Overrepresentation of pregnancies conceived by artificial reproductive technology in prenatally identified fetuses with Beckwith-Wiedemann syndrome

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Abstract

Objectives In vitro fertilization (IVF) has been linked to an increased risk for imprinting disorders in offspring. The data so far have predominantly been retrospective, comparing the rate of IVF conceptions in affected patients with controls. We describe a series of fetuses with omphalocele that were tested for Beckwith-Wiedemann syndrome (BWS) and subsequently ascertained as to whether pregnancies were conceived by assisted reproductive technologies (ART).

Methods Fetuses were tested for BWS by Southern blot, PCR based methods, and methylation analysis to identify the imprinting status at primarily the IC2 locus, KCNQ1OT1, as well as IC1, H19/IGF-2. Some fetuses were also tested for uniparental disomy of chromosome 11p.

Results We tested 301 fetuses with omphalocele for BWS. Forty samples were positive. Sixteen were from IVF pregnancies, for an overall rate of 40%. Such a high proportion of IVF pregnancies in a series of BWS-positive fetuses has not been described previously. Possible factors such as twinning and ascertainment bias are discussed.

Conclusion We found about a 20-fold overrepresentation of IVF cases in fetuses with BWS/omphalocele when compared with the rate of ART pregnancies in the USA ($p < .0001$). Our series provides support for an association of IVF and BWS. Patients should be counseled about these risks and made aware of the availability of prenatal diagnosis for detection.

Keywords Omphalocele · Beckwith-Wiedemann syndrome · Methylation · Imprinting · IVF

Introduction

In the USA, approximately 1–3% of births involve the use of assisted reproductive technology (ART). In 2002, Cox et al. [1] reported two Angelman syndrome (AS) patients from their

clinic who happened to be born of pregnancies conceived by artificial reproductive techniques (ART). Both were positive for abnormal SNRPN methylation [2] and did not have chromosome 15q11 deletions or maternal uniparental disomy (UPD) of chromosome 15q (presumed imprinting error). Both were born following intracytoplasmic sperm injection (ICSI) due to oligospermia and poor motility.

Following this report, it has been postulated that imprinting disorders may be more prevalent in children born after ART which is biologically plausible as in the mouse model embryo culture media has been shown to affect gene imprinting [2–4]. To date, there have been numerous observations suggesting an association between ART and Beckwith-Wiedemann syndrome (BWS) as well as AS. Orstavik et al. [3] also observed a case of Angelman syndrome associated with ART, specifically ICSI, with laboratory identical to what Cox et al. had reported. ICSI was done following failed traditional in vitro fertilization (IVF) though neither parent had obvious causes for infertility.

The work reported here is the result of routine provision of medical attention at the standard of care, with data collected solely for patient care purposes. No additional data were collected or medical intervention undertaken for the purposes of this study. As such, a Research Involving Human Subjects Committee was not convened.

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To further evaluate a possible association of ART with an imprinting disorder, DeBaun et al. [4] in 2003 studied another imprinting syndrome, BWS, by inquiring their BWS patient registries located in two centers. In one center, they found 7 cases, five conceived by ICSI. Of these, 6 were tested by molecular means, and 4 had alterations of IC1, 1 of both IC1 and IC2, and one tested normal [5]. For four of the cases from one registry, the denominator was unknown. From the other center, 3 of 65 (4.6%) cases in their registry were conceived by ART. The population frequency of ART at that time was 0.76%, so the increased risk was approximately 6-fold.

Maier et al. [6] studied their BWS clinic patients from 1995 to 2000 ($n = 149$). They found that 6 of the 149 were conceived by ART: 3 by standard IVF and 3 by ICSI. During the study period, there were 43,037 births in the area, about 1% conceived by ART. From this, they would have expected only 1.7 cases of ART in the 149 BWS patients. The difference was significant by Poisson distribution, $p = .018$, with an estimated increased risk of BWS following IRT of 1.5- to 8.8-fold.

Similarly, Giquel et al. [7] examined their BWS patient cohort of 149 and found 6 who were conceived by ART, with 1.3% being the population incidence at the time. By their calculations, they would have expected 1.54 BWS patients with a history of ART, and the difference from the expectation was significant at .018. The odds ratio from the study predicts a 3.2-fold increase in BWS following ART.

Population surveys followed these anecdotal and small BWS patient series. Halliday et al. [8] studied all births in Australia between 1983 and 2003. There were 1,316,500 births in this period, with 37 babies having BWS. This yielded a population prevalence of BWS of 1/35,580 births. Of these BWS cases, 4 were conceived by IVF. They matched 148 control infants and found 1 positive for IVF. The comparison of 1/148 with 4/37 showed an odds ratio for an increased risk of BWS following IVF of 17.8-fold, with a very wide confidence interval of 1.8–437.9. All of the 37 patients were evaluated clinically and were proven by molecular methods, KVDMM (IC2) and H19 (IC1) [5].

Chang et al. [9] reported on 19 BWS/ART patients. Of 12 further evaluated, 5 were from ICSI pregnancies, and 5 were from standard IVF. The other 2 were products of in utero insemination. There did not seem to be an effect of culture media or embryo transfer method upon the incidence of BWS following ART. The causes of infertility in the involved couples were varied.

Sutcliffe et al. [10] contacted families having children with four imprinting syndromes: BWS, AS, Prader-Willi syndrome, and transient neonatal diabetes mellitus. They found that 2.9%, 95% confidence interval (CI) of 1.4–6.4%, were conceived by ART, as compared with a population ART incidence of 0.8%.

Lidegaard et al. [11] reviewed 6052 ART births in Denmark. They did not find a case of an imprinting syndrome following ART.

Doonbos et al. [12] evaluated their Dutch imprinting syndrome (AS, BWS) clinic patients. They found 220, all molecularly proven. Those born via ART represented 6.4% of the group. This compares with a population rate of 2.1%. Of 71 BWS patients, 4 were conceived by ART. The rate of births following infertility was 6.8% in the BWS group and 3.4% in the general population. However, correcting for the infertility, there did not appear to be an increased risk of BWS following ART.

Bowdin et al. [13] evaluated IVF births in England by sending out a survey with about a 60% return rate. Of 1524 questionnaires, the group was able to evaluate 47. They found one BWS case in this small group.

Kallen et al. [14] studied 31,850 births following ART from 1986 to 2006. They found 4 cases of PWS, with an expected population prevalence of 1/15,000 to 1/22,000 births [15, 16]. In their sample, they would have expected 1 case. Three of the PWS births were conceived by ICSI and 1 from standard IVF. Two cases of Russell-Silver syndrome were observed, with an expected incidence of only 1/30,000 to 1/100,000 [17]. One case was born after ICSI and one IVF. Finally, one BWS case (ICSI) was found. Therefore, seven imprinting syndrome cases were found in the sample of about 32,000 ART births (1/4570). However, no molecular data were available to verify if all cases resulted from primary imprinting errors.

In Italy, Mussa et al. [18] analyzed a total of 379,872 live births delivered between 2005 and 2014 for incidence of BWS. They reported a significantly greater proportion of ART conceived births (7 of 7884 or 1:1126) than in those conceived naturally (31 of 371,957, 1:12,254). The significance of this difference is $p < .001$, with a relative risk of 10.7 (95% CI, 4.7 to 24.2).

The prenatal literature on this topic is scarce. Wilson-Haug and colleagues [19] studied a group of 30 fetuses with omphalocele. Six had BWS clinically or by DNA testing. Three were conceived by IVF and the difference between the unaffected and the BWS group for IVF reached a p value $< .05$.

The available case reports, registry studies, population studies, and questionnaire studies suggest a link between ART and increased incidence of BWS and AS and other hypomethylation syndromes (see below). The predicted increased incidence varies from none to 18-fold. Given the disparity in the above results and the wide confidence intervals, it is difficult to define what the increased risk of BWS following ART really is.

To further investigate this question, we tested by molecular means fetuses with prenatally diagnosed omphalocele. In a majority of BWS cases, omphalocele is observed, approximately 80% [20]. When omphalocele is observed prenatally, and trisomies have been excluded by chromosome or array testing, BWS is an important consideration meriting prenatal diagnosis if desired by the parents [5, 19]. A significant percentage of fetuses with omphalocele have BWS [19].

We retrospectively studied prenatal samples sent to our laboratory between 2002 and 2015 for BWS testing. We identified 301 pregnancies affected by omphalocele and found 40 (13.3%) positive cases. The genetic mechanism causing BWS was abnormal imprinting of *KCNQ1OT1* (IC2) in 36 cases, *H19* (IC1) in 2 cases, or both loci due to paternal uniparental isodisomy 11 (patUPD11) in 2 cases. Among the 40 fetuses that tested positive for BWS, 16 (40.0%) were conceived by IVF. This compares with a population incidence of about 1–2% for IVF pregnancies [21]. This study provides supporting evidence to the existing anecdotal and registry data suggesting an increased risk of BWS following IVF.

Methods and materials

Beckwith syndrome genetics are complex [5]. The majority of cases result from either overexpression of *IGF2* (usually paternal expressed only) or reduced expression of *CDKN1C* (usually maternally expressed). About 15–20% results from mosaic paternal uniparental disomy (UPD) 11p, which causes abnormalities of both imprinted regions. Other cases result from *CDKN1C* mutations (perhaps 5–10%, more commonly in familial cases) and 1–2% from chromosomal rearrangements. Between 2002 and 2011, our laboratory tested only for abnormal imprinting of *LIT1/KCNQ1OT1* (IC2) [22–24], which detects primary imprinting errors at that locus. Overall, detection rate of BWS is approximately 70–75% with assays for the *LIT1/KCNQ1OT1* locus. An abnormal result is usually caused by a primary imprinting error at this locus, but with a possibility of associated paternal UPD 11 (patUPD11). As described below, since 2011, we have added *H19/IC1* testing which allows for both, detection of isolated gain of methylation of *H19* (IC1) on the maternal chromosome (approximately 5% of BWS) and of patUPD11 when combined with *KCNQ1OT1/IC2* analysis.

The Shodair Genetics Laboratory is a clinical laboratory certified by the Centers for Medicare and Medicaid Services (CMS) through Clinical Laboratory Improvement Amendments (CLIA; certificate number: 27D0652530). Between 2002 and 2015, we tested 301 prenatal samples obtained by CVS or amniocentesis from pregnancies affected by fetuses with omphalocele and normal chromosomes or chromosomal microarrays sent to our laboratory for BWS testing. The work reported here is the result of routine provision of medical attention at the standard of care, with data collected solely for patient care purposes. No additional data were collected, or medical intervention undertaken for the purposes of this study. As such, a Research Involving Human Subjects Committee was not convened.

Since initiation of clinical testing for BWS, methods in our laboratory have evolved. Initially, Southern blot analysis was performed. For Beckwith-Wiedemann syndrome, isolated

fetal DNA was digested with *Bam*HI/*Not*I and probed with a *LIT1/KCNQ1OT1* sequence [22–24] (IC2, Fig. 1a). Later, we added a bisulfite-treated methylation PCR assay adapted from Coffee et al. [25] (Fig. 1b). For details, see Johnson et al. [26]

Subsequently, we replaced Southern blot with a restriction digest PCR (RDPCR). As this is not described elsewhere, we will describe the technique here. In this 4-reaction procedure, sample DNA is either digested with *Eae*I, which is methylation sensitive (not cutting methylated CpG in the T/C GGCC A/G recognition sequence), with *Lpn*PI, which preferentially recognizes methylated CpG at the center of a 32-base pair sequence, with both enzymes, or with neither (Mock digest). PCR for the *KCNQ1OT1* locus is then performed. Expected results include roughly equal maternal and paternal allele quantitative PCR results (analyzed using either a Light Scanner 32 [Idaho Technologies, Salt Lake City, UT] or a CFX 96 real-time PCR machine [Bio-Rad, Hercules, CA]) for the two digests, indicating a methylation index [27] near 0.5. For the undigested sample, the PCR reaches maximum product one cycle before the single digests (which amplify only one intact copy), and a product amplifying much later is seen in the double digest samples (Fig. 1c).

As noted above, in 2011, we added *H19* (IC1) methylation PCR [28] and a similar RDPCR test. We use similar PCR methods for the *H19* assay. The use two different assays for the above tests is to improve estimates of the MI. Samples tested prior to 2011 were initially only tested for the *KCNQ1OT1* (IC2) locus ($n = 182$). Among those, 18 of the 23 samples that tested positive for the *KCNQ1OT1* (IC2) locus were re-analyzed on the *H19* (IC1) locus for this study to evaluate for possible paternal UPD11. For 4 of these samples, the *H19* (IC1) locus could not be analyzed due to insufficient DNA.

Results

Between 2002 and 2015, our laboratory performed methylation testing for Beckwith-Wiedemann syndrome (BWS) in 185 amniocentesis and 102 CVS samples from pregnancies affected by fetal omphalocele. During the same time period, 9 samples from isolated fetal DNA, and 5 from fetal losses, were analyzed (Table 1).

Of the 301 samples, 40 tested positive for BWS (Table 1). We found 36 positives by Southern blot and/or methylation PCR assays for *LIT1/KCNQ1OT1*. Two samples revealed isolated gain of methylation of *H19* (IC1), whereas another 2 samples showed abnormal methylation of both *LIT1/KCNQ1OT1* (IC2) and *H19* (IC1) loci consistent with paternal uniparental disomy 11 (patUPD11). Methylation of *H19* (IC1) was normal in 32 of the 36 IC2-positive BWS samples and could not be verified in 4 samples due to insufficient DNA (Table 2). Among the 40 BWS-positive samples, 32 were

Fig. 1 Methods and results of fetal DNA analysis for Beckwith-Wiedemann syndrome. **a** Southern blot results on leukocyte DNA digested with BamHI and NotI hybridized with a LIT1/KCNQ1OT1 probe. Note the absence or reduced intensity of the maternal fragment in the BWS positive (arrow). **b** Methylation-sensitive (MS) polymerase chain reaction for LIT1/KCNQOT1 (IC2) and H19 (IC1). DNA was treated with bisulfite and then amplified. Normal results show roughly equal size/area of the methylated (Meth) and unmethylated (UnMeth) alleles (upper lane). Patients with mosaic hypomethylation of the (maternal) LIT1/KCNQOT1 (IC2) locus show skewed results (lower lane), consistent with mosaic Beckwith-Wiedemann syndrome (BWS).

c Restriction digest PCR (RDPCR) of the LIT1/KCNQOT1 (IC2) locus shows that DNA digested with methylation-sensitive or methylation-dependent enzymes amplifies similarly (upper panel) and that in an affected patient, the sample digested with a methylation-dependent enzyme amplifies like a control sample (undigested), whereas the sample digested with a methylation-sensitive enzyme digests normally (the DNA is not methylated) and amplifies late like a double digest control (bottom panel). Blue, DNA not digested (Mock digest); green, DNA digested with a methylation-dependent restriction enzyme; red, DNA digested with a methylation-sensitive enzyme; black, DNA double digested with both enzymes

cultured amniocytes and 5 were cultured CVS. One of the POC as well as 2 isolated fetal DNA samples tested positive as well (Table 1).

Maternal age, available for 231 amniocentesis/CVS samples, was <35 years in 137 samples and ≥35 years for 94

samples (Table 3). The average maternal age for the CVS and amniocentesis samples was 34 and 32.5, respectively. Of the 231 samples for which maternal age was available, 19/137 samples with maternal age <35 years (13.9%) and 14/94 (14.9%) with maternal age 35 years and older tested positive

Table 1 Prenatal BWS test results of 301 fetuses with omphalocele

Sample type	Total number	BWS positive ^a	BWS negative ^b
Amniocytes (cultured)	184	32	152
Amniocytes (direct)	1	0	1
CVS (cultured)	101	5	96
CVS (direct)	1	0	1
POC/fetal tissue (cultured)	5	1	4
Extracted DNA (CVS or amniocytes)	9	2	7
Total	301 (100%)	40 (13.3%)	261 (86.7%)

BWS Beckwith-Wiedemann syndrome

^a Among the 40 positive samples, 23 were initially only tested for the IC2 locus and IC1 methylation was determined retrospectively for this study; of these, 4 did not have sufficient DNA to analyze the IC1 locus

^b Of the 261 negative samples, 103 were assessed for both IC2 and IC1 loci, whereas 158 were tested for the IC2 locus only

indicating no significant difference in the positive rate between these age groups. Note that only for 33 of the 40 BWS-positive samples, maternal age was available (Table 3).

Twenty-four of the 301 analyzed samples were from pregnancies conceived by ART (Table 4), and 13 were from twin pregnancies (Table 5). Among the 40 BWS-positive samples, 16 (40.0%) were from IVF pregnancies (Table 4). Of these, 4 were cultured CVS samples subsequently confirmed by amniocentesis in 3 cases, and the remaining 12 samples were cultured amniocytes. Hypomethylation of the LIT1/KCNQ1OT1 (IC2) locus was present in 15 of the 16 positive samples from IVF pregnancies, whereas one was found positive due to patUPD showing a paternal methylation pattern for both IC2 (hypomethylation) and IC1 (hypermethylation). None of the samples from pregnancies conceived with ART showed isolated hypermethylation of H19 (IC1) as genetic

Table 2 Genetic mechanisms among BWS-positive samples

Causes of BWS by genetic mechanism	Total number (percentage)	ART pregnancies number (percentage)	Non-ART pregnancies number (percentage)
Loss of methylation of IC2 on maternal chromosome	36 ^a	15	21
Gain of methylation of IC1 on maternal chromosome	2	0	2
Paternal UPD11	2	1	1
Total	40 (100%)	16 (40.0%)	24 (60.0%)

BWS Beckwith-Wiedemann syndrome

^a In 4 of 36 samples, only IC2 was analyzed

mechanism for BWS (Table 2). Among the BWS 261 negative samples, 8 (3.1%) were from pregnancies conceived by ART (Table 4).

Seven of the 40 BWS-positive samples were from twin pregnancies (17.5%) of which 5 were conceived by ART. Thus, twinning was present in 5 of the 16 BWS-positive samples from IVF pregnancies (31.3%), compared to 2 of the 24 BWS-positive samples conceived without ART (8.3%). In comparison, 6 of the 261 BWS-negative samples were from twin pregnancies (2.3%) (Table 5).

Discussion

Assisted reproductive technologies (ART) including in vitro fertilization (IVF) are generally considered safe infertility treatments but have been associated with birth defects and adverse pregnancy outcomes in some studies. Specifically, an increased risk following ART of fetal imprinting disorders including BWS and Angelman syndrome has been suggested. Postulated biological explanations range from direct effects of ART caused by embryo culture techniques, culture media, or the use of intracytoplasmic sperm injection (ICSI), to heritable traits associated with subfertility itself that are bypassed through ART as further discussed below.

Omphalocele is a major manifestation of BWS readily identifiable on prenatal ultrasound. Here, we reviewed prenatal cases with fetal omphalocele referred to our laboratory for molecular testing of BWS between 2002 and 2015. The percentage of fetuses with omphalocele found to have BWS was 13.3% based on the diagnostic methods used during that period. Considering that H19 (IC1) methylation testing was not established in our laboratory until 2011 and given that analysis of CDKN1C was not included, our testing up until 2011 would have missed cases caused by gain of methylation at H19 (IC1) estimated to make up 5% of alterations in BWS, and those cases caused by maternal pathogenic variants (5% of BWS cases in the absence of a family history). In contrast, loss of methylation at IC2 (maternal) and patUPD11 accounting for approximately 50% and 20% of BWS, respectively,

Table 3 Maternal age distribution among BWS-positive samples

Samples	Number of samples by maternal age ^a	
	<35	≥35
CVS	50	36
Amniocentesis	87	58
CVS and amniocentesis	137	94
Positive (percentage)	19 (13.9%)	14 (14.9%)

BWS Beckwith-Wiedemann syndrome

^a Number of samples for which maternal age data was available

Table 4 Pregnancies conceived by ART among BWS-positive and BWS-negative prenatal cases

Sample type	ART pregnancies	
	BWS-positive samples (<i>n</i> = 40)	BWS-negative samples (<i>n</i> = 261)
Amniocytes	12	2
CVS	4	6
POC/fetal tissue	0	0
Total	16 (40.0%)	8 (3.1%)

ART assisted reproductive technology, BWS Beckwith-Wiedemann syndrome

would have been detected. Also, microdeletions and duplications that alter the methylation status of the IC2 (since 2011 also IC1) would have been detected. Overall, our diagnostic rate is comparable with previously reported prenatal series [19].

What is striking about our results is the high percentage of BWS-positive fetuses who were conceived by IVF (Table 4). We reviewed charts and called referring centers to verify that 16 of 40 BWS-positive fetuses (40.0%) were conceived by IVF. In contrast, only 8 of 261 BWS-negative fetuses (3.1%) were conceived using ART. For comparison, the population incidence for IVF conception in the USA is 1–2%. Thus, while the rate of ART-related pregnancies observed among BWS-negative fetuses with omphalocele was similar to the rate of ART-related pregnancies in the general population, fetuses conceived by IVF were overrepresented among BWS-positive fetuses with omphalocele. Using a 1–2% estimate for ART conception in the USA, the increase in risk with ART could approximate 20-fold, the highest estimate so far and the first from a prenatal series. This would create an overall risk of BWS with omphalocele following IVF of about 1/850 (BWS/omphalocele prevalence assumed at 1/17,000, about 80% of 1/13,700 [5]). The chi-square difference of proportions calculator indicates the difference in the observed (.366) vs expected number (.02) for this sample size reaches significance at $p < .0001$ [29].

Table 5 Twin pregnancies by BWS diagnosis and ART status

BWS result and ART status	Twin pregnancies (percentage) ^a
BWS positive (<i>n</i> = 40)	7 (17.5%)
• Conceived with ART (<i>n</i> = 16)	5 (31.3%)
• Conceived without ART (<i>n</i> = 24)	2 (8.3%)
BWS negative (<i>n</i> = 261)	6 (2.3%)
Total (<i>n</i> = 301)	13 (4.3%)

ART assisted reproductive technology, BWS Beckwith-Wiedemann syndrome

^a The twinning rate in the USA in 2015 was 33.5 per 1000 deliveries corresponding to 3.35% (Martin et al. 2018)

The BWS-positive fetuses conceived with (16) and without (24) ART each included one case caused by patUPD11 and provide no observable evidence for a relationship between conception method and genetic mechanism, although our numbers are too small to allow for statistical comparison (Table 2). Of note, an association of UPD 11p and omphalocele/BWS has previously been suggested [30].

A contributing factor to the disproportionately high number of BWS-positive IVF pregnancies could be an overrepresentation of twin gestations in that group (Table 5): In our entire cohort of 301 fetuses with omphalocele, 13 (4.3%) were from twin gestations compared to a twinning rate of 3.35% in the USA in 2015. [31] Five of the 15 BWS-positive cases conceived by ART were twin gestations (31.3%) compared to 2 of 26 (8.3%) non-ART pregnancies that tested positive for BWS. However, the difference of 23.6% did not quite reach statistical significance ($p = .055$, 95% CI −0.7 to 48.6 [32]) indicating that the high twinning rate alone is unlikely to be the only factor driving the overrepresentation of ART cases among the BWS positives. In contrast, 6 twin pregnancies were among the 261 BWS-negative fetuses with omphalocele (2.3%), similar to the twinning rate in the USA.

Many BWS twins have been described (summarized in Elaloui SC et al. [33]). The majority of BWS twins are female MZ discordant, and most of them present hypomethylation of IC2. This has led to the hypothesis that the methylation defect and the twinning process are correlated. [34] In a cohort of 250 BWS patients described by Weksberg et al., the frequency of monozygotic (MZ) twinning was 8% as compared with 1% dizygotic (DZ) twinning. [35] Bliok et al. observed an excess of monozygotic twins among BWS patients with IC2 hypomethylation in their cohort of 400 BWS patients: 3% were twin pairs of which about two-thirds were MZ twins. [36] The high twinning rate of a third among our BWS-positive cases conceived by IVF suggest additional factors at play that might in part derive from the ART process. If methylation failure precedes and possibly triggers the twinning process as previously suggested, [34, 36] ART might create conditions that allow for a higher rate of aberrant methylation.

Manipalviratn et al. [37] and Hiura et al. [38] discussed possible mechanisms within ART procedures which may lead to an increased incidence of imprinting syndromes. These include the hormones given to the prospective mother, oocyte collection and maturation in vitro, possible use of immature sperm, the injection procedure (ICSI), and embryo culture. Perhaps relevant are the observations that the early embryo undergoes a wave of demethylation and then the parental patterns are reset [39]. A maternal hypomethylation syndrome has been observed [40] with abnormal methylation at multiple known imprinting sites with varying manifestations of the associated syndromes, first described in infants with transient neonatal diabetes syndrome. Finally, the methylation status of early embryos and placentas revealed multiple variable

findings at different loci and different developmental stages following ART in a recent study [41]. Lastly, the underlying problem which causes couples to seek out ART, infertility, may be a primary driver for the increased risk of associated imprinting syndromes[42].

Our study has several weaknesses. The referrals were likely non-random and the overall numbers are small. It is possible that prenatal centers were more likely to request BWS testing because of IVF conception. We think that the main reason for referral was simply the fetal omphalocele but cannot be certain. In our postnatal series of affected patients, a significant number have resulted from IVF pregnancies.

In summary, it is apparent there are no specific mechanisms known to disturb imprinting in ART embryos and no consistent estimates of the increased risk of associated syndromes (primarily BWS and AS). However, from our data and review of the literature, we recommend that patients be counseled about the increased risks for these imprinting syndromes and that prenatal testing be considered at the parents' request.

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