

A Retrospective Analysis of Placentas From Twin Pregnancies Derived From Assisted Reproductive Technology

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There are conflicting studies associating twin pregnancies derived from assisted reproductive technology (ART) with preterm birth, low birthweight, and other negative outcomes. This work investigates whether ART is linked with any placental pathology, given that placentation significantly influences fetal development. A 5-year, retrospective cohort study was conducted on placentas from twin pregnancies. The placental information from 417 patients was divided into two groups: placentas derived from ART and placentas derived from spontaneous pregnancies (non-ART). Available clinical information and pathologic findings from both groups then were compared. There was no statistical difference in the prevalence of placental pathology between the non-ART and ART cohorts (i.e., cord insertion, single umbilical artery, cord knot, retroplacental hemorrhage, infarction, vasculopathy, vascular anastomoses, chorangiosis, villitis, deciduitis, chorioamnionitis, meconium staining). However, 8% of ART multiple pregnancies were monochorionic. While monochorionicity is a known risk factor for adverse obstetric and neonatal outcomes, the rate of monochorionic placentation did not increase as a result of ART. Nevertheless, it is interesting to note that this small percentage of monochorionic placentation occurred in the ART cohort despite the implantation of individual embryos. Overall, the data suggests that ART does not have a role in the pathologic placentation of twin pregnancies.

More than a quarter of a century has passed since the first successful in vitro fertilization-derived pregnancy. Since 1978, the number of patients undergoing treatment for infertility by assisted reproductive technology (ART) has steadily increased. In the United States, 115,392 ART procedures were reported to the Centers for Disease Control and Prevention in 2002 (Wright et al., 2005). In 2003, this number rose to 122,872 ART procedures resulting in 48,756 infants or approximately 1% of all infants born in the United States (Wright et al., 2006).

Despite wide usage, ART carries the risk of multiple gestations. Of the live-born infants conceived from ART in 2003, 45% were twins; and 6% were triplets or higher-order deliveries (Wright et al., 2006). Moreover, 16% of all twin births in that year were from ART.

It is recognized that multiple gestations are associated with increased morbidity and mortality when compared to singleton births (Kato & Matsuda, 2006; Norwitz et al., 2005; Rodrigues et al., 2005; Scholz et al., 1999). However, given the connection between ART and multiple births, the role that ART has in the development of such complications is controversial. For example, some investigators have linked ART-derived twin pregnancies with greater incidences of preterm birth, delivery by cesarean section, low birthweight, and respiratory complications when compared to spontaneous twin pregnancies (Liang et al., 2002; McDonald et al., 2005; Narine et al., 2003; Nassar et al., 2003; Wang et al., 2005). Conversely, there are other groups that do not associate ART with such negative outcomes (Bernasko et al., 1997; Fitzsimmons et al., 1998; Minakami et al., 1998; Zaib-un-Nisa et al., 2003). In light of these contrasting studies, the effect of ART on twin morbidity and mortality remains equivocal, except for the known risk of prematurity (Bergh et al., 1999; Nassar et al., 2003).

The placentation of twins is important in affecting fetal development and subsequent neonatal outcome (Benirschke, 1995). For example, chorionic development has bearing on fetal morbidity and mortality (Ferreira et al., 2005; Hatkar & Bhide, 1999). Monochorionic placentas, compared to dichorionic placentas, have been associated with preterm birth (Hatkar & Bhide, 1999; Leduc et al., 2005; Penava & Natale, 2004), low birthweight (Leduc et al., 2005),

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Table 1

Comparison of Maternal and Neonatal Characteristics

	Non-ART		ART		<i>p</i> value
		<i>N</i>		<i>N</i>	
Diamniotic, dichorionic					
Maternal age (years)	30 (6)	225	35 (6)	70	< .0001
Gravidity	3 (2)	208	3 (2)	68	.4582
Parity	1 (1)	208	1 (1)	68	.1846
Gestational age (weeks)	34 (4)	225	34 (4)	69	.4127
Total neonatal weight (kg)	4.4 (1.5)	174	4.4 (1.2)	56	.7280
Diamniotic, monochorionic					
Maternal age (years)	30 (6)	104	35 (6)	5	.0529
Gravidity	3 (1)	87	3 (2)	5	.6009
Parity	1 (1)	86	0 (0)	5	.0542
Gestational age (weeks)	32 (5)	97	29 (8)	5	.2699
Total neonatal weight (kg)	3.5 (1.5)	67	3.3 (2.2)	3	.7280

Note: The available clinical data from the ART and non-ART cohorts is listed with their samples sizes. Values in parentheses are standard deviations. The total neonatal weight is the sum of both twin birthweights.

ART = assisted reproductive technology, *N* = sample size.

p values were calculated between non-ART and ART-derived pregnancies using an unpaired two-tailed *t* test.

fetal entanglement of umbilical cords (Nyberg et al., 1984), fetal thrombosis (Sato & Benirschke, 2006), neuromorbidity (Adegbite et al., 2004), and the twin-twin transfusion syndrome (Galea et al., 2005).

The aim of this study is to identify whether ART affects the placentation of twin pregnancies, which consequently would influence neonatal outcome. A 5-year, retrospective analysis was conducted on twin placentas to investigate whether ART has an effect on placental pathology. Additionally, the prevalence of monozygosity in the ART cohort was compared to the non-ART cohort.

Materials and Methods

Placentas

A database search was conducted for all twin placental examinations from January 2000 to August 2005 at the University of California, San Diego Medical Center. A

total of 417 placentas from twin deliveries were identified ($n = 341$ spontaneous twin pregnancies, $n = 76$ twin pregnancies by assisted reproductive technology). Other clinical information (i.e., maternal age, gravidity, parity, gestational age, neonatal birthweight, infant sex) obtained from the medical records was recorded when available. All placentas were reviewed and diagnosed by the same pathologist (K.B.), and pathologic data was obtained grossly and microscopically. The following data were recorded for each placenta: amnionicity, chorionicity, placental weight, umbilical cord length, umbilical cord insertion, single umbilical artery, umbilical cord knot, maternal surface calcification, retroplacental hemorrhage, infarction, vasculopathy, vascular anastomoses, chorangioma, chorangiomatosis, chorangioma, increased syncytial knots, villitis, deciduitis, chorioamnionitis, and meconium-staining. Patient identities were not divulged, and confidentiality was respected at all times. The protocol was approved by the University of California, San Diego Human Research Protections Program (Project #051036X).

Assisted Reproductive Technology

ART represents any therapeutic, laboratory-based manipulation of sperm and eggs to promote a pregnancy. A placental examination was placed into the ART cohort if the pregnancy was facilitated by any ART procedures as recorded in the patient's medical records (i.e., in vitro fertilization, intracytoplasmic sperm injection, 'assisted reproductive technology'). Pregnancies from ovulation induction or ovarian hyperstimulation without a subsequent in vitro procedure were placed into the non-ART cohort when this information was available in the patient data. Of note, in order to facilitate egg retrieval, mothers

Table 2

Comparison of Placental Amnionicity and Chorionicity

	Non-ART (<i>N</i> = 341)		ART (<i>N</i> = 76)	
	#	%	#	%
Diamniotic, dichorionic	225	66	70	92
Diamniotic, monochorionic	104	30	5	7
Monoamniotic, monochorionic	12	4	1	1

Note: *p* value < .0001

Amnionicity and chorionicity were determined histologically.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, *N* = sample size of twins, % = percentage of total placentas.

The *p* value was calculated between non-ART and ART-derived pregnancies using a two-sided chi-square test.

Table 3

Calculation of Monozygosity via the Weinberg Method

	Non-ART (N = 298)		ART (N = 71)	
	#	%	#	%
Like-sex twins	222	74	42	59
Unlike-sex twins	76	26	29	41
Monozygotic twins	146	49	13	18

Note: *p* value = .01

Weinberg method: number of monozygotic twins = number of like-sex twins – number of unlike-sex twins.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, N = sample size of twins with available clinical data, % = percentage of total number of twins (rounded to nearest whole number).

The *p* value was calculated between non-ART and ART-derived pregnancies using a two-sided chi-square test.

with ART-related pregnancies likely received ovulation induction or ovarian hyperstimulation prior to in vitro manipulation of sperm and eggs.

Computer and Statistical Analysis

The pathologic diagnoses and clinical information were catalogued and analyzed using Microsoft Access (Microsoft Corporation, 2003; professional edition 2003). Two-sided chi-square tests and unpaired, two-tailed *t* tests were calculated using GraphPad Prism (GraphPad Software, 2005; version 4.00). *p* values less than or equal to .05 were considered statistically significant.

Results

At the University of California, San Diego Medical Center from January 2000 to August 2005, there were 417 placental examinations of twin deliveries. These were divided into two cohorts: placentas that were from spontaneous pregnancies (non-ART) and those that were derived from ART. The maternal and neonatal data of the two groups were summarized in Table 1. The mothers from the ART cohort tended to be older than the mothers from the non-ART cohort (statistical

significance achieved in the diamnionic, dichorionic group). All other maternal and neonatal characteristics (gravidity, parity, gestational age, total neonatal weight) did not show any statistical differences.

Placental amnionicity and chorionicity were varied between the non-ART and ART cohorts (see Table 2). For both groups, the majority of placentas were dichorionic. Interestingly, a small subset of the placentas from the ART cohort was monochorionic (8%) despite the implantation of individual embryos by ART.

Since ART has been associated with monozygotic twinning (Schachter et al., 2001), the prevalence of monozygotic twins was calculated for our ART and non-ART cohorts using the Weinberg method (Weinberg, 1901). With this method, the occurrence of male fetuses is assumed to be equal to that of female fetuses for dizygotic twinning. Then, any remaining increase in like-sex pairs would be due to monozygotic twins. Therefore, according to Weinberg, the number of monozygotic twins is equal to the difference between the numbers of like-sex pairs and unlike-sex pairs. Application of the Weinberg method to the ART cohort of this study yielded a monozygotic twin prevalence of 18% (see Table 3). The monozygotic prevalence in the non-ART cohort was 49%. Although less frequent than in naturally occurring twins, monozygosity is present in a large number of ART-derived twins.

Between the non-ART and the ART-derived placentas, there were no differences in placental weight (see Table 4), umbilical cord length (see Table 4), or cord insertion type (see Table 5) for both diamnionic, dichorionic (DiDi) and diamnionic, monochorionic (DiMo) placentas. Comparisons of the pathologic characteristics of the placentas were also conducted. In general, there was no difference in the prevalence of placental pathology between the non-ART and ART groups. These findings are summarized for both DiDi (see Table 6) and DiMo (see Table 7) placentas. Curiously, the only statistical difference between the non-ART and ART groups was

Table 4

Comparison of Placental Weights and Cord Lengths

	Non-ART		ART		<i>p</i> value
	<i>N</i>		<i>N</i>		
Diamnionic, dichorionic					
Placental weight (g)	651 (213)	221	673 (187)	70	.4376
Cord length (cm)	63 (22)	226	61 (21)	70	.5646
Diamnionic, monochorionic					
Placental weight (g)	618 (219)	102	511 (210)	4	.3428
Cord length (cm)	55 (21)	101	50 (32)	4	.5956

Note: Standard deviations are in parentheses.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, *N* = sample size of twins with available clinical data.*p* values were calculated between non-ART and ART-derived pregnancies using an unpaired two-tailed *t* test.

observed for maternal surface calcification (see Table 6). ART placentas had approximately half the prevalence of calcification than spontaneous pregnancies, and this difference was observed only in DiDi placentas.

Discussion

With the widespread use of ART and the high risk of multiple gestations that it carries, this retrospective cohort study investigates whether ART has an effect on twin placentation since placentation has significant bearing on fetal maturation and neonatal outcome (Benirschke, 1995). Indeed, compared to the non-ART placentas of this study, ART affected the proportion of placental amnionicity and chorionicity, where diamnionic, dichorionic placentas comprised almost all of the ART cohort (see Table 2). This may not entirely be surprising since in ART, there are multiple, separate embryos that are being transferred, which would develop presumably into fused or separate dichorionic placentas of dizygous twins. However, what is potentially remarkable is the occurrence of a small percentage of monochorionic placentas in the ART group (total of 8%, see Table 2). Other investigators have identified similar findings; Narine et al. (2003) reported 25% monochorionic placentation in their ART cohort of 20 twin pairs.

Two interpretations may be made from this observation. First, there may be a low level of monozygous twinning with ART to yield monochorionic placentas,

Table 5
Comparison of Umbilical Cord Insertion Types

	Non-ART		ART	
	<i>N</i>	%	<i>N</i>	%
Diamnionic, dichorionic				
Cord insertion				
Central	54	13	18	14
Eccentric	277	64	71	55
Marginal	59	14	23	18
Velamentous	42	10	17	13
Furcate	1	0.2	1	0.8
<i>p</i> value = .3305				
Diamnionic, monochorionic				
Cord insertion				
Central	22	11	1	14
Eccentric	87	45	4	57
Marginal	51	26	1	14
Velamentous	35	18	1	14
Furcate	0	0	0	0
<i>p</i> value = .8710				

Note: Individual umbilical cords for each placenta were enumerated.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, *N* = sample size of individual placentas, % = percentage of total umbilical cords.

p values were calculated between non-ART and ART-derived pregnancies using a two-sided chi-square test.

Table 6
Comparison of Pathologic Characteristics: Diamnionic, Dichorionic Placentas

	Non-ART (<i>N</i> = 226)		ART (<i>N</i> = 70)		<i>p</i> value
	#	%	#	%	
Single umbilical artery	6	1.3	1	0.7	.5576
Umbilical cord knot	2	0.4	1	0.7	.6923
Maternal surface calcification	186	41	31	22	< .0001
Retroplacental hemorrhage	14	3.1	5	3.6	.7809
Infarction	131	29	41	29	.9949
Vasculopathy	11	4.9	5	7.1	.4619
Vascular anastomoses	2	0.9	0	0	.4297
Chorangiosis	1	0.4	1	1.4	.3789
Chorangiomas	0	0	0	0	> .05
Chorangioma	1	0.4	0	0	.5772
Increased syncytial knots	12	2.7	2	1.4	.4041
Villitis	5	2.2	2	2.9	.7564
Deciduitis	5	2.2	1	1.4	.6843
Chorioamnionitis/meconium	11	4.9	1	1.4	.2024

Note: Vasculopathy includes atherosclerosis and thrombosis.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, *N* = sample size of twins, # = number of instances (single umbilical artery, umbilical cord knot, calcification, retroplacental hemorrhage, infarction, and increased syncytial knots are counted per individual placenta. The remaining pathologic conditions are counted per twin placenta.), % = percentage of total.

p values were calculated between non-ART and ART-derived pregnancies using a two-sided chi-square test.

Table 7

Comparison of Pathologic Characteristics: Diamniotic, Monochorionic Placentas

	Non-ART (N = 104)		ART (N = 5)		p value
	#	%	#	%	
Single umbilical artery	6	2.9	1	10	.2125
Umbilical cord knot	0	0	0	0	> .05
Maternal surface calcification	63	30	1	10	.1688
Retroplacental hemorrhage	11	5.3	0	0	.4555
Infarction	51	25	0	0	.0735
Vasculopathy	16	15	0	0	.3424
Vascular anastomoses	75	72	4	80	.6998
Chorangiosis	6	5.8	0	0	.5806
Chorangiomas	0	0	0	0	> .05
Chorangioma	1	1	0	0	.8257
Increased syncytial knots	12	5.8	0	0	.4346
Villitis	7	6.7	1	17	.3621
Deciduitis	8	7.7	1	20	.3287
Chorioamnionitis/meconium	4	3.8	1	20	.0917

Note: Vasculopathy includes atherosclerosis and thrombosis.

Non-ART = spontaneous pregnancies, ART = assisted reproductive technology-derived pregnancies, N = sample size of twins, # = number of instances (single umbilical artery, umbilical cord knot, calcification, retroplacental hemorrhage, infarction, and increased syncytial knots are counted per individual placenta. The remaining pathologic conditions are counted per twin placenta.), % = percentage of total.

p values were calculated between non-ART and ART-derived pregnancies with a two-sided chi-square test.

despite the placement of individual, distinct embryos. A second interpretation is that these monochorionic placentas result from the fusing of dizygous twins, which is an extremely rare phenomenon that has been recently reported in ART-derived twins (Miura & Niikawa, 2005; Souter et al., 2003).

Regarding the first interpretation, some investigators have reported that ART promotes monozygous twinning (Alikani et al., 2003; Behr et al., 2000; Blickstein et al., 1999; Derom et al., 2001; Milki et al., 2003; Schachter et al., 2001), reporting values roughly two to 12 times the expected 0.4% monozygous twinning rate of all births from the general population (Bulmer, 1970). To explain the role of ART in monozygous twinning, various mechanistic hypotheses have been put forth, such as oral contraceptive use (Bressers et al., 1987; Macourt et al., 1982; Rothman, 1977), gonadotrophin therapy for ovarian hyperstimulation (Schachter et al., 2001), calcium reduction (Steinman, 2002; Steinman & Valderrama, 2001), zona pellucida micromanipulation (Alikani et al., 1994; Saito et al., 2000; Schieve et al., 2000; Slotnick & Ortega, 1996), blastocyst embryo transfer (Behr et al., 2000; da Costa et al., 2001; Milki et al., 2003), and the transfer of a high number of embryos (Sills et al., 2000). A definitive mechanism has not been elucidated, and it remains an area of active discussion.

In the current study, to examine the issue of monozygous twinning with ART, monozygosity was first estimated using the Weinberg method (Weinberg, 1901), a process corroborated by Husby et al. (1991)

and Vlietinck et al. (1988). As determined by this method, monozygous twinning did occur in the ART cohort (see Table 3) despite the transfer of distinct, individual embryos.

While our data supports the occurrence of monozygous twinning with ART, it does not demonstrate that this rate is greater than the spontaneous rate. Table 3 indicates that the frequency of monozygous twinning in the spontaneous, non-ART cohort was greater than that of the ART group. Of note, in the non-ART cohort, monozygous twins comprised 49% of the births (see Table 3), which is higher than the 1/3 monozygous, 2/3 dizygous proportion that has been reported for natural twin births (Moore & Persaud, 1998; Thompson et al., 1991). Thus, as a caveat for our data, this increased monozygous twinning in the current report may be due to a selection bias at our tertiary care center, where there may be more higher risk, monochorionic/monozygous twin deliveries due to referrals of such patients.

Regarding the second, previously discussed interpretation of monochorionic placentation in our ART cohort, it is possible that the twins of the monochorionic placentas (see Table 2) are actually dizygous instead of monozygous. Previously, it was thought that monochorionic twins were exclusively monozygous. However, Souter et al. (2003) described sex-discordant (therefore dizygous), monochorionic twins conceived by in vitro fertilization. Other investigators soon reported cases of dizygous, monochorionic twins; and some of the births also were derived from assisted reproductive technology

(Ginsberg et al., 2005; Miura & Niikawa, 2005; Williams et al., 2004; Yoon et al., 2005).

Given the potential for dizygous, monozygotic twinning with ART, monozygosity may not be a fail-safe method to assess the frequency of monozygosity as done in Table 3. Therefore, with this idea in mind, the 8% of monozygotic placentas observed in the ART cohort (see Table 2) actually may be monozygous and/or dizygous. Of note, all of these monozygotic twins (see Table 2) were of similar sex (data not shown); therefore, dizygosity could not be determined by gender. Additionally, cytogenetic information (i.e., microsatellite markers) to assess zygosity for these twins, unfortunately, was not available. Therefore, due to the rare possibility that monozygotic twins may be dizygous, the data suggests, but does not prove, that ART is associated with some monozygous twinning.

While the role of ART in monozygous twinning still requires analysis, this study does characterize other features of the ART and non-ART placentas. ART did not significantly alter average placental weights or umbilical cord lengths in our cohort (see Table 4). Additionally, there was no statistically different effect on umbilical cord insertion type (Table 5) and no change in the rate of significant placental pathology (see Tables 6 and 7).

Only for the DiDi placentas did the prevalence of maternal surface calcification differ between the ART and non-ART groups (see Table 6). Calcification has been suggested to be associated with growth restriction and pregnancy-induced hypertension (McKenna et al., 2003, 2005). Interestingly, in our study, the proportion of placentas with maternal surface calcification in the ART group was lower than the non-ART group. Therefore, the data still supports the idea that ART does not promote placental pathology when compared to non-ART placentas.

We find similar results with previous studies describing the lack of disparity in placental features between ART and non-ART pregnancies. Gavriil et al. (1993) observed no significant difference in fetal weight or cord insertion after examining 75 ART and non-ART twin pregnancies. Daniel et al. (2001) examined 105 ART and non-ART twin placentas and also found no difference in cord insertion type. This group also noted that ART-derived placentas had no difference in the rate of chorioamnionitis, increased syncytial knots, or villous lesions when compared to non-ART placentas.

However, prior studies also have demonstrated contrasting results between the two cohorts. In the previously mentioned study by Daniel et al. (2001), ART-derived placentas weighed less (approximately 30–50 grams less) and had more infarcts (approximately 10–16% more). However, in our study, we could not corroborate this finding. Interestingly, dissimilar results were not only observed in twin studies. For singleton pregnancies, analyses comparing ART

and non-ART-derived placentas also show some variability in results (Daniel et al., 1999; Englert et al., 1987; Gavriil et al., 1993; Lalosevic et al., 2003). The reasons for the differences between these individual studies are not clear; however, it was our hope to add our institutional experience, sample size, and comprehensive placental examination to the literature for others to analyze.

From the results of the current investigation, ART does not increase pathology in the placenta. Therefore, any role that ART may have in twin neonatal outcome does not appear to derive from placentation. The number of women who will experience infertility by the year 2025 is projected to be 5.4 to 7.7 million (Stephen & Chandra, 1998). Likewise, the number of ART procedures surely will be commensurate with infertility. This study has examined the role of ART in abnormal twin placentation and found no difference in the prevalence of pathologic features. For future work in this area, we recommend that investigators examine whether differences in placental pathology are noted with particular ART techniques. Additionally, having accurate information of zygosity in monozygotic placentas (i.e., confirmation with microsatellite markers ascertained from fibrous tissue) also would prove beneficial to assess if ART is linked with monozygous twinning.

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