Vanishing Twin Syndrome

Maria Carla Monni¹, Ambra Iuculano², Cristina Peddes³, Giovanni Monni⁴

ABSTRACT

The vanishing twin syndrome (VTS) is defined as a spontaneous demise of one twin or one gestational twin sac in a multiple pregnancy in the first trimester. The incidence of VTS is increased in assisted reproduction techniques (ART) pregnancies, fundamentally due to the increased twin pregnancy rate rather than ART *per se*. Vanishing twin syndrome seems to be associated with adverse outcomes affecting the remaining survivor both in spontaneously conceived pregnancies and ART pregnancies. Vanishing twin syndrome can influence the measurement of invasive and noninvasive screening techniques.

Conclusion: To date, the evidence of the short and long-term effects of VTS on the surviving fetus is discordant. We can reduce its incidence by correcting some predisposing factors. Early detection of VTS and meticulous ultrasound monitoring of the alive twin for the entire pregnancy is mandatory to identify possible adverse obstetric outcomes.

Keywords: First trimester, Multiple pregnancy, Ultrasound, Vanishing twin syndrome.

Donald School Journal of Ultrasound in Obstetrics and Gynecology (2021): 10.5005/jp-journals-10009-1693

INTRODUCTION

Vanishing twin syndrome (VTS) was first described by Levi¹ and it is defined as a spontaneous demise of one twin or one gestational twin sac in a multiple pregnancy in the first trimester (Fig. 1).²

The main causes would seem to be chromosomal aberrations of the demised twin (known as a risk of premature abortion) and placental pathologies, probably due to early changes in implantation that would result in an unfit uteroplacental exchange.³⁻⁵

Other possible mechanisms involved are intrauterine hemorrhages, placental crowding, and chronic maternal diseases such as diabetes during pregnancy and hypertension, which can cause disorders of the placenta and uterine environment by themselves.⁶⁻¹²

Most cases of VTS are asymptomatic, although some patients report mild vaginal bleeding.^{13,14}

The incidence of VTS is estimated to be in about 50% of pregnancies that start with three or more gestational sacs, and about 36% in twin pregnancies¹⁵ but the exact prevalence in spontaneously conceived pregnancies remains uncertain.^{4,16,17} The incidence of VTS in *in vitro* fertilization (IVF) pregnancies with or without intracytoplasmic sperm injection (ICSI) is 12–30%.^{3,18,19}

Vanishing Twin Syndrome in Assisted Reproduction Technique Pregnancies

Regarding pregnancies following assisted reproduction techniques (ART), the higher the number of embryos transferred at IVF cycles, the higher the rate of multiple pregnancies, which is currently estimated at 19.2% in Europe and 43.6% in the USA.^{20,21}

The great advances in laboratory protocols, culture media, and incubation techniques have allowed to extend cell culture duration from cleavage-stage to blastocyst-stage and to improve embryo selection to reduce the number of transferred embryos into the uterine cavity. Until not long ago, the cleavage stage was considered to be the standard stage for embryo transfer (ET) because of the still limited knowledge about the stage-specific culture media and the low embryo survival after this stage.^{22,23} Despite this, the number of single ET is still relatively low (e.g., 21.4% in 2013 in the United States, 30% in 2010 globally).^{21,24}

¹⁻⁴Department of Prenatal and Preimplantation Genetic Diagnosis and Fetal Therapy, Microcitemico Pediatric Hospital "A. Cao", Cagliari, Italy

Corresponding Author: Giovanni Monni, Department of Prenatal and Preimplantation Genetic Diagnosis and Fetal Therapy, Microcitemico Pediatric Hospital "A. Cao", Cagliari, Italy, Phone: +39-070-52965546, e-mail: prenatalmonni@tiscali.it

How to cite this article: Monni MC, Iuculano A, Peddes C, *et al.* Vanishing Twin Syndrome. *Donald School J Ultrasound Obstet Gynecol* 2021;15(2):134–142.

Source of support: Nil

Conflict of interest: None

Several studies have reported that about one in ten singleton pregnancies after IVF treatment starts as a twin pregnancy.²⁵ However, it is important to note that most of these studies involve cases of cleavage-stage ET and it would seem that this estimate is significantly lower in the case of blastocyst-stage ET.^{26,27}

Despite evidence of lower incidence of early VTS in blastocyst-stage ET compared to cleavage-stage ET, it appears that the possible adverse effects on the surviving twins are almost the same in both cases.²⁷ It is essential to underline the need for single ET in IVF/ICSI cycles to reduce risks associated with twin pregnancies, both in case of survival of all fetuses and in case of VTS (Figs 2 to 5).

It should also be pointed out that the increased rate of VTS in ART pregnancies is fundamentally due to the increased twin pregnancy rate rather than ART *per se*. Indeed, according to Marton *et al.*, fetal loss within 14 weeks is statistically greater in spontaneously conceived twin pregnancies than in IVF/ICSI twin pregnancies.²⁸ Given the close correlation between VTS and chromosomal abnormalities,^{29,30} it is likely that the lower incidence of VTS in pregnancies from IVF/ICSI is due to the morphological selection of embryos chosen for transfer to the uterine cavity.

The percentage of VTS in IVF/ICSI pregnancies can also be related to the transfer of intermediate quality embryos,³⁰ to laboratory techniques,³¹ and to artificial modifications of the endometrium.³² Even though a mildly thick endometrium with a triple-line pattern is correlated with a good implant outcome, the

Figs 1A and B: Twin pregnancy of 12 weeks with the early spontaneous loss of heartbeat of one twin. (Courtesy: reprinted with permission of KV Sridevi, Pinnacle Hospital)

Fig. 2: Trichorionic triamniotic triplets with a partial demise of one twin at 9 weeks. (Courtesy: reprinted with permission of KV Sridevi, Pinnacle Hospital)

Fig. 3: HD live imaging of bichorionic diamniotic triplets at 13 weeks with spontaneous heartbeat interruption of the two fetuses in the monochorionic sac at 9 weeks. (Courtesy: reprinted with permission of KV Sridevi, Pinnacle Hospital)

Fig. 4: Early ultrasound scan at 6 weeks with one sac low implantation and less chorionic fluid. Absent cardiac activity was found in a repeated scan after a week. (Courtesy: reprinted with permission of KV Sridevi, Pinnacle Hospital)

Fig. 5: Early ultrasound scan at 6 gestational weeks with one vanishing gestational twin sac

decidualized endometrium has an important role as a biosensor of embryo quality, and its interaction with an inadequate quality embryo can influence the appearance of a VTS.³³

Furthermore, as already known, adverse obstetric outcomes in ART pregnancies, such as abortion in singletons and twin pregnancies, are more related to the status of infertility/subfertility per se, to advanced maternal age, and to chronic pathologies linked to advanced maternal age, rather than to ART. These can determine low oocyte quality and, consequently, low embryonic quality too.^{31,34}

The high frequency of VTS in IVF/ICSI pregnancies and its possible effects on embryos³ have become the subject of numerous debates among ART experts in recent years. Despite conducting numerous studies, the results are contradictory.

Some studies conclude that VTS is associated with adverse obstetric outcomes for the remaining survivor,^{19,34–39} while others show that similar obstetric events are also present in IVF/ICSI singletons.²

So far, the fundamental differences among existing studies must be considered, such as maternal lifestyle characteristics; the type of study performed (e.g., case-control, cohort study); chorionicity which often is not evaluated; and gestational age at fetal death that is not always limited to the first trimester.

Vanishing twin syndrome seems to be associated with adverse outcomes affecting the remaining survivor both in spontaneously conceived pregnancies and ART pregnancies,^{34,40} including increased risk of low birth weight, preterm birth, small-for-gestational-age, and congenital malformations. However, several studies in this regard cannot exclude the possibility of confounding factors linked to maternal characteristics. A sibling comparison approach (which confronts brothers from the same mother) is useful to assess the importance of maternal factors not yet evaluated that remain stable among deliveries.⁴¹

In 2017, a study by Magnus et al. evaluated the association between VTS and adverse events at birth in singleton ART pregnancies. More than 20,000 singleton ART pregnancies were included in this survey based on the Medical Birth Registry of Norway, containing information about all births after 16 weeks of gestation since 1984. This study concluded that pregnancies with VTS had a higher incidence of low birth weight and small-for-gestational-age compared to other singleton pregnancies following ART. These data were also evaluated among siblings, thus limiting maternal confounding factors.¹⁵

Multiple pregnancies involve an increased risk of adverse outcomes at birth due in part to placentation⁴² and related consequences may explain this data in singleton ART pregnancies with VTS. It appears that complications in pregnancies with VTS can be attributed to the absorption of necrotic fetoplacental tissues which can lead to an increased release of cytokines and prostaglandins with a consequent determination of an inflammatory process.³⁷ The cytokines released by the missing twin can affect the placental function and influence the growth of the surviving fetus.⁴⁰

A recent review carried out on behalf of the Embryology Special Interest Group for Early Pregnancy⁴³ suggested that the increase in adverse obstetric outcomes can be attributed to the placental crowding, that is an early alteration of the implant that would result in a uterus-placental insufficiency.

Chasen et al. observed that this altered placental growth would also be at the base of the increased rate of preeclampsia during pregnancies with VTS.⁴⁴

The characteristics of the adverse events detected in the study conducted by Magnus et al. were similar to those of reduced growth in the case of maternal smoking during pregnancy. They can be indicators of a uterine environment that can determine long-term health effects. Children with low birth weight or small for gestational age are at higher risk of chronic diseases such as asthma and allergies, neuronal disorders, and metabolic dysfunctions related to cardiovascular disease.⁴⁵

In a meta-analysis published by Sun et al. in 2017 on ART pregnancies, >400 cases of VTS were compared with nearly 3,000 singleton control pregnancies: no difference was found between the two groups regarding duration of pregnancy, rate of preterm birth, low birth weight, and rate of small for gestational age.³

Effects of Vanishing Twin Syndrome on Surviving Twin

Considering the numerous studies reporting the presence of adverse pregnancy outcomes in the case of VTS, it is important to clarify some aspects.

First of all, the stage of pregnancy when fetal death occurred is not defined in some of these studies although it is closely related to the prognosis of VTS.¹⁹

The loss of a twin in the second trimester is associated with an increased risk for the surviving twin in terms of growth restriction, premature delivery, and perinatal mortality compared to the first trimester VTS. This is most likely linked to the fact that an inflammatory response occurs after one twin's demise because cytokines and prostaglandins released from fetoplacental necrotic tissues increase with pregnancy advancing and fetus growing.⁴⁶

The meta-analysis by Sun et al. included studies that defined VTS as the disappearance of one empty gestational twin sac. In these cases, the gestational sac is smaller when compared to a gestational sac containing an embryo with cardiac activity. Hence, fetoplacental necrotic tissues are of less quantity and therefore the absorption of inflammatory substances is lower. If this hypothesis is correct, obstetric implications in the case of VTS with confirmed embryonic cardiac activity could be worse as compared to VTS with an empty gestational sac (Figs 6 to 8).

In addition, Sun's meta-analysis does not take into account some maternal characteristics that are known to influence studied outcomes, such as body mass index, weight gain during pregnancy, and presence of maternal diseases during pregnancies affecting placental function.

Fig. 6: Twin pregnancy of 8 weeks with the early spontaneous loss of cardiac activity of one of the two twins

Figs 7A and B: Two-dimensional and three-dimensional ultrasound images of 13-week twin pregnancy with one empty gestational twin sac undergoing VTS for maternal age

Figs 8A to D: HD live and three-dimensional HD silhouette images of two 10-week twin pregnancy with one partially demised empty gestational twin sac. (Courtesy: reprinted with permission of RK Pooh, Clinical Research Institute of Fetal Medicine PMC)

Several studies^{19,46,47} suggest that, in the case of late VTS, the greater amount of fetoplacental tissues requires longer elimination time and this can result in unfavorable obstetric outcomes. After VTS is manifested, we can observe placental remodeling which can result in greater blood supply to the demised twin decomposition products and thereafter in temporary reduction of blood toward the surviving twin, with induction of a relative placental insufficiency at the base of a possible intrauterine growth restriction.²⁸

There are other meta-analyses of adverse obstetric outcomes in the case of VTS, but they also take into account spontaneously

conceived monochorionic twin pregnancies (excluded by Sun's study) which notoriously involve a greater incidence of adverse obstetric outcomes.^{48–50}

In conclusion, we notice an association between VTS and obstetric outcomes of the surviving twin but it is currently not well defined.

As regarding other possible VTS complications, it is necessary to mention the case report by Dällenbach et al. describing a rare case of repeated and potentially fatal hemorrhages during pregnancy caused by fetoplacental tissues believed to originate from a VTS diagnosed early in the first trimester.⁵¹

Early ultrasound scan showed a dichorionic twin pregnancy with both fetal heart pulses. An early embryonic loss of one of the twins occurred in the first trimester.

An ultrasound scan was performed at 25 and 27 weeks of gestation when mild vaginal bleeding occurred. An intense arteriovenous flow of low impedance was detected with Doppler sonography distant from the normal placenta of the surviving fetus. An urgent cesarean section was performed after a severe 27-week hemorrhage with hypovolemic shock. Heavily bleeding fragments were found in the correspondence of the intense flow area seen on ultrasound. The removal of these tissues was sufficient to stop the profuse bleeding and to prevent hysterectomy. They clinically corresponded to necrotic placental retained products with dilated vessels, later confirmed histologically.

Probably the subinvolution of the disappeared twin tissues led to the development of an arteriovenous fistula that determined the ultrasound evidence during pregnancy. Although rare, this complication must be considered in case of bleeding in pregnancies with VTS.

Influence of Vanishing Twin Syndrome on Maternal Serum Markers

Vanishing twin syndrome can influence the measurement of maternal serum markers used in association with fetal nuchal translucency (NT) and maternal age for first-trimester prenatal screening of aneuploidies in singleton pregnancies.⁵²

Controversial results were reported in previous studies: some authors found differences in measurements of serum markers between pregnancies started as singleton and those of singleton pregnancies following VTS. This is probably related to the well-known differences of the serum marker levels between singleton and twin pregnancies and first-trimester screening must be adjusted for a twin pregnancy and chorionicity to not reduce its reliability.⁵²

First-trimester screening is one of the most effective methods for screening of Down syndrome (trisomy 21), with a detection rate of 90% and a false-positive rate of <5%. It also allows the identification of a large portion of the other major aneuploidies such as trisomies 13 and 18, triploidies, and different aneuploidies of sex chromosomes.⁵³ The dosed markers for this screening are pregnancy-associated plasma protein (PAPP-A) and free beta-human chorionic gonadotropin (free β -hCG).

Second-trimester screening has that same purpose and uses additional maternal serum markers: alpha-fetoprotein (AFP), dimeric inhibin A (DIA), and unconjugated estriol (uE3).

Chasen et al. reported in their study of 41 cases of VTS that no changes in PAPP-A and free β -hCG if VTS occurs >4 weeks before the first-trimester screening; however, if VTS occurs <4 weeks before both markers are altered.⁵⁴

Gjerris et al. evaluated the same parameters in IVF pregnancies and found no differences as compared to pregnancies started as singleton.⁵⁵

Spencer et al. found an increase in PAPP-A of about 30% and levels of free β -hCG almost unchanged on a large sample of pregnancies with VTS of a measurable craniocaudal length (CRL) embryo in the first trimester; while there was a difference in the case of VTS of an empty gestational sac as compared to singleton pregnancies.⁵³

In 2015, Huang et al. published a study about the impact of VTS on screening both in the first and second trimester in spontaneously

conceived twin pregnancies. PAPP-A, AFP, and DIA in the second trimester were increased, while free β -hCG in the first trimester, uE3 in the second trimester, and total hCG remained unchanged.⁵²

The low impact of the demise of a twin on free β -hCG levels is not surprising given its very rapid clearance (<24 hours).⁵³ On the other hand, high PAPP-A values similar to those seen in twin pregnancies were observed up to 8 weeks after the disappearance of one twin.⁵⁶

Previous studies of the increase of PAPP-A were contradictory. This is probably due to the differences in studied populations, like gestational age at blood sampling for the first-trimester screening, the period between the disappearance of one embryo and the blood sampling, lack of discernment between VTS of an embryo with measurable CRL, and an empty twin sac. The study of Chasen's group was criticized about the failure to correct the results for IVF (64% of pregnancies in the VTS group were obtained with IVF).⁵³

Several studies results support the recommendation to exclude PAPP-A from the screening algorithm and employ NT measurement only or associated with free β -hCG for pregnancies with VTS.⁵⁷

Second-trimester screening of pregnancies with VTS may be inaccurate therefore it is currently not recommended.⁵²

Influence of Vanishing Twin Syndrome on Non-invasive Prenatal Screening

Noninvasive prenatal screening (NIPS) also appears to be influenced by VTS, particularly those using methods that can evaluate total DNA but cannot determine the presence of additional haplotypes.

These last methods allow the determination of the number of copies of fetal chromosomes by comparing the absolute number of the sequences read in chromosomes in question (e.g., chromosome 21) and a reference number, deducing a fetal trisomy when this number is greater than a certain threshold value.⁵⁸ Therefore, by counting the high frequency of chromosomal abnormalities in VTS, Down syndrome can lead to false-positive results when the non-determination of additional haplotypes method is used.

Furthermore, in the case of VTS with the gender discrepancy, an incorrect sex identification can be observed (e.g., when a male fetus VTS occurs, a female fetus can be identified as a male fetus in case of NIPS). This is particularly important for diseases such as X-linked monogenic disorders.⁵⁹

The use of a new analysis approach based on single-nucleotide polymorphism (SNP) seems to reduce this potential source of false positives. Indeed, this method allows detecting the presence of any twins not previously found thanks to the identification of additional fetal haplotypes.

The presence of additional fetal haplotypes in the case of singleton pregnancy may therefore suggest a VTS. In these cases, there are several diagnostic options: NIPS repetition, wait-and-see approach, follow-up diagnostic tests to exclude trisomies, and, finally, the use of invasive tests (chorionic villous sampling and amniocentesis) that have to be evaluated regarding other indications and risks for the mother and the fetus.⁶⁰ Once the possibility of trisomy is discarded, the possibility of a VTS with an early disappeared embryo cannot be ruled out.

Most VTS occur in the first trimester, so the clinical diagnosis largely depends on the age of the first obstetric ultrasound and the time of fetal disappearance.⁶¹ For this reason, in the case of NIPS, it is advisable to perform an early ultrasound scan to determine the fetus's number and to identify the presence of VTS.

The high incidence of VTS means that this possibility must not be neglected in the case of positive NIPS results. This is particularly important seeing the increased incidence of twin pregnancies in developed countries, which in turn is fundamentally linked to the increased maternal age and the consequent use of ART.⁶² As already mentioned, the twin pregnancy rate is higher in ART pregnancies and consequently, the VTS rate increased too. This seems indirectly confirmed also by the significantly higher maternal age in the case of NIPS with demonstrated VTS.

The weight of VTS on false positives at NIPS is still controversial.

The cumulative risk of false positives at NIPS is 1%.⁶³ The risk of aneuploidy in VTS is about 60%,^{64,65} and about 0.11% of NIPS are calculated to be cases of VTS with chromosomal abnormalities. According to other authors, this estimate is 0.42–0.6%.⁵⁸ However, VTS would appear to be in 42.1% of confirmed false-positive NIPS results.^{58,66}

Furthermore, it is still unclear for how long fetal DNA remains in maternal blood after twin disappearance in case of VTS. This time interval is probably related to the number of placental tissues from the demised twin and the gestational age at the time of disappearance. Some studies about singleton pregnancies have shown that levels of circulating fetal DNA are five times greater at the time of spontaneous abortion as compared to evolutionary pregnancies at the same gestational age.⁶⁷ These values remain increased up to 7 days after diagnosis of abortion. Furthermore, this effect is more pronounced in spontaneous abortions with chromosomal abnormalities than in those with normal karyotype.⁶⁸ Because of this, it is likely that in multiple pregnancies with VTS, NIPS results for the surviving twin are strongly compromised immediately after the disappearance of the vanishing twin. In several studies concerning the impact of VTS on NIPS,^{58,59} fetal DNA circulating from the demised twin was detected up to 8 weeks after disappearance, suggesting that the reliability of NIPS in these cases is long-term compromised as well as maternal serum markers of the first- and second-trimester screening.

The early detection of VTS is undoubtedly important and positive results obtained by non-SNP-based NIPS should be carefully evaluated to avoid anxiety in parents and unnecessary and potentially dangerous invasive diagnostic procedures.

Influence of Vanishing Twin Syndrome on Invasive Prenatal Diagnosis

Vanishing twin syndrome can be a cause of misdiagnosis in chorionic villus sampling (CVS).

The incidence of CVS “false-negative” results is very low, approximately <1%.

Very few studies are reported in literature^{36,69–71} about the association between VTS and misdiagnosis at CVS. They are essentially case reports in which a non-diagnosis of VTS has been ascertained. In these cases, the villous sampling was carried out by residual chorion frondosum coming from twin or from fetal tissues that previously disappeared.

Nowadays, in most cases, the first obstetric ultrasound scan is performed between 6 weeks and 8 weeks so that most twin pregnancies with VTS are identified even when they affect only the gestational sac.

Vanishing twin syndrome tissues may no longer be visible when CVS is usually performed, between 11 weeks and 13 weeks. However, as previously mentioned, these tissues may persist for several weeks after the other twin has vanished and they can be

inadvertently acquired during the CVS procedure and result in a misdiagnosis.

In the case of a dubious result, it is possible to discern VTS from other diagnostic hypotheses by using specific chromosomal markers. In the case of VTS, the possibility that these markers are identical is <25% meanwhile they will be identical if the discrepancy found between karyotypes is due to a poor chromosomal disruption.⁶⁹

Considering that about 70% of twin pregnancies are dizygotic and because of the increase in the incidence of twin pregnancies mentioned above, the possibility of CVS misdiagnosis by VTS is not negligible. Nonetheless, the low series of VTS-related CVS misdiagnosis reported in literature makes this risk reasonably unimportant, but it must be taken into account when a contrasting CVS result occurs.

In 2001, Lloveras et al. published a case report of amniocentesis performed because of maternal age which showed a karyotype 46,XX in 35 cells and a karyotype 48,XY + 8 + 10 in 3 cells. Subsequent ultrasound scan checks throughout all pregnancy resulted in a normal and phenotypically female fetus, which was then confirmed after birth. The small percentage of Y chromosome found at amniocentesis, once excluded technical errors such as maternal contamination and exchange of samples, suggested an early VTS, indirectly supported also by the fact that double trisomies are often aborted at the very early age of the pregnancy.⁷² It is, therefore, necessary to take VTS into account as a possible cause of mosaicism detected in cells sampled in amniotic fluid.

How Early Can the Suspicion of Vanishing Twin Syndrome be Posed?

To find an answer to this question some authors evaluate the growth trend of β -hCG, which is classically used to monitor the progression of early pregnancy, to identify as early as possible couples to be directed toward counseling and appropriate clinical options. A 53% increase of β -hCG in 48 hours was accepted as the minimum increase to identify a physiological intrauterine pregnancy, while lower values are indicated as suspected of an ectopic or non-evolutionary pregnancy. More recent studies suggest that a more stringent cut-off (35%) could minimize errors in the identification of developmental pregnancies.^{73,74}

The study of Kelly et al. found an increase of β -hCG in 40 twin pregnancies dosed at 12 days and 52 days after induced ovulation and 1 and 3 days after these two samples. The β -hCG increase was significantly higher in evolutionary twin pregnancies than in those with VTS.⁷⁵ Brady et al. evaluated the increase in β -hCG dosed at 7 and 22 days after ET and 1 and 7 days after these two samples in all pregnancies obtained from IVF/ICSI in Brigham and Women's Hospital from 1998 to 2010. They found that this increase was significantly lower in pregnancies with VTS when compared to singleton and twin evolutionary pregnancies, and the earlier one of the pregnancies was interrupted, the lower the increase.⁷⁶

Considering that the majority of pregnancies with abnormal growth of β -hCG result in non-evolutionary pregnancy, the possibility of VTS should not be omitted in the evolutionary ones to carry out a possible early diagnosis.

CONCLUSION

To date, the evidence of the short and long-term effects of VTS on the surviving fetus is discordant. The incidence of this event can be

reduced by correcting maternal predisposing factors and reducing the risk of twin pregnancies obtained from ART by the improvement of embryo selection techniques which can allow single ET. Early detection of VTS and monitoring of fetal and placental residues disappearance are crucial for the prognosticating of VTS's possible effects on the surviving twin. The use of noninvasive screening techniques such as maternal serum markers and NIPS remains controversial.

Meticulous ultrasound monitoring of the alive twin for the entire pregnancy is mandatory to identify possible adverse obstetric outcomes.

This paper was previously published in Kurjak A, Chervenak FA. Donald School. Embryo as a Person and as a Patient. New Delhi: Jaypee Brothers Medical Publishers, 2019: 121–130.

REFERENCES

- Levi S. Ultrasonic assessment of the high rate of human multiple pregnancy in the first trimester. *J Clin Ultrasound* 1976;4(1):3–5. DOI: 10.1002/jcu.1870040104.
- La Sala GB, Villani MT, Nicoli A, et al. Effect of the mode of assisted reproductive technology conception on obstetric outcomes for survivors of the vanishing twin syndrome. *Fertil Steril* 2006;86(1):247–249. DOI: 10.1016/j.fertnstert.2005.11.073.
- Sun L, Jiang LX, Chen HZ. Obstetric outcome of vanishing twins syndrome: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2017;295(3):559–567. DOI: 10.1007/s00404-017-4289-9.
- Landy HJ, Keith L. The vanishing twin: a review. *Hum Reprod Update* 1998;4(2):177–183. DOI: 10.1093/humupd/4.2.177.
- Patrizio P, Bianchi V, Lalioti MD, et al. High rate of biological loss in assisted reproduction: it is in the seed, not in the soil. *Reprod Biomed Online* 2007;14(1):92–95. DOI: 10.1016/s1472-6483(10)60769-9.
- Barton SE, Missmer SA, Hornstein MD. Twin pregnancies with a 'vanished' embryo: a higher risk multiple gestation group? *Hum Reprod* 2011;26(10):2750–2753. DOI: 10.1093/humrep/der221.
- Greenwold N, Jauniaux E. Collection of villous tissue under ultrasound guidance to improve the cytogenetic study of early pregnancy failure. *Hum Reprod* 2002;17(2):452–456. DOI: 10.1093/humrep/17.2.452.
- Jauniaux E, Van Oppenraaij RH, Burton GJ. Obstetric outcome after early placental complications. *Curr Opin Obstet Gynecol* 2010;22(6):452–457. DOI: 10.1097/GCO.0b013e3283404e44.
- Oloyede OA, Iketubosin F, Bamgbopa K. Spontaneous foetal reduction and early pregnancy complications in multiple pregnancies following in vitro fertilisation. *Int J Gynaecol Obstet* 2012;119(1):57–60. DOI: 10.1016/j.ijgo.2012.05.021.
- Pinborg A, Wennerholm UB, Romundstad LB, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013;19(2):87–104. DOI: 10.1093/humupd/dms044.
- Rizzo G, Capponi A, Pietrolucci ME, et al. First trimester placental volume and three dimensional power doppler ultrasonography in type I diabetic pregnancies. *Prenat Diagn* 2012;32(5):480–484. DOI: 10.1002/pd.3848.
- Loeken MR. Advances in understanding the molecular causes of diabetes induced birth defects. *J Soc Gynecol Investig* 2006;13(1):2–10. DOI: 10.1016/j.jsig.2005.09.007.
- De Sutter O, Bontnick J, Schutyers V, et al. First trimester bleeding and pregnancy outcome in singletons after assisted reproduction. *Hum Reprod* 2006;21(7):1907–1911. DOI: 10.1093/humrep/del054.
- Jauniaux E, Elkazen N, Leroy F, et al. Clinical and morphological aspects of the vanishing twin phenomenon. *Obstet Gynecol* 1988;72(4):577–581.
- Magnus MC, Ghaderi S, Morken NH, et al. Vanishing twin syndrome among ART singletons and pregnancy outcomes. *Hum Reprod* 2017;32(11):2298–2304. DOI: 10.1093/humrep/dex277.
- Depp R, Macones GA, Rosenn MF, et al. Multifoetal pregnancy reduction: evaluation of foetal growth in the remaining twins. *Am J Obstet Gynecol* 1996;174(4):1233–1238. DOI: 10.1016/s0002-9378(96)70666-x.
- Sampson A, de Crespigny LC. Vanishing twins: the frequency of spontaneous foetal reduction of a twin pregnancy. *Ultrasound Obstet Gynecol* 1992;2(2):107–109. DOI: 10.1046/j.1469-0705.1992.02020107.x.
- Gjerris AC, Tabor A, Loft A, et al. First trimester prenatal screening among women pregnant after IVF/ICSI. *Hum Reprod Update* 2012;18(4):350–359. DOI: 10.1093/humupd/dms010.
- Pinborg A, Lidegaard O, la Cour Freiesleben N, et al. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod* 2005;20(10):2821–2829. DOI: 10.1093/humrep/dei142.
- Kupka MS, D'Hooghe T, Ferraretti AP, et al. European IVF-monitoring consortium (EIM) European Society of Human Reproduction and Embryology (ESHRE). *Hum Reprod* 2016;31(2):233–248. DOI: 10.1093/humrep/dev319.
- Sunderam S, Kissin DM, Crawford SB, et al. Assisted reproductive technology surveillance, United States, 2012. *MMWR Surveill Summ* 2015;64(11):1–29. DOI: 10.15585/mmwr.ss6411a1.
- Glujovsky D, Blake D, Farquhar C, et al. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev* 2012;7:CD002118.
- Glujovsky D, Farquhar C. Cleavage-stage or blastocyst transfer: what are the benefits and harms? *Fertil Steril* 2016;106(2):244–250. DOI: 10.1016/j.fertnstert.2016.06.029.
- Dyer S, Chambers GM, de Mouzon J, et al. International committee for monitoring assisted reproductive technologies world report: assisted reproductive technology 2008, 2009 and 2010. *Hum Reprod* 2016;31(7):1588–1609. DOI: 10.1093/humrep/dew082.
- Rudnicki M, Vejerslev LO, Junge J. The vanishing twin: morphologic and cytogenetic evaluation of an ultrasonographic phenomenon. *Gynecol Obstet Invest* 1991;31(3):141–145. DOI: 10.1159/000293133.
- Fernando D, Halliday JL, Breheny S, et al. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. *Fertil Steril* 2012;97(3):579–584. DOI: 10.1016/j.fertnstert.2011.12.032.
- Pereira N, Pryor KP, Petrini AC, et al. Perinatal risks associated with early vanishing twin syndrome following transfer of cleavage- or blastocyst-stage embryos. *J Pregnancy* 2016;2016:1245210. DOI: 10.1155/2016/1245210.
- Márton V, Zádori J, Kozinszky Z, et al. Prevalences and pregnancy outcome of vanishing twin pregnancies achieved by in vitro fertilization versus natural conception. *Fertil Steril* 2016;106(6):1399–1406. DOI: 10.1016/j.fertnstert.2016.07.1098.
- Jauniaux E, Ben-Ami I, Maymon R. Do assisted-reproduction twin pregnancies require additional antenatal care? *Reprod Biomed Online* 2013;26(2):107–119. DOI: 10.1016/j.rbmo.2012.11.008.
- La Sala GB, Nicoli A, Villani MT, et al. Spontaneous embryonic loss rates in twin and singleton pregnancies after transfer of top-versus intermediate-quality embryos. *Fertil Steril* 2005;84(6):1602–1605. DOI: 10.1016/j.fertnstert.2005.05.045.
- Schieve LA, Meikle SF, Ferre C, et al. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;346(10):731–737. DOI: 10.1056/NEJMoa010806.
- Jauniaux E, Farquharson RG, Christiansen OB, et al. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Hum Reprod* 2006;21(9):2216–2222. DOI: 10.1093/humrep/del150.
- Zhao J, Zhang Q, Li Y. The effect of endometrial thickness and pattern measured by ultrasonography on pregnancy outcomes during IVF-ET cycles. *Reprod Biol Endocrinol* 2012;10(1):100. DOI: 10.1186/1477-7827-10-100.
- Evron E, Sheiner E, Friger M, et al. Vanishing twin syndrome: is it associated with adverse perinatal outcome? *Fertil Steril* 2015;103(5):1209–1214. DOI: 10.1016/j.fertnstert.2015.02.009.

35. Almog B, Levin I, Wagman I, et al. Adverse obstetric outcome for the vanishing twin syndrome. *Reprod Biomed Online* 2010;20(2):256–260. DOI: 10.1016/j.rbmo.2009.11.015.
36. Shebl O, Ebner T, Sommergruber M, et al. Birth weight is lower for survivors of the vanishing twin syndrome: a case-control study. *Fertil Steril* 2008;90(2):310–314. DOI: 10.1016/j.fertnstert.2007.06.048.
37. Mansour R, Serour G, Aboulghar M, et al. The impact of vanishing fetuses on the outcome of ICSI pregnancies. *Fertil Steril* 2010;94(6):2430–2432. DOI: 10.1016/j.fertnstert.2010.02.058.
38. Sun L, Chen Z, Liu J, et al. Obstetric and neonatal outcomes of vanishing twin syndrome. *Nan Fang Yi Ke Da Xue Xue Bao* 2014;34(10):1537–1540.
39. Tabanelli C, Ferraretti AP, Feliciani E, et al. Vanishing twins in IVF/ICSI pregnancies: a case-control study. *Mol Hum Reprod* 2009;24:i130–i131.
40. Zhou L, Gao X, Wu Y, et al. Analysis of pregnancy outcomes for survivors of the vanishing twin syndrome after in vitro fertilization and embryo transfer. *Eur J Obstet Gynecol Reprod Biol* 2016;203:35–39. DOI: 10.1016/j.ejogrb.2016.04.014.
41. Frisell T, Oberg S, Kuja-Halkola R, et al. Sibling comparison designs: bias from non-shared confounders and measurement error. *Epidemiology* 2012;23(5):713–720. DOI: 10.1097/EDE.0b013e31825fa230.
42. Oepkes D, Suetters M. Antenatal fetal surveillance in multiple pregnancies. *Best Pract Res Clin Obstet Gynaecol* 2017;38:59–70. DOI: 10.1016/j.bpobgyn.2016.09.004.
43. Van Oppenraaij RH, Jauniaux E, Christiansen OB, et al. ESHRE special interest group for early pregnancy (SIGEP). Predicting adverse obstetric outcome after early pregnancy events and complications: a review. *Hum Reprod Update* 2009;15(4):409–421. DOI: 10.1093/humupd/dmp009.
44. Chasen ST, Luo G, Perni SC, et al. Are in vitro fertilization pregnancies with early spontaneous reduction high risk? *Am J Obstet Gynecol* 2006;195(3):814–817. DOI: 10.1016/j.ajog.2006.06.022.
45. Murray E, Fernandes M, Fazel M, et al. Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. *BJOG* 2015;122(8):1062–1072. DOI: 10.1111/1471-0528.13435.
46. Bass NH, Oliver JB, Srinivasan M. Persistently elevated AFP and AChE in amniotic fluid from a normal fetus following the demise of its twin. *Prenat Diagn* 1986;6(1):33–35. DOI: 10.1002/pd.1970060105.
47. Pinborg A, Lidsgaard O, Freisleben N, et al. Vanishing twins: a predictor of small-for-gestational age in IVF singletons. *Hum Reprod* 2007;22(10):2707–2714. DOI: 10.1093/humrep/dem225.
48. Shek NW, Hillman SC, Kilby MD. Single-twin demise: pregnancy outcome. *Best Pract Res Clin Obstet Gynaecol* 2014;28(2):249–263. DOI: 10.1016/j.bpobgyn.2013.11.003.
49. Hillman SC, Morris RK, Kilby MD. Co-twin prognosis after single fetal death: a systematic review and meta-analysis. *Obstet Gynecol* 2011;118(4):928–940. DOI: 10.1097/AOG.0b013e31822f129d.
50. Ong SS, Zamora J, Khan KS, et al. Prognosis for the co-twin following single-twin death: a systematic review. *BJOG* 2006;113(9):992–998. DOI: 10.1111/j.1471-0528.2006.01027.x.
51. Dällenbach P, Pelte MF, Irion O. Life-threatening third-trimester hemorrhage following a vanishing twin phenomenon in early pregnancy. *Ultrasound Obstet Gynecol* 2005;26(2):196–197. DOI: 10.1002/uog.1946discussion 197.
52. Huang T, Boucher K, Aul R, et al. First and second trimester maternal serum markers in pregnancies with a vanishing twin. *Prenat Diagn* 2015;35(1):90–96. DOI: 10.1002/pd.4492.
53. Spencer K, Staboulidou I, Nicolaides KH. First trimester aneuploidy screening in the presence of a vanishing twin: implications for maternal serum markers. *Prenat Diagn* 2010;30(3):235–240. DOI: 10.1002/pd.2445.
54. Chasen ST, Perni SC, Predanic M, et al. Does a “vanishing twin” affect first-trimester biochemistry in Down syndrome risk assessment? *Am J Obstet Gynecol* 2006;195(1):236–239. DOI: 10.1016/j.ajog.2006.01.044.
55. Gjerris AC, Loft A, Pinborg A, et al. The effect of a ‘vanishing twin’ on biochemical and ultrasound first trimester screening markers for Down’s syndrome in pregnancies conceived by assisted reproductive technology. *Hum Reprod* 2009;24(1):55–62. DOI: 10.1093/humrep/den362.
56. Abbas A, Sebire NJ, Johnson M, et al. Maternal serum concentrations of pregnancy associated placental protein A and pregnancy specific B1 glycoprotein in multifetal pregnancies before and after fetal reduction. *Hum Reprod* 1996;11(4):900–902. DOI: 10.1093/oxfordjournals.humrep.a019275.
57. Sankaran S, Rozette C, Dean J, et al. Screening in the presence of a vanished twin: nuchal translucency or combined screening test? *Prenat Diagn* 2011;31(6):600–601. DOI: 10.1002/pd.2735.
58. Curnow KJ, Wilkins-Haug L, Ryan A, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. *Am J Obstet Gynecol* 2015;212(1):79.e1–9. DOI: 10.1016/j.ajog.2014.10.012.
59. Masala M, Saba L, Zoppi MA, et al. Pitfalls in noninvasive fetal RhD and sex determination due to a vanishing twin. *Prenat Diagn* 2015;35(5):506–508. DOI: 10.1002/pd.4489.
60. American College of Obstetricians and Gynecologists. Invasive prenatal testing for aneuploidy. ACOG practice bulletin no. 88. *Obstet Gynecol* 2007;110(6):1459–1467. DOI: 10.1097/01.AOG.0000291570.63450.44.
61. Manzur A, Goldsman MP, Stone SC, et al. Outcome of triplet pregnancies after assisted reproductive techniques: how frequent are the vanishing embryos? *Fertil Steril* 1995;63(2):252–257. DOI: 10.1016/s0015-0282(16)57350-6.
62. Centers for Disease Control and Prevention ASFRM, Technology SFRM. 2010 Assisted reproductive technology national summary report. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention 2012.
63. Niles KM, Murji A, Chitayat D. Prolonged duration of persistent cell free fetal DNA from a vanishing twin. *Ultrasound Obstet Gynecol* 2018(4). DOI: 10.1002/uog.19004.
64. Lathi RB, Gustin SL, Keller J, et al. Reliability of 46, XX results on miscarriage specimens: a review of 1,222 first-trimester miscarriage specimens. *Fertil Steril* 2013;101(1):178–182. DOI: 10.1016/j.fertnstert.2013.09.031.
65. Levy B, Sigurjonsson S, Pettersen B, et al. Genomic imbalance in products of conception: single nucleotide polymorphism chromosomal microarray analysis. *Obstet Gynecol* 2014;124(2 Pt 1):202–209. DOI: 10.1097/AOG.0000000000000325.
66. Hartwig TS, Ambye L, Sorensen S, et al. Discordant noninvasive prenatal testing (NIPT) - a systematic review. *Prenat Diagn* 2017;37(6):527–539. DOI: 10.1002/pd.5049.
67. Yin A, Ng EH, Zhang X, et al. Correlation of maternal plasma total cell-free DNA and fetal DNA levels with short term outcome of first-trimester vaginal bleeding. *Hum Reprod* 2007;22(6):1736–1743. DOI: 10.1093/humrep/dem058.
68. Lim JH, Kim MH, Han YJ, et al. Cell-free fetal DNA and cell-free total DNA levels in spontaneous abortion with fetal chromosomal aneuploidy. *PLoS ONE* 2013;8(2):e56787. DOI: 10.1371/journal.pone.0056787.
69. Reddy KS, Petersen MB, Antonarakis SE, et al. The vanishing twin: an explanation for discordance between chorionic villus karyotype and fetal phenotype. *Pren Diagn* 1991;1(9):679–684. DOI: 10.1002/pd.1970110903.
70. Tharapel AT, Elias S, Shulamm LP, et al. Resorbed co-twin as an explanation for discrepant chorionic villus results: non-mosaic 47,XX, + 16 in villi (direct and culture) with normal (46,XX) amniotic fluid and neonatal blood. *Prenat Diagn* 1989;9(7):467472. DOI: 10.1002/pd.1970090703.
71. Ledbetter DH, Martin AO, Verlinsky Y, et al. Cytogenetic results of chorionic villus sampling: high success rate and diagnostic accuracy in the United States collaborative study. *Am J Obstet Gynecol* 1990;146(2):495–501. DOI: 10.1016/0002-9378(90)90419-8.
72. Lloveras E, Lecumberri JM, Pérez C, et al. A female infant with a 46,XX/48,XY+8+10 karyotype in prenatal diagnosis: a ‘vanishing

- twin' phenomenon? *Prenat Diagn* 2001;21(10):896–897. DOI: 10.1002/pd.174.
73. Barnhart KT, Sammel MD, Rinaudo PF, et al. Symptomatic patients with an early viable intrauterine pregnancy: HCG curves redefined. *Obstet Gynecol* 2004;104(1):50–55. DOI: 10.1097/01.AOG.0000128174.48843.12.
 74. Morse CB, Sammel MD, Shaunik A, et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: exceptions to the rules. *Fertil Steril* 2012;97(1):101–106. DOI: 10.1016/j.fertnstert.2011.10.037.
 75. Kelly MP, Molo MW, Maclin VM, et al. Human chorionic gonadotropin rise in normal and vanishing twin pregnancies. *Fertil Steril* 1991;56(2):221–224. DOI: 10.1016/s0015-0282(16)54475-6.
 76. Brady PC, Correia KF, Missmer SA, et al. Early beta-human chorionic gonadotropin trends in vanishing twin pregnancies. *Fertil Steril* 2013;100(1):116–121. DOI: 10.1016/j.fertnstert.2013.02.057.