in the first case, there was miscarriage within 1 week of laser surgery, in the second case, the MC pair died within 2 weeks after laser and this was followed by miscarriage of the separate triplet 3 weeks later, and in the third case, the pregnancy progressed uneventfully after laser surgery at 17 weeks' gestation, but all three fetuses died after prelabor amniorrhexis and chorioamnionitis at 28 weeks.

MCTA Triplet Pregnancies

In the first 4 cases of the series, there was laser coagulation of the communicating vessels between the recipient triplet and the other two fetuses. In the subsequent 7 cases, after completion of the first step as above, the fetoscope was advanced through the inter-twin membrane into the sac of one of the other fetuses to coagulate the vascular connections between them.

Table 1. Outcome of our triplet pregnancies treated by endoscopic laser surgery

Outcome measure	DCTA, FFTS (n = 33)	DCTA, sFGR (n = 14)	MCTA, FFTS (n = 11)
GA at laser surgery, weeks	18 (15–23)	18 (16–24)	18 (16–23)
Survival			
Three	15 (45.5)	1 (7.1)	1 (9.1)
Two	10 (30.3)	9 (64.3)	2 (18.2)
One	7 (21.2)	1 (7.1)	6 (54.5)
> one	32 (97.0)	11 (78.6)	9 (81.8)
Overall	72 (72.7)	22 (52.	13 (39.
GA at delivery of live births, weeks	29 (24–36)	32 (28–36)	32 (28–42)
<32 weeks, n/total n (%)	22/32 (68.8)	5/11 (45.5)	6/9 (66.7)

Values are medians (ranges) or n (%), unless otherwise specified. GA = Gestational age.

Endoscopic laser surgery was performed at a median gestational age of 18 (range 16–23) weeks (table 1). There was survival to discharge from hospital in 39.4% babies, and there was at least one survivor in 81.8% of the pregnancies. The median gestational age at delivery of live births was 32 weeks, and 66.7% were born at <32 weeks.

In 1 pregnancy, all three babies survived after delivery at 30 weeks' gestation following laser surgery at 19 weeks. In 2 pregnancies, the donor triplet died within 2 weeks of laser surgery at 16 and 20 weeks' gestation, respectively, but the other two babies were live born 14 and 8 weeks later and survived. In 6 pregnancies, there was only one survivor; donor and recipient triplets died within 4 weeks after laser surgery. In 2 pregnancies, all three babies died; there was miscarriage within 1 week of laser surgery.

Comparison of Survival between the Triplet Pregnancies

Survival of at least one baby in the DCTA pregnancies with FFTS (97.0%) was not significantly different from that in DCTA pregnancies with sFGR (78.6%; $p = 0.790$) or MCTA pregnancies with FFTS (81.8%; $p = 0.276$). Overall survival for all fetuses in the DCTA pregnancies with FFTS (72.7%) was significantly higher than in DCTA pregnancies with sFGR (52.4%; $p = 0.003$) or MCTA pregnancies with FFTS (33.3%; $p < 0.0001$).

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Table 2. Studies reporting on endoscopic laser surgery for FFTS in DCTA triplet pregnancies

Study	Period	Median GA at laser surgery (range), weeks	Total, n	Stage 3/4, n (%)	Survival of >1 baby, n/total n (%, 95% CI)	Overall survival, n/total n (%, 95% CI)	Live birth at <32 weeks, n/total n (%, 95% CI)
Van Schoubroeck et al. [9], 2004	1996–2002	20 (18–23)	5	5 (100)	5/5 (100, 47.8–100)	12/15 (80.0, 51.9–95.7)	4/5 (80.0, 28.4–99.5)
De Lia et al. [11], 2009	1992–2008	21 (18–25)	8	7 (87.5)	8/8 (100, 63.1–100)	20/24 (83.3, 62.6–95.3)	2/8 (25.0, 3.2–65.1)
Chmait et al. [12], 2010	1998–2008	20 (16–24)	40	**26** (65.	**37**/40 (92. –98.4)	**92**/120 (76 –83.9)	not known
Diemert et al. [13], 2010	2004–2008	20 (17–23)	13	**9** (69.2	**11**/13 (84. –98.1)	27/36 (75.0, 57.8–87.9)	7/11 (63.6, 30.8–89.1)
Peeters et al. [14], 2012	2000–2012	18 (15–25)	8	4 (50.0)	8/8 (100, 63.1–100)	19/24 (79.2, 57.8–92.9)	4/8 (50.0, 15.7–84.3)
Argoti et al. [15], 2014	2005–2011	20 (15–25)	16	**13** (81.	16/16 (100, 79.4–100)	**39**/48 (81. –91.1)	not known
Ishii et al. [16], 2014	2007–2013	21 (16–25)	9	6 (66.7)	9/9 (100, 66.4–100)	20/27 (74.1, 53.7–88.9)	4/9 (44.4, 13.7–78.8)
Our study[a]	1996–2015	18 (15–23)	33	**30** (90.	32/33 (97.0, 84.2–100)	**72**/99 (72. –81.2)	**22**/32 (68. –83.9)
Pooled analysis			132		**126**/132 (9 –97.6)	**301**/393 (7 –80.2)	**43**/73 (56. –69.8)
Cochran's Q (p value)					4.3230 (0.7419)	2.0919 (0.9546)	6.8491 (0.2321)
I^2 statistic, % (95% CI)					0 (0–56.3)	0 (0–56.3)	27 (0–70.7)

GA = Gestational age. [a] Including data from Sepulveda et al. [10].

Table 3. Studies reporting on endoscopic laser surgery for FFTS in MCTA triplet pregnancies

Study	Period	Median GA at laser surgery (range), weeks	Total, n	Stage 3/4, n (%)	Survival of >1 baby, n/total n (%, 95% CI)	Overall survival n/total n (%, 95% CI)	Live birth at <32 weeks, n/total n (%, 95% CI)
De Lia et al. [11], 2009	1992–2008	21 (18–25)	2	1 (50.0	**1**/2 (50.0 –98.7)	3/6 (50.0, 11.8–88.2)	1/2 (50.0, 1.3–98.7)
Chmait et al. [12], 2010	1998–2008	19 (17–22)	6	**2** (33.3	**5**/6 (83.3, –99.6)	11/18 (61.1, 35.7–82.7)	4/5 (80.0, 28.4–99.5)
Diemert et al. [13], 2010	2004–2008	19 (18–20)	3	1 (33.3	**2**/3 (66.7 –99.2)	5/9 (55.6, 21.2–86.3)	1/2 (50.0, 1.3–98.7)
Ishii et al. [16], 2014	2007–2013	20 (17–22)	7	**4** (57.1	7/7 (100, 59.0–100)	18/21 (85.7, 63.7–97.0)	5/7 (71.4, 29.0–96.3)
Our study[a]	1996–2015	18 (16–23)	11	11 (100.0)	9/9 (81.8, 48.2–97.7)	11/33 (33.3, 18.0–51.8)	6/9 (66.7, 29.9–92.5)
Pooled analysis			29		24/29 (80.6, 64.9–92.4)	48/87 (57.5, 36.2–77.4)	17/25 (65.4, 47.7–81.1)
Cochran's Q (p value)					4.3804 (0.3570)	15.9245 (0.0031)	0.9285 (0.9204)
I^2 statistic, % (95% CI)					8.7 (0–67.1)	74.9 (6.1–87.9)	0 (0–64.4)

GA = Gestational age. [a] Including data from Sepulveda et al. [10].

Synthesis with Results from Previous Studies

The literature search identified 8 previous studies reporting data on DCTA pregnancies with FFTS treated by endoscopic laser surgery, and the combined data from these studies with ours are shown in table 2 and figure 1 [9–16]. In a total of 132 such pregnancies, the pooled survival rate of at least one baby was 94.4% (95% CI 90.1–97.6), and the overall survival for all babies was 76.1% (95% CI 71.9–80.2); there was no significant heterogeneity between the studies. Delivery in live births at <32 weeks' gestation occurred in 56.7% (95% CI 43.1–69.8) of cases.

The literature search identified 5 previous studies reporting data on MCTA pregnancies with FFTS treated by endoscopic laser surgery, and the combined data from these studies with ours are shown in table 3 and

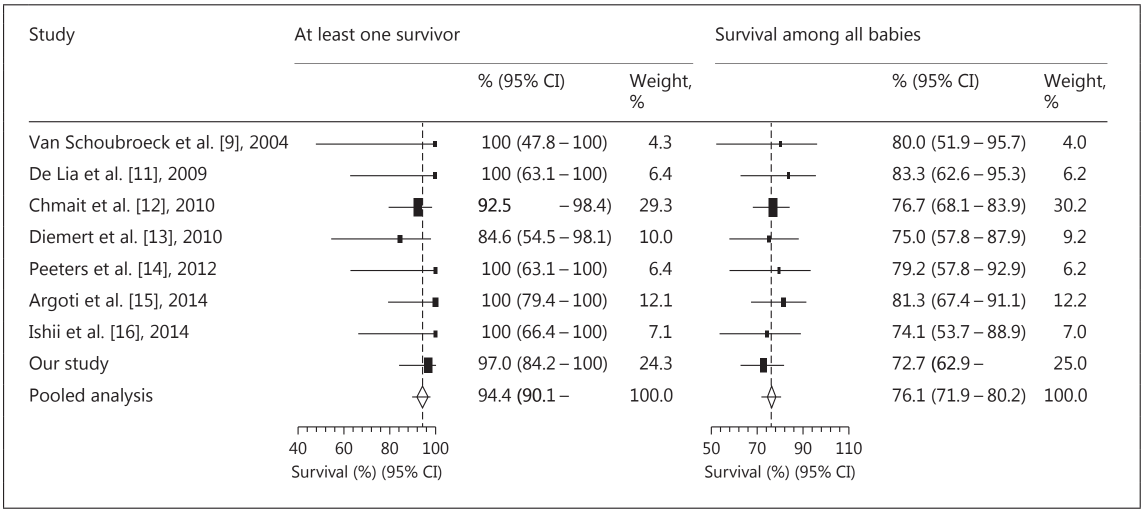

Fig. 1. Pooled estimates of rates of at least one survivor and survival among all babies in DC triplet pregnancies treated with laser coagulation of placental vessels for FFTS.

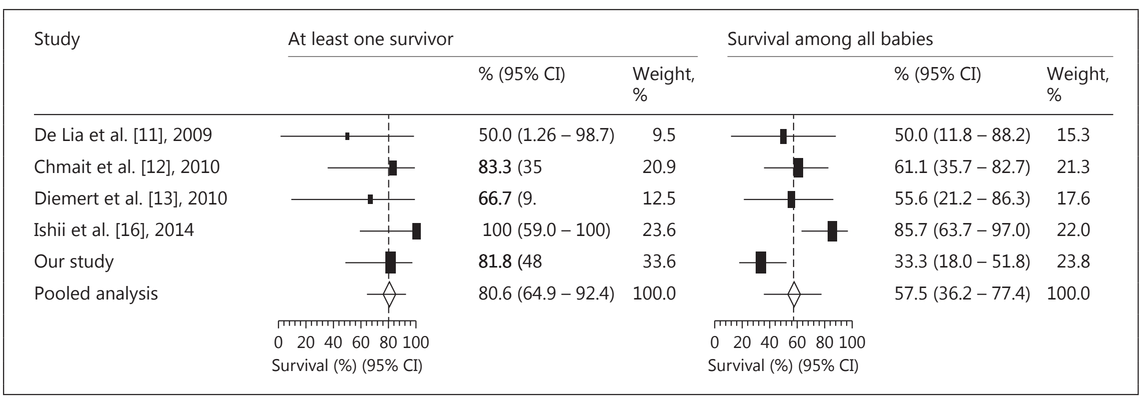

Fig. 2. Pooled estimates of rates of at least one survivor and survival among all babies in MC triplet pregnancies treated with laser coagulation of placental vessels for FFTS.

figure 2 [10–13, 15]. In a total of 29 such pregnancies, the pooled survival rate of at least one baby was 80.6% (95% CI 64.9–92.4), and the overall survival for all babies was 57.5% (95% CI 36.2–77.4); there was significant heterogeneity between the studies. Delivery in live births at <32 weeks' gestation occurred in 65.4% (95% CI 47.7–81.1) of cases.

Discussion

The findings of this study demonstrate the feasibility of endoscopic laser coagulation of communicating placental vessels in the treatment of MC and DC triplet pregnancies complicated by severe FFTS or sFGR. In DC triplets, the separate fetus can pose some technical problems in selecting the appropriate site of entry of the fetoscope, but in general, these problems can easily be overcome; consequently, the outcome of affected pregnancies either by FFTS or sFGR is similar to that in MC twins but with an inevitable higher incidence of early preterm birth. In contrast, in MC triplets, endoscopic laser surgery can be technically difficult because of the necessity to coagulate the communicating placental vessels between all three fetuses; as a result of such technical problems or the inability to achieve the objective of complete separation between all fetuses, the outcome is poorer than in DC triplets.

In the combined data from this and previous studies, in DC triplets with FFTS, there was survival of at least one baby in about 95% of pregnancies and survival of 75% of all babies. These results are compatible with those of endoscopic laser surgery in MC twins with severe FFTS. However, in the triplet pregnancies, a very high proportion of survivors, about 60%, were born at <32 weeks' gestation. We have previously reported that in trichorionic triplet pregnancies with three live fetuses at 10–14 weeks' gestation that were managed expectantly, 35% delivered at <33 weeks; the rate in DC triplet pregnancies managed expectantly was 46% [27]. The higher rate of preterm birth in DC triplets complicated by severe FFTS and treated by endoscopic laser surgery is not surprising.

In our DC triplets with sFGR type II, there was survival of at least one baby in 79% of pregnancies and survival of 52% of all babies. These results are compatible with findings in our series of 547 MC twin pregnancies with sFGR type II treated with endoscopic laser surgery; there was survival of at least one baby in 72% of pregnancies, and 54% of all babies survived [7]. However, survival of the growth-restricted fetus in our DC triplets (7%) was lower than in our MC twin pregnancies with sFGR (40%), possibly because the placental territory of the growth-restricted fetus in DC triplets may be smaller than in MC twins.

In the DC triplets with sFGR type II, 46% of survivors were born at <32 weeks' gestation. A likely explanation for this lower rate of early preterm birth than in our DC triplets with FFTS treated with endoscopic laser (46 vs. 69%) is that following laser surgery, 93% of pregnancies with sFGR continued to delivery with only one or two live fetuses, whereas in those with FFTS, the equivalent rate was 54%; consequently, in relation to preterm birth, only 7% in the sFGR group versus 46% in the FFTS group behaved as triplet pregnancies.

In MC triplets with severe FFTS, in comparison to DC triplets with FFTS, survival was poorer. In our MC triplets, at least one baby survived in 82 of the pregnancies, and 39% of all babies survived, compared to the respective rates of 97 and 73% in our series of DC triplets. The number of MC triplets is too small for definite conclusions to be drawn, but it is certain that endoscopic laser surgery in these pregnancies is considerably more difficult than in DC triplets.

The results from endoscopic laser surgery in triplet pregnancies in our series and those from previous smaller studies allow some general conclusions to be drawn on the effectiveness of such therapy and overall survival rates. However, the total number of cases is still too small to allow accurate assessment of outcome according to subgroups of triplets, including MC and DC complicated by FFTS and/or sFGR, different stages of FFTS, gestational age at therapy or techniques of endoscopic laser surgery.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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STUDY 3

Chaveeva P, **Peeva G**, Gil Pugliese S, Shterev A, Nicolaides KH: Intrafetal laser for embryo reduction from dichorionic triplets to dichorionic twins. Ultrasound Obstet Gynecol 2017; Aug 9. doi: 10.1002/uog.18834

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Intrafetal laser ablation for embryo reduction from dichorionic triplets to dichorionic twins

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KEYWORDS: dichorionic triplets; embryo reduction; first trimester; laser surgery; miscarriage; preterm birth; triplet pregnancy

ABSTRACT

Objective *To report the outcome of dichorionic (DC) triplet pregnancies reduced to DC twins by laser ablation of the pelvic vessels of one of the monochorionic (MC) twins.*

Methods *Intrafetal laser embryo reduction (ER) from DC triplets to DC twins was carried out in 61 pregnancies at 11 + 0 to 14 + 3 weeks' gestation. Pregnancy outcome was examined.*

Results *Intrafetal laser was successfully carried out in all cases, but ultrasound examination within 2 weeks of the procedure demonstrated that the MC cotwin had died in 28 (45.9%) cases and was alive in the other 33 (54.1%). In the DC group, there was one miscarriage at 23 weeks, one neonatal death after delivery at 26 weeks and in the other 31 cases there were two live births at a median gestational age of 35.3 (range, 30.4–38.4) weeks. In the 28 cases in which both MC fetuses died, there was one miscarriage at 16 weeks and in the other 27 cases the separate triplet was liveborn at a median gestation of 38.2 (range, 32.2–42.1) weeks. The overall rate of miscarriage was 3.3% (2/61) and that of preterm birth (PTB) at < 33 weeks was 6.8% (4/59).*

Conclusions *In the management of DC triplet pregnancies, ER to DC twins by intrafetal laser ablation is associated with lower rates of miscarriage or early PTB, compared with expectant management or ER by fetal intracardiac injection of potassium chloride. However, about half of the pregnancies result in the birth of one rather than two babies. Copyright © 2017 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

In triplet pregnancies diagnosed during the first trimester, management options include continuing with the whole pregnancy or embryo reduction (ER) to twins or singletons. In trichorionic (TC) triplets, ER is achieved by fetal intracardiac injection of potassium chloride (KCl). The beneficial consequence of ER is decrease in the rate of early preterm birth (PTB) at < 33 weeks' gestation; a large study and review of the literature of TC triplets diagnosed at 10–14 weeks reported that PTB at < 33 weeks occurred in 35% of those managed expectantly and this was reduced to 13% after ER to twins and 9% after ER to singletons[1]. However, such benefit was at the expense of the rate of miscarriage at < 24 weeks, which increased from 3% with expectant management to 7% after ER to twins and 12% after ER to singletons.

In dichorionic (DC) triplet pregnancies, ER by KCl involves either the DC fetus or both monochorionic twins; the injected KCl to only one of the monochorionic twins could be transferred to the cotwin through the intertwin placental vascular anastomoses or death of one fetus could lead to hemorrhage from the cotwin into the dead fetoplacental unit with consequent death or neurodevelopmental impairment in the survivor. In DC triplet pregnancies, compared with TC triplets, there is a higher rate of miscarriage both with expectant management (9% *vs* 3%)[1–5] and after ER to twins (13% *vs* 7%)[1], which could, at least in part, be attributed to complications arising from intertwin placental vascular communications and/or unequal sharing of the placenta in the monochorionic pair.

We have proposed a new approach for ER of DC triplet pregnancies to DC twins by ultrasound-guided laser ablation of the pelvic vessels of one of the monochorionic twins[6]. In a series of 22 DC triplet pregnancies undergoing intrafetal laser, ultrasound examination within 2 weeks of the procedure demonstrated that the cotwin had died in 11 cases and was alive in the other 11; in the DC group there was one miscarriage at 23 weeks and in the other 10 cases there were two live births at a median gestational age of 35 weeks, whereas in the 11 cases in which both monochorionic fetuses died, the separate triplet was liveborn at a median gestation of 38 weeks.

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The objective of this study of 61 DC triplet pregnancies undergoing intrafetal laser ablation was to provide an update on the outcome of such pregnancies.

METHODS

Intrafetal laser ablation for ER from DC triplets to DC twins was carried out in 61 pregnancies referred to one of two fetal medicine units between 2004 and 2016. In all cases, ultrasound examination was carried out at 10–14 weeks' gestation to demonstrate three live fetuses, determine chorionicity from examination of the inter-triplet membranes[7] and calculate gestational age from the crown–rump length (CRL) of the largest fetus[8]. Parents were counseled regarding the options of expectant management or ER. Maternal demographic characteristics, ultrasound findings and, in those undergoing ER, details of the procedure were recorded in a database. Pregnancy outcomes were collected in the same database when they became available from the referring hospitals, general practitioners or the patients themselves.

Intrafetal laser ER was carried out as previously described[6]. Color flow Doppler was used to visualize the internal iliac arteries and intra-abdominal umbilical vein of one of the monochorionic twins. Local anesthesia was administered to the maternal abdomen. Under continuous ultrasound visualization, an 18-G needle was inserted into the fetal abdomen adjacent to the pelvic vessels, a 400-μm laser fiber was then inserted into the needle and advanced to a couple of millimeters beyond the tip of the needle and laser coagulation was performed using an Nd:YAG laser (Dornier Med Tech, Wessling, Germany) with 40 W. This resulted in hyperechogenicity of tissues in the lower abdomen and cessation of blood flow in the iliac arteries and umbilical vein. Fetal heart activity continued for several minutes. After a period of rest for about 60 min, another ultrasound examination was carried out to confirm death of one monochorionic twin and survival of a DC pair. The patient was discharged and a further appointment was made for an ultrasound examination within 2 weeks of the procedure.

RESULTS

In the study population of 61 DC triplet pregnancies undergoing ER to twins by intrafetal laser ablation, conception was spontaneous in eight (13.1%) and by *in-vitro* fertilization in 53 (86.9%). The median maternal age was 34 (range, 19–46) years and the median gestational age at ER was 12.3 (range, 11.0–14.3) weeks.

Ultrasound examination had demonstrated that all three fetuses in each pregnancy were alive. There were no obvious defects in any of the separate fetuses. In the monochorionic twins, one fetus had diaphragmatic hernia, one had a major cardiac defect and one had acrania; in 18 (29.5%) cases there were early signs of twin-to-twin transfusion syndrome or selective fetal growth restriction with large discordance in CRL (> 10%) and/or reversed a-wave in the ductus venosus in one of the fetuses.

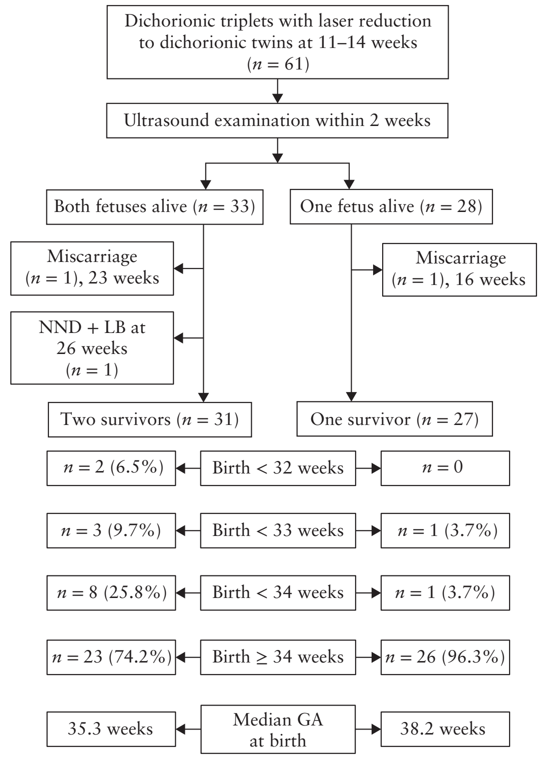

Figure 1 Flowchart summarizing pregnancy outcome of dichorionic triplet pregnancies reduced to dichorionic twins with ultrasound-guided intrafetal laser ablation. GA, gestational age; LB, livebirth; NND, neonatal death.

At the end of the ER procedure and 1 h later both remaining DC twins were alive and there was no blood flow within the dead fetus. Ultrasound examination within 2 weeks of the procedure demonstrated that both fetuses were alive in 33 (54.1%) cases and that the monochorionic cotwin had died in 28 (45.9%) (Figure 1). In the DC group, there was one miscarriage at 23 weeks due to cervical incompetence, one neonatal death after spontaneous delivery at 26 weeks and in the other 31 cases there were two live births at a median gestational age of 35.3 (range, 30.4–38.4) weeks. In the 28 cases in which both monochorionic fetuses died, there was one miscarriage at 16 weeks and in the other 27 cases the separate triplet was liveborn at a median gestation of 38.2 (range, 32.2–42.1) weeks. None of the neonates had any obvious defect or neurological deficit. In the total group, the rates of miscarriage and PTB at < 33 weeks were 3.3% (2/61) and 6.8% (4/59), respectively.

DISCUSSION

Principal findings of this study

The findings of this study demonstrate that, in the management of DC triplet pregnancies, ER to DC twins by intrafetal laser ablation is an additional option to the traditional ones of expectant management, ER by intrafetal injection of KCl to monochorionic twins or ER by KCl to singleton. In 87% of our DC triplet pregnancies, conception was by *in-vitro* fertilization. The risk of monozygotic twinning in assisted conception is more than five times as high as the 0.4% rate in natural conceptions[9–12]. The increased rate of embryo splitting has been attributed to prolonged embryo culture and delayed transfer at the blastocyst rather than cleavage stage and, possibly, zona pellucida manipulation, oocyte age and type of culture medium[11,12].

The technique of ER utilizing interstitial laser is similar to that used in interrupting the blood supply of the acardiac twin in twin reversed arterial perfusion sequence[13]. In our cases of DC triplet pregnancy treated by intrafetal laser, although all monochorionic cotwins were alive 1 h after the procedure, 46% of these twins died within the subsequent 2 weeks. The most likely explanation for the deaths was retrograde hemorrhage into the placenta of the dead fetus or the fetus itself if the occlusion of the fetal pelvic vessels was incomplete.

In the DC triplet pregnancies undergoing intrafetal laser, the rate of miscarriage (3%) was considerably lower than the reported rate in DC triplets managed expectantly (9%)[1–5], and those having ER by KCl to monochorionic twins (13%)[1] or singleton (18%)[1–3,5]. The rate of PTB at < 33 weeks' gestation was considerably lower with intrafetal laser (7%) than with expectant management (35%)[1,3] or ER by KCl to monochorionic twins (13%)[1], and it was similar to that of ER to singleton (9%)[1,3].

Strengths and limitations

The strengths of this study relate to the large number of pregnancies treated by intrafetal laser, the narrow gestational range at which treatment was undertaken and the complete follow-up of cases.

A limitation of any study of this nature is the positive bias in terms of favorable outcome in comparison with historical controls managed expectantly or by intracardiac injection of KCl. Ultimately, accurate comparisons can be provided from randomized trials comparing different management options, but it is very unlikely that such studies will ever be undertaken.

Comparison with previous studies

One other study has reported on the outcome of a series of DC triplet pregnancies managed by occlusion of the blood supply to one of the monochorionic pair[3]. In this multicenter study, ultrasound-guided laser or radiofrequency ablation of pelvic vessels was carried out in 12 DC triplet pregnancies at 10–14 weeks' gestation. Two (17%) pregnancies miscarried and in the remaining 10 there were two survivors in seven and one survivor in three; PTB at < 33 weeks occurred in 40% (4 of 10) of cases.

Implications for practice

In DC triplet pregnancies, counseling of the parents concerning management options should include intrafetal laser reduction to DC twins. Intrafetal laser is associated with a substantially lower rate of miscarriage, compared with that with expectant management or ER to twins or singleton by fetal intracardiac injection of KCl. The rate of PTB at < 33 weeks' gestation is also considerably lower than with expectant management or ER by KCl to monochorionic twins, but is similar to that of ER to singleton. However, with intrafetal laser ablation, about half of the pregnancies will result in the birth of one rather than two babies.

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3. SUMMARY

STUDY 1

Peeva G, Bower S, Orosz L, Chaveeva P, Akolekar R, Nicolaides KH: Endoscopic placental laser coagulation in monochorionic diamniotic twins with type II selective fetal growth restriction. Fetal Diagn Ther 2015; 38: 86-93.

The objective of this study was to determine predictors of survival in monochorionic diamniotic twins with selective fetal growth restriction type II (sFGR-II), with or without twin-to-twin transfusion syndrome, treated by endoscopic placental laser coagulation.

Laser surgery was performed at 20 (15-27) weeks' gestation in 405 cases of sFGR-II with and in 142 without coexisting TTTS. Multivariable logistic regression analysis was performed to determine significant predictors of survival to discharge from hospital from the following variables: degree of discordance in estimated fetal weight, coexisting TTTS, abnormal Doppler findings in the umbilical artery and ductus venosus of the smaller and larger twins, placental location, gestational age and cervical length at laser.

There was survival of the small twin in 216 (39.5%) and of the large twin in 379 (69.3%) cases. Significant predictors of survival of both the small and larger twin were ductus venosus Doppler findings in the small twin, gestational age at laser and cervical length, but not the presence of TTTS or Doppler findings in the large twin.

It was concluded that in sFGR-II survival after laser surgery is primarily dependent on the condition of the small twin.

STUDY 2

Peeva G, Chaveeva P, Gil Guevara E, Akolekar R, Nicolaides KH: Endoscopic placental laser coagulation in dichorionic and monochorionic triplet pregnancies. Fetal Diagn Ther 2016; 40: 174-180.

The objective of this study was to determine the outcome of monochorionic and dichorionic triamniotic triplet pregnancies treated with endoscopic laser coagulation of communicating placental vessels for severe feto-fetal transfusion syndrome and selective fetal growth restriction.

Laser surgery was performed at 18 (15-24) weeks´ gestation in 11 MCTA and 33 DCTA pregnancies complicated by FFTS and 14 DCTA pregnancies complicated by sFGR. Data from our study and previous reports were pooled using meta-analytic techniques.

Survival of at least one baby and survival among all fetuses was 97.0% and 72.7% in DCTA pregnancies with FFTS, 78.6% and 52.4% in DCTA pregnancies with sFGR and 81.8% and 39.4% in MCTA pregnancies with FFTS. The literature search identified eight previous studies reporting data on DCTA pregnancies with FFTS treated by endoscopic laser surgery and five previous studies reporting data on MCTA pregnancies with FFTS treated by endoscopic laser surgery. In the combined data from our study and previous reports, the pooled survival rates in 132 DCTA pregnancies with FFTS were 94.4% and 76.1% and in 29 MCTA pregnancies with FFTS were 80.6% and 57.5%.

It was concluded that survival after laser surgery is higher in DC triplets with FFTS than those with sFGR and in DC than MC triplets with FFTS.

STUDY 3

Chaveeva P, **Peeva G**, Gil Pugliese S, Shterev A, Nicolaides KH: Intrafetal laser for embryo reduction from dichorionic triplets to dichorionic twins. Ultrasound Obstet Gynecol 2017; Aug 9. doi: 10.1002/uog.18834

The objective of this study was to determine the outcome of dichorionic triplet pregnancies reduced to dichorionic twins by laser ablation of the pelvic vessels of one of the monochorionic twins.

Intrafetal laser embryo reduction from dichorionic triplet to dichorionic twins was carried out in 60 pregnancies at 11^{+0} - 14^{+3} weeks' gestation. Pregnancy outcome was examined and compared to previous studies in DCTs managed expectantly or by ER to twins or singleton with fetal intracardiac injection of potassium chloride.

Intrafetal laser was successfully carried out in all cases, but ultrasound examination within two weeks of the procedure demonstrated that the co-twin had died in 28 (46.7%) cases and was alive in the other 32 (53.3%). In the dichorionic group there was one miscarriage at 23 weeks due to cervical incompetence, one neonatal death after spontaneous delivery at 26 weeks and in the other 30 cases there were two live births at a median gestational age of 35.8 (range 30.4-38.4) weeks. In the 28 cases where both monochorionic fetuses died there was one miscarriage at 16 weeks and in the other 27 cases the separate triplet was live born at a median gestation of 38.3 (range 32.2-42.1) weeks. In DCTs intrafetal laser was associated with lower rate of miscarriage (3%), compared to that with expectant management (9%) or ER by intracardiac injection of KCl to monochorionic twins (13%) or singleton (18%). The rate of PTB at <33 weeks' gestation (9%) was also lower than with expectant management (38%) or ER by intracardiac KCl to monochorionic twins (23%), but the same as with ER to singleton (9%).

It was concluded that in the management of DCT pregnancies, ER to dichorionic twins by intrafetal laser is associated with lower rates of miscarriage or early PTB, compared to expectant management or ER by intrafetal injection of KCl. However, about half of the pregnancies result in the birth of one rather than two babies.

4. DISCUSSION

The studies in this thesis have to a large extent fulfilled the objectives as outlined in chapter 1.6.

Study 1 of a large series of 547 MC diamniotic twin pregnancies with sFGR type II, with or without TTTS, treated by endoscopic placental laser coagulation has shown that survival of the small twin was about 40% and of the large twin was 70%. Significant predictors of survival of both the small and larger twin were ductus venosus Doppler findings in the small twin, gestational age at laser and cervical length, but not the presence of TTTS or Doppler findings in the large twin. Consequently, in sFGR type II survival after laser surgery is primarily dependent on the condition of the small twin.

Study 2 of 11 MC and 47 DC triplet pregnancies complicated by severe feto-fetal transfusion syndrome and / or sFGR treated by endoscopic placental laser coagulation has shown that survival was higher in DC triplets with FFTS than those with sFGR and in DC than MC triplets with FFTS. There was survival of at least one baby in 97% of DC triplets with FFTS, 79% of DC triplets with sFGR and 82% of MC triplets with FFTS.

Study 3 of 60 DC triplet pregnancies undergoing ER to DC twins by intrafetal laser has demonstrated the feasibility of this technique. Such intervention is associated with substantially lower rates of miscarriage and early preterm birth, compared to expectant management or ER by intrafetal injection of KCl. However, about half of the pregnancies result in the birth of one rather than two babies.

5. CONCLUSIONS

ENGLISH

Conclusions of the first study

Endoscopic placental laser coagulation was carried in 547 monochorionic diamniotic twins with sFGR-II, including 405 with coexisting TTTS. There was survival of the small twin in 40% and of the large twin in 69% of cases. Significant predictors of survival of both the small and larger twin were ductus venosus Doppler findings in the small twin, gestational age at laser and cervical length, but not the presence of TTTS or Doppler findings in the large twin. Consequently, in sFGR-II survival after laser surgery is primarily dependent on the condition of the small twin.

Conclusions of the second study

Endoscopic placental laser coagulation was carried in 11 MCTA and 33 DCTA pregnancies complicated by FFTS and 14 DCTA pregnancies complicated by sFGR. In DC triplets with FFTS, there was survival of at least one baby in 97% of pregnancies and survival of 73% of all babies; these results are compatible with those of endoscopic laser surgery in MC twins with severe FFTS. In MC triplets with severe FFTS at least one baby survived in 82% of pregnancies and 39% of all babies survived; these results are worse than in DC triplets with FFTS because endoscopic laser surgery in MC triplets is considerably more difficult than in DC triplets. In our DC triplets with sFGR type II there was survival of at least one baby in 79% of pregnancies and survival of 52% of all babies; these results are compatible with findings in our series of 547 MC twin pregnancies with sFGR type II treated with endoscopic laser surgery.

Conclusions of the third study

Intrafetal laser ER from dichorionic triplet to dichorionic twins was carried out in 60 pregnancies at 11^{+0} - 14^{+3} weeks' gestation and pregnancy outcome was compared to previous studies in DCTs managed expectantly or by ER to twins or singleton with fetal intracardiac injection of KCl. The results showed that ER by intrafetal laser is associated with lower rates of miscarriage (3%) or early preterm birth at <33 weeks (9%), compared to expectant management or embryo reduction by intrafetal injection of potassium chloride. However, about half of the pregnancies result in the birth of one rather than two babies.

Final conclusions

Endoscopic placental laser coagulation and intrafetal laser can be successfully applied in the management of highly complicated monochorionic twin and triplet pregnancies.

ESPAÑOL

Conclusiones del primer estudio

Se realizó coagulación láser endoscópica de la placenta en 547 gemelos monocoriales biamnliónicas (MCBA) con crecimiento intrauterino restringido selectivo tipo II (CIRs-II), incluyendo 405 con síndrome de transfusión feto-fetal coexistente. El gemelo pequeño sobrevivió en el 40% de los casos y el grande en el 69% de los casos. Los factores predictivos significativos para la supervivencia de ambos gemelos, grande y pequeño, fueron los hallazgos del Doppler del ductus venoso en el gemelo pequeño, la edad gestacional al momento del láser y la longitud cervical, sin embargo, ni la presencia de STFF o los hallazgos del Doppler en el gemelo grande se vieron relacionados. Por lo tanto, en el CIRs-II la supervivencia tras cirugía láser depende esencialmente de la condición del gemelo pequeño.

Conclusiones del segundo estudio

Se realizó coagulación láser endoscópica de la placenta en 11 embarazos MCTA y en 33 embarazos BCTA complicados con STFF y en 14 embarazos BCTA complicados con CIRs. En gestaciones BCTA con STFF la supervivencia de al menos un feto fue del 97% y de todos los fetos en el 73%; estos resultados son compatibles con los de la coagulación láser endoscópica en gemelos MC con STFF severo. En gestaciones MCTA con STFF severo sobrevivió al menos un feto en el 82% de los embarazos y todos los fetos en el 39; estos resultados son peores que en las gestaciones DCTA con STFF porque la cirugía laser endoscópica en MCTA es considerablemente más complicada que en las gestaciones DCTA. En nuestra serie de gestaciones triples BC con CIRs-II la supervivencia de al menos un feto fue del 79% y de todos los fetos del 52%; estos resultados son compatibles con los hallazgos en nuestra serie de 547 gemelares MC con CIRs-II tratado con cirugía laser endoscópica.

Conclusiones del tercer estudio

Se realizó reducción embrionaria (RE) con laser intrafetal de gestación triple BC a gemelar BC en 60% embarazos a las 11 (11-14) semanas de gestación. El resultado del embarazo fue comparado con estudios previos en triples BC manejados de manera expectante o con RE a gestación gemelar o a única con inyección intracardiaca fetal de cloruro de potasio (KCl). Los resultados mostraron que la RE con láser intrafetal se asocia a tasas más bajas de aborto espontáneo (3%) o parto prematuro < 33 semanas de gestación (9%), comparado con el manejo expectante o reducción embrionaria mediante inyección intrafetal de KCl. Sin embargo, alrededor de la mitad de los embarazos resultan en el nacimiento de un único feto en lugar de dos.

Conclusiones finales

La coagulación láser endoscópica de la placenta y el láser intrafetal pueden emplearse con éxito en el manejo de gestaciones gemelares MC y gestaciones triples complicadas

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