Clinical and molecular characterization of patients affected by Beckwith-Wiedemann spectrum conceived through assisted reproduction techniques

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Abstract
The prevalence of Beckwith–Wiedemann spectrum (BWSp) is tenfold increased in children conceived through assisted reproductive techniques (ART). More than 90% of ART-BWSp patients reported so far display imprinting center 2 loss-of-methylation (IC2-LoM), versus 50% of naturally conceived BWSp patients. We describe a cohort of 74 ART-BWSp patients comparing their features with a cohort...
of naturally conceived BWSp patients, with the ART-BWSp patients previously described in literature, and with the general population of children born from ART. We found that the distribution of UPD(11)pat was not significantly different in ART and naturally conceived patients. We observed 68.9% of IC2-LoM and 16.2% of mosaic UPD(11)pat in our ART cohort, that strongly differ from the figure reported in other cohorts so far. Since UPD(11)pat likely results from post-fertilization recombination events, our findings allows to hypothesize that more complex molecular mechanisms, besides methylation disturbances, may underlie BWSp increased risk in ART pregnancies. Moreover, comparing the clinical features of ART and non-ART BWSp patients, we found that ART-BWSp patients might have a milder phenotype. Finally, our data show a progressive increase in the prevalence of BWSp over time, paralleling that of ART usage in the last decades.

KEYWORDS
assisted reproductive technologies, Beckwith-Wiedemann spectrum, hypomethylation, imprinting disorders, uniparental disomy

1 | INTRODUCTION

Birth from assisted reproductive technology (ART) account for approximately 3.1% of all births in Europe and are known to be associated with pregnancy complications, preterm delivery and related problems, increased birth defects rate, long-term effects on health, and genetic/epigenetic risk. Most of such adverse events are indeed connected with characteristics of the couples that undergo ART, including age, health condition, and subfertility. Several studies documented an increased risk of DNA methylation anomalies in children born from ART, some resulting in an higher incidence of human imprinting disorders. It is unclear whether such epigenetic anomalies are the direct result of ART itself or, rather, connected with genetic/environmental factors causing parental subfertility.

The most common human imprinting disorder is Beckwith-Wiedemann spectrum (BWSp), a congenital overgrowth condition with cancer predisposition and a prevalence of 1:10340 in naturally conceived births, and 1:1126 in the population born from ART. BWSp is characterized by a variable association of neonatal macrosomia, postnatal overgrowth, hyperinsulinemic hypoglycemia, abdominal wall defects, macroglossia, lateralized overgrowth, organomegaly, auricular abnormalities, nevus flammeus at the glabella, nephrourological abnormalities and predisposition to the development of embryonal tumors. Over 80% of patients affected by BWSp harbor an epigenetic defect of the imprinted chromosomal region 11p15.5, including hypomethylation of Imprinting Center 2 (IC2-LoM, nearly 50% of cases), chromosome 11 paternal uniparental disomy (UPD[11]pat, 20% of cases), gain-of-methylation of the imprinting center 1 (IC1-GoM, 10% of cases). More rare are the genetic defects leading to BWSp, such as loss-of-function variants of CDKN1C or chromosomal rearrangements of the 11p15.5 region. Each genotype is characterized by a specific phenotype and tumor risk.

The proportion of children with BWSp that are conceived through ART is well above that of the general population, ranging from 4%–6% in the earliest reports dating back two decades ago, to 15% in the most recent ones. Patients with BWSp conceived through ART have been reported to display typically IC2-LoM (>90% of cases), suggesting that a defect in imprint establishment or maintenance is underlying the association between BWSp and ART.

To further investigate into this issue, here we describe the genotypic and phenotypic features of a large Italian cohort of patients with BWSp conceived through ART and compare them with (a) a cohort of naturally conceived patients with BWSp, (b) previously reported ART-BWSp cohorts, and (c) the general population of children born after ART.

2 | METHODS

This is a retrospective observational study that was conducted on a sample of patients affected by BWSp born after ART diagnosed and followed in 15 pediatric clinical genetic centers in Italy and with the help of the Italian Association of patients affected by BWSp (AIBWS, www.aibws.org). Written informed consent for the study was obtained from patients or guardians for the study, according to the local ethic committee’s policy. The study was approved by the ethics committee of the Città della Salute e della Scienza University Hospital of Torino, Italy (IRB approval protocol 0052021–0052712 with ID 155/2022, May 2022).

Two criteria were considered for patients’ inclusion: diagnosis of BWSp (i.e., with positive molecular tests and/or with clinical diagnosis made with the specific score of the 2018 International Consensus, with ≥4 points), and conception through ART, including ovarian stimulation, intrauterine insemination (IUI), in vitro fertilization (IVF), or intracytoplasmatic sperm injection (ICSI). Clinical and molecular data
were obtained directly from the clinical center where the patients were diagnosed or followed-up.

Methylation analysis of the chromosomal region 11p15.5 was performed by CoBRA (Combined Bisulfite Restriction Analysis), or MS-MLPA (Methylation-Sensitive Multiple Ligation-dependent Probe Amplification, MRC Holland, Amsterdam, Netherlands). In patients with IC2-LoM and IC1-GoM, UPD(11)pat was confirmed by either high-resolution polymorphism or microsatellite analysis. Patients scoring negative for 11p15.5 methylation defects underwent CDKN1C Sanger sequencing.

For comparison, three cohorts were used: a literature-derived cohort of ART-BWSp patients, a naturally conceived (non-ART)-BWSp cohort, and a non-BWSp ART cohort. The literature derived ART-BWSp cohort was obtained merging previously described ART-BWSp cohorts: the literature search on Pubmed was conducted to identify publications reporting case series of patients with BWSp conceived through ART (original search string “Beckwith-Wiedemann Syndrome” [Mesh] and “Reproductive Techniques, Assisted” [Mesh], then refined adding references from retrieved papers). The naturally conceived BWSp cohort was our historical one (n = 318) was derived from the one previously described by our group, after exclusion of the conceived through ART (n = 14). For comparison between our naturally conceived BWSp cohort and ART-BWSp cohort, we did not use the data of patients scoring negative to the molecular tests in the current ART-BWSp cohort, as our historical non-ART-BWSp cohort included only molecularly confirmed patients and was published before the definition of BWSp diagnostic criteria. To compare our study group with the cohort of children born in Italy after ART (ART-nonBWSp), we used the data reported in the National Registry of Medically Assisted Procreation (www.iss.it/rART, accessed September 15, 2021, covering the years 2005–2019).

Data were compared using the $\chi^2$ test for distribution analysis of variables greater than 200, the $\chi^2$ test with Yates’s correction for variables between 40 and 200, and Fisher’s exact test for variables <40. Comparison between continuous variables was performed with Student’s $t$ test for variables with normal distribution or Mann-Whitney’s U test for those distributed non-normally, after checking for homoscedasticity of the sample with Shapiro-Wilk test. Correlation between continuous variables was confirmed with Pearson’s method. The $p$-values less than 0.05 were considered statistically significant.

### RESULTS

Our ART-BWSp cohort included 74 patients, 40 females (54.1%) and 34 males (45.9%), all molecularly tested on blood-extracted DNA: among them, 65 (87.8%) had a molecular anomaly consistent with BWSp and 9 (12.2%) were negative with a clinical score $\geq 4$. In Table 1 we report their clinical characteristics, and in Table 2 family history, type of ART, and pregnancy, sorted by molecular subtype. All these data were compared between the IC2-LoM and UPD(11)pat

| TABLE 1 | Clinical characteristics of the ART-BWSp patients’ group |
|---------|-----------------|
|         | IC2-LoM | IC1-GoM | UPD(11)pat | Negative | Total | $p$-value$^a$ |
| $n$     | 51 (68.9%) | 2 (2.7%) | 12 (16.2%) | 9 (12.2%) | 74 | - |
| Females | 28 (54.9%) | 2 (100%) | 6 (50%) | 4 (44.4%) | 40 (54.1%) | 0.759 |
| Males   | 23 (45.1%) | 0 (0%) | 6 (50%) | 5 (55.6%) | 34 (45.9%) | 0.356 |
| BWSp score$^{19}$ | $5.5 \pm 2.1$ | $6.5 \pm 0.7$ | $6.2 \pm 2.4$ | $5.1 \pm 1.6$ | $5.6 \pm 2.1$ | 0.356 |
| Neonatal hypoglycemia | 19 (37.3%) | 1 (50%) | 5 (41.7%) | 6 (66.7%) | 31 (41.9%) | 0.777 |
| Neonatal hyperinsulinism | 1 (2.0%) | 1 (50%) | 1 (8.3%) | 0 (0.0%) | 3 (4.1%) | 0.257 |
| Macroglossia | 42 (82.4%) | 1 (50%) | 1 (8.3%) | 0 (0.0%) | 56 (75.7%) | 0.072 |
| Abdominal wall defects | 34 (66.7%) | 1 (50%) | 7 (58.3%) | 6 (66.7%) | 49 (66.2%) | 0.586 |
| Omphalocele | 7 (13.7%) | 0 (0.0%) | 2 (16.7%) | 0 (0.0%) | 9 (12.5%) | 0.793 |
| Umbilical hernia or diastasis recti | 27 (52.9%) | 1 (50%) | 5 (41.7%) | 9 (100%) | 40 (54.1%) | 0.482 |
| Lateralized overgrowth | 26 (50.1%) | 2 (100%) | 11 (91.7%) | 3 (33.3%) | 42 (56.8%) | 0.001$^b$ |
| Organ enlargement | 9 (17.6%) | 1 (50%) | 3 (25%) | 1 (11.1%) | 14 (18.9%) | 0.559 |
| Ear pits or creases | 16 (31.3%) | 1 (50%) | 7 (58.3%) | 6 (66.7%) | 30 (40.5%) | 0.081 |
| Angioma at the glabella | 27 (52.9%) | 1 (50%) | 4 (33.3%) | 5 (55.6%) | 37 (50%) | 0.222 |
| Polyhydramnios | 8 (15.7%) | 0 (0.0%) | 2 (16.7%) | 0 (0.0%) | 10 (13.5%) | 0.993 |
| Neonatal macrosomia | 22 (43.1%) | 0 (0.0%) | 4 (33.3%) | 2 (22.2%) | 28 (37.8%) | 0.535 |
| Postnatal overgrowth | 20 (39.2%) | 1 (50.0%) | 4 (33.3%) | 0 (0.0%) | 25 (33.8%) | 0.650 |
| Malignant tumors | 0 (0.0%) | 0 (0.0%) | 2 (16.7%) | 0 (0.0%) | 2 (2.7%) | 0.003$^b$ |
| Renal anomalies | 5 (9.8%) | 1 (50.0%) | 5 (41.7%) | 1 (11.1%) | 12 (16.2%) | 0.007$^a$ |

$^a$The reported $p$-value represents the result of a comparison between the subgroup with IC2-LoM and UPD(11)pat.

$^b$Statistically significant.
We observed that the subgroup with mosaic UPD(11)pat showed a higher frequency of lateralized overgrowth (91.7% vs. 50.1%, $p = 0.001$), malignancies (16.7% vs. 0%, $p = 0.003$), and renal anomalies (41.7% vs. 9.8%, $p = 0.007$). Also, more ART attempts before obtaining a pregnancy were made in the UPD(11)pat than in the IC2-LoM subgroup (2.6 ± 2.1 vs. 1.1 ± 1.4, $p = 0.036$).

### TABLE 2

Family history, kind of technology used, and pregnancy characteristics of the patients with Beckwith-Wiedemann spectrum conceived after assisted reproduction technology (ART-BWSp)

|                | IC2-LoM (n = 51) | IC1-GoM (n = 2) | UPD(11)pat (n = 12) | Negative (n = 9) | Total (n = 74) | $p^a$ |
|----------------|------------------|----------------|---------------------|-----------------|---------------|------|
| Presence of siblings | 18 (35.3%)      | 1 (50%)        | 6 (50%)             | 3 (75%)         | 28 (37.8%)   | 0.345 |
| Time of pregnancy attempts (years) | 4.5 ± 3.5      | 1.5            | 4.1 ± 2.9           | 0.5 ± 0.7       | 4.1 ± 3.3    | 0.707 |
| Average number of abortions | 0.8 ± 1.2     | 0              | 0.4 ± 0.5           | 1.8 ± 1.3       | 0.8 ± 1.1    | 0.311 |
| Number of previous ART attempts | 1.1 ± 1.4      | 0              | 2.6 ± 2.1           | 1.0 ± 1.4       | 1.5 ± 1.7    | 0.036 |
| Cause of infertility | Maternal | 10 (19.6%) | 0 | 4 (40%) | 1 (11.1%) | 15 (20.3%) | 0.303 |
| | Paternal | 6 (11.8%) | 1 (50%) | 4 (40%) | 1 (11.1%) | 12 (16.2%) | 0.066 |
| | Both | 10 (19.6%) | 1 (50%) | 2 (20%) | 0 | 13 (17.6%) | 0.815 |
| | Unknown | 25 (49%) | 0 | 0 | 7 (77.8%) | 34 (45.9%) | - |
| Abnormal sperm count | 9 (17.6%) | 1 (50%) | 4 (33.3%) | 1 (50%) | 15 (20.3%) | 0.227 |
| Maternal mean age at ART (years) | 36.5 ± 4.6 | 38 | 35.4 ± 4.0 | 34.1 ± 4.5 | 35.9 ± 4.4 | 0.479 |
| Mean paternal age at ART (years) | 38.6 ± 5.1 | 45 | 38.6 ± 4.1 | 41.3 ± 8.3 | 39.1 ± 5.4 | 0.985 |
| Average number of oocytes retrieved | 8.4 ± 5.2 | 4 | 8.9 ± 3.0 | 8.0 ± 7.1 | 8.3 ± 4.7 | 0.822 |
| Gamete freezing | 3/25 (12.0%) | 1/2 (50%) | 0/9 | 0/2 | 4/38 (10.5%) | 0.276 |
| Embryo freezing | 11/25 (44.0%) | 1/2 (50%) | 2/9 (22.2%) | 1/2 (50%) | 15/38 (39.5%) | 0.249 |
| Average number of embryos obtained | 4.5 ± 4.2 | 2 | 4.8 ± 2.6 | 3.5 ± 3.5 | 4.4 ± 3.7 | 0.866 |
| Technique used | Stimulation only | 1 (2%) | 0 | 0 | 0 | 1 (1.4%) | 1 |
| | IUI | 1 (2%) | 0 | 0 | 0 | 1 (1.4%) | 1 |
| | IVF | 12 (23.5%) | 1 (50%) | 6 (50%) | 1 (25%) | 20 (27.0%) | 0.068 |
| | ICSI | 23 (45%) | 1 (50%) | 5 (41.7%) | 3 (75%) | 32 (43.2%) | 0.830 |
| | Not available | 14 (27.5%) | 0 | 1 (8.3%) | 0 | 20 (27%) | - |
| Gamete origin | Homologous | 23 (45.1%) | 1 (50%) | 8 (66.7%) | 2 (22.2%) | 34 (45.9%) | 0.178 |
| | Heterologous | 4 (7.8%) | 1 (50%) | 1 (8.3%) | 1 (11.1%) | 7 (9.5%) | - |
| | Not available | 24 (47.1%) | 0 | 3 (25%) | 6 (66.7%) | 33 (44.6%) | - |
| Number of embryos transferred | 1.7 ± 0.7 | 1.5 ± 0.7 | 2.1 ± 0.6 | 1.0 ± 0.0 | 1.8 ± 0.7 | 0.127 |
| Twin pregnancy | Twin at conception | 15 (29.4%) | 1 (50%) | 1 (8.3%) | 2 (22.2%) | 19 (25.7%) | 0.131 |
| | Twin at birth | 10 (19.6%) | 1 (50%) | 0 | 0 | 11 (14.9%) | 0.186 |
| | Monozygote | 2/15 (13.3%) | 1/1 (100%) | 0/1 | 0/2 | 3/19 (15.8%) | 0.696 |
| | Dizygote | 13/15 (86.7%) | 0/1 | 1/1 (100%) | 2/2 (100%) | 16/19 (84.2%) | - |
| Pregnancy complications | 19 (37.6%) | 1 (50%) | 3 (25%) | 2 (22.2%) | 25 (33.8%) | 0.423 |
| Abnormal prenatal ultrasound | 15 (29.4%) | 0 | 4 (33.3%) | 0 | 19 (25.7%) | 0.071 |
| Gestational age | 36.4 ± 2.6 | 37.2 ± 1.2 | 37.0 ± 4.1 | 35.4 ± 3.1 | 36.4 ± 2.9 | 0.546 |
| Weight at birth (SDS) | 1.3 ± 1.7 | 0.0 ± 1.0 | 1.2 ± 1.7 | 1.1 ± 2.9 | 1.2 ± 1.9 | 0.908 |
| Lenght at birth (SDS) | 1.1 ± 1.5 | 1.0 ± 0.9 | 0.7 ± 1.3 | 0.0 ± 1.4 | 0.8 ± 1.5 | 0.443 |
| Head circumference at birth (SDS) | 0.5 ± 1.5 | 0.0 ± 1.6 | 0.0 ± 0.9 | 0.3 ± 1.8 | 0.4 ± 1.4 | 0.391 |
| Birth complications | 8 (15.7%) | 0 | 1 (8.3%) | 0 | 9 (12.2%) | 0.513 |

Abbreviations: IC1-GoM, imprinting center 1 gain of methylation; IC2-LoM, imprinting center 2 loss of methylation; SDS, standard deviation score; UPD(11)pat, chromosome 11 paternal uniparental disomy.

The $p$-value refers to the comparison between the subgroups with IC2-LoM and UPD(11)pat.

Data available only in 38 patients.

Statistically significant.
Table 3 summarizes the studies retrieved from literature providing genotype and phenotype data of ART-BWSp patients (n = 168).10,20–23,27–37

Table 3 Studies in the literature analyzing the association between the Beckwith-Wiedemann spectrum (BWSp) and assisted reproductive techniques (ART)

| Study                          | ART-BWSp cases | Molecular defects found in ART-BWSp | Kind of ART used |
|-------------------------------|----------------|-------------------------------------|-----------------|
| DeBaun et al., 200321         | 7/0            | 4 IC2-LoM, 1 IC2-LoM + IC1-GoM, 1 negative | IVF, ICSI       |
| Maher et al., 200330          | 6/149          | 2 IC2-LoM                           | IVF, ICSI       |
| Gicquel et al., 200322        | 6/149          | 6 IC2-LoM                           | IVF, ICSI       |
| Halliday et al., 200410       | 4/37           | 3 IC2-LoM                           | IVF, ICSI       |
| Rossignol et al., 200623      | 11/40          | 11 IC2-LoM                          | IVF, ICSI       |
| Sutcliffe et al., 200634      | 11/79          | 8 IC2-LoM                           | ICSI, IVF, ovulation induction |
| Bowdin et al., 200735         | -              | 1 IC2-LoM                           | IVF, ICSI       |
| Doombos et al., 200736        | 6/71           | 4 IC2-LoM                           | IVF, ICSI       |
| Lim et al., 200937            | 25/112         | 24 IC2-LoM, 1 negative              | IVF, ICSI       |
| Hiura et al., 201227          | 6/70           | 1 IC2-LoM                           | ICSI            |
| Tee et al., 201328            | 14/187         | 14 IC2-LoM                          | –               |
| Tenorio et al., 201641        | 17/156         | 15 IC2-LoM, 2 negative              | IVF, ICSI       |
| Johnson et al., 201829        | 16/40          | 15 IC2-LoM, 1 UPD(11)pat            | IVF             |
| Duffy et al., 201923          | 40/208         | 34 IC2-LoM, 3 IC1-GoM, 3 UPD(11)pat | IUI, IVF, ICSI  |
| Hattori et al., 201930        | 7/117          | 3 IC2-LoM, 1 IC1-GoM, 1 negative    | Ovulation induction, IVF, ICSI |
| Hara-Isono et al., 202031     | 8/31           | 6 IC2-LoM and 2 IC1-GoM             | IVF, ICSI, FER  |
| Eltan et al., 202032          | 1/0            | 1 IC2-LoM                           | IVF             |
| Total                         | 186/1446       | 152 IC2-LoM, 5 UPD(11)pat, 6 IC1-GoM, 5 negative | –               |

Abbreviations: ART, artificial reproduction techniques; BWSp, Beckwith-Wiedemann spectrum; IC1-GoM, imprinting center 1 gain of methylation; IC2-LoM, imprinting center 2 loss of methylation; ICSI, intracytoplasmatic sperm injection; IUI, intrauterine insemination; IVF, in-vitro fertilization; UPD(11) pat, chromosome 11 paternal uniparental disomy.

“UPD(11)pat was excluded.

Figure 1 reports the distribution of the molecular subgroups in our non-ART-BWSp and ART-BWSp cohorts, and in the literature-derived ART-BWSp cohort, including only patients with positive molecular test. IC2-LoM cases were 78.5% versus 93.2% in the literature-derived ART-BWSp (p < 0.001) and 59.2% in our non-ART-BWSp cohort, respectively. The distribution of the molecular subtypes in our ART-BWSp cohort was significantly different from that of the literature-derived ART-BWSp cohort. This difference with previous literature is due to a higher fraction of UPD(11)pat cases in our ART-BWSp cohort compared to the literature (18.5% vs. 3.4%, p < 0.001). Overall, both the distribution of the molecular subgroups in the ART-BWSp cohort was different from that of the non-ART cohort (p = 0.018), and this was mostly due to a higher fraction of
| Condition                                      | IC2-LoM ART | IC2-LoM not-ART | p     | UPD(11)pat ART | UPD(11)pat non-ART | p     | Total ART | Total not-ART | p     |
|-----------------------------------------------|-------------|----------------|-------|---------------|-------------------|-------|-----------|---------------|-------|
| Neonatal hypoglycemia/hyperinsulinism         | 20 (39.2%)  | 55 (30.6%)     | 0.244 | 6 (50.0%)     | 29 (34.9%)        | 0.312 | 34 (46.0%)| 96 (31.6%)    | 0.020 |
| Macroglossia                                  | 42 (82.5%)  | 158 (87.8%)    | 0.316 | 7 (58.3%)     | 56 (67.5%)        | 0.531 | 56 (75.7%)| 249 (81.9%)   | 0.223 |
| Abdominal wall defects                        | 34 (66.7%)  | 118 (65.6%)    | 0.883 | 7 (58.3%)     | 41 (49.4%)        | 0.563 | 49 (66.2%)| 188 (61.8%)   | 0.485 |
| Omphalocele                                   | 7 (13.7%)   | 52 (28.9%)     | 0.028 | 2 (16.7%)     | 6 (7.2%)          | 0.271 | 9 (12.2%) | 68 (22.4%)    | 0.051 |
| Umbilical hernia/diastasis recti             | 27 (52.9%)  | 66 (36.7%)     | 0.036 | 5 (41.7%)     | 35 (42.2%)        | 0.974 | 40 (54.1%)| 119 (39.1%)   | 0.020 |
| Lateralized overgrowth                        | 26 (51.0%)  | 85 (47.2%)     | 0.635 | 11 (91.7%)    | 69 (83.1%)        | 0.449 | 42 (56.8%)| 168 (55.3%)   | 0.817 |
| Organ enlargement                             | 9 (17.6%)   | 49 (27.2%)     | 0.164 | 3 (25.0%)     | 30 (36.1%)        | 0.449 | 44 (18.9%)| 101 (33.2%)   | 0.016 |
| Ear pits or creases                           | 16 (31.4%)  | 90 (50.0%)     | 0.018 | 7 (58.3%)     | 33 (39.8%)        | 0.223 | 30 (40.5%)| 136 (44.7%)   | 0.514 |
| Angioma at the glabella                       | 27 (52.9%)  | 87 (48.3%)     | 0.561 | 4 (33.3%)     | 28 (33.3%)        | 0.978 | 37 (50.0%)| 128 (42.1%)   | 0.219 |
| Polyhydramnios                                | 8 (15.7%)   | 26 (14.4%)     | 0.825 | 2 (16.7%)     | 11 (13.3%)        | 0.748 | 10 (13.5%)| 48 (15.8%)    | 0.626 |
| Neonatal macrosomia                           | 22 (43.1%)  | 102 (56.7%)    | 0.087 | 4 (33.3%)     | 53 (63.9%)        | 0.044 | 28 (37.8%)| 189 (62.2%)   | <0.001*|
| Malignant tumors                              | 0 (0%)      | 3 (1.7%)       | 0.989 | 2 (16.7%)     | 13 (15.7%)        | 0.929 | 2 (2.7%)  | 23 (7.6%)     | 0.131 |
| Twin delivery                                 | 10 (19.6%)  | 10 (5.6%)      | 0.002 | 0             | 0                 | -     | 11 (14.9%)| 10 (3.3%)     | <0.001*|
| Gestational age                               | 36.4 ± 2.6  | 37.1 ± 2.5     | 0.082 | 37.0 ± 4.1    | 38.1 ± 1.7        | 0.290 | 36.4 ± 2.9| 37.2 ± 2.5    | 0.017*|
| Weight at birth (SDS)                         | 1.2 ± 1.6   | 1.8 ± 1.5      | 0.049 | 12 ± 1.7      | 2 ± 1.5           | 0.093 | 1.2 ± 1.9 | 2.1 ± 1.8     | <0.001*|
| Length at birth (SDS)                         | 1.1 ± 1.5   | 1.5 ± 1.6      | 0.112 | 0.7 ± 1.3     | 1.4 ± 1.4         | 0.106 | 0.8 ± 1.5 | 1.6 ± 1.6     | <0.001*|
| Head circumference at birth (SDS)             | 0.5 ± 1.5   | 1.0 ± 1.3      | 0.020 | 0.0 ± 0.9     | 0.8 ± 1.1         | 0.018 | 0.4 ± 1.4 | 1 ± 1.3       | <0.001*|

Abbreviations: IC1-GoM, imprinting center 1 gain of methylation; IC2-LoM, imprinting center 2 loss of methylation; SDS, standard deviation score; UPD(11)pat, chromosome 11 paternal uniparental disomy.

*Statistically significant.
IC2-LoM cases ($p = 0.004$); conversely, the UPD(11)pat frequency in our ART-BWSp cohort (18.5%) was not significantly different from that observed in the non-ART BWSp cohort (27.3%), but it significantly higher than that reported in the literature ART-BWSp one (3.0%, $p < 0.001$). IC1-GoM was underrepresented in both the ART cohorts compared to the naturally conceived BWSp cohort (8/230, 3.5% vs. 31/304, 10.2%, $p = 0.004$). No patient with CDKN1C mutation was observed in the ART cohorts.

Table 4 reports the comparison of the clinical features of cases with IC2-LoM and UPD(11)pat in the ART-BWSp and naturally conceived BWSp patients. We observed that omphalocele was less frequent in the IC2-LoM ART-BWSp subgroup than that in the naturally conceived patients with the same molecular defect (13.7% vs. 28.9%, $p = 0.028$); however, minor abdominal wall defects were more common in the former than in the latter group (52.9% vs. 36.7%, $p = 0.036$). Moreover, neonatal overgrowth was less common and birth parameters were lower in the ART-BWSp patients than in those conceived naturally (33.3% vs. 63.9%, $p = 0.044$). Also the auricular anomalies were less represented in the ART-IC2-LoM group than in the non-ART-IC2-LoM one (31.4% vs. 50.0%, $p = 0.018$). Overall, twin births were more common in the ART-BWSp than in the naturally conceived BWSp patients (14.9% vs. 3.3%, $p < 0.001$); among the twins, there was a significant difference between the IC2-LoM patients conceived after ART (19.6%) and those conceived naturally (5.6%, $p = 0.002$). Finally, gestational age was lower among the ART-BWSp patients than those conceived naturally (36.4 ± 2.9 vs. 37.2 ± 2.5 weeks, $p = 0.017$).

Since its institution in 2005 to the last registry data release in 2019, 172,568 children from ART were registered in the Italian ART Registry, including the 67 patients with BWSp of our cohort. This allowed to calculate a minimum prevalence of BWSp of 1 in 2575 live births. Over this period, the number of patients with BWSp conceived though ART born each year showed a constant increase over the years ($r^2 = 0.657$, $p < 0.001$), paralleling that of births after ART (Figure 2). The comparison between our ART-BWSp cohort and the children conceived after ART in the 2005 to 2018 time-period showed that the patients with ART-BWSp were more commonly premature (42.5%) than both those from the ART Italian Registry (20.8%, $p = 0.022$) and the naturally conceived BWSp children (28.6%, $p < 0.001$). The rate of twin births in our ART-BWSp cohort (14.9%) and in the Italian ART Registry (16.7%) was similar, but both were higher than those observed in the naturally conceived BWSp patients (3.3%, $p = 0.524$).

### 4 DISCUSSION

Since its first report, many data on the association between ART and BWSp have accumulated: most studies concluded that the ART rate in the BWSp cohorts is higher than in the general population indicating that the risk of BWSp in the children born after ART is 10-folds than those conceived naturally. To gain further insights into the relationship between ART and BWSp, here we report the molecular and clinical features of a large cohort of patients with ART-BWSp and compare this cohort with our historical cohort of non-ART-BWSp, with an ART-BWSp derived from published data, and with the non-BWSp-ART children of the Italian ART Registry.

These results contrast with previous reports stating that the molecular abnormality of ART-BWSp patients is almost exclusively (>90%) IC2-LoM. Instead, in our ART-BWSp cohort the fraction of cases with IC2-LoM was 78.5%, much closer to that of the naturally conceived patients in our (59%) and other cohorts (50%–60%). The differences in the molecular breakdown between our ART-BWSp cohort and the cohort of the literature largely result from a higher prevalence of UPD(11)pat: this molecular defect is nearly 16% in our ART-BWSp group, but only 3% previously reported ART-BWSp cases. This discrepancy could be due to a selection bias (i.e., previous studies mostly focused on methylation anomalies) or lower sensitivity of diagnostic tests in the older studies. For instance, a possible explanation could be the age at patients’ evaluation: earlier studies and cohorts from laboratory referrals might have likely investigated younger patients (perhaps after birth) and not based on a lasting follow-up: this might have led to underdiagnose cases presenting later in childhood, as typically happens in patients with mild UPD(11)pat. Another possibility is that mosaic UPD(11)pat might have been incorrectly diagnosed as IC2-LoM in the older studies. Copy-neutral segmentally restricted and mosaic UPD is thought to arise post-zygotically from homologous recombination due to repair of double-stranded DNA breaks. This type of UPD is quite rare in congenital diseases and in BWS is associated with cell growth advantage due to duplication of the paternal and loss of the maternal imprinted 11p15.5 genes. It is possible that the characteristics of the parents undergoing ART
(e.g., health conditions, cause of subfertility) may predispose to this mitotic error in the embryo.

Many of the genotype-phenotype correlations previously reported in the BWSp\textsuperscript{13,15,17,23,38} were grossly confirmed in our ART-BWSp cohort as well. In particular, lateralized overgrowth and tumors were more common in the subgroup with UPD(11)pat, renal/ureteral anomalies less common in the IC2-LoM group, as observed previously.\textsuperscript{39,40} However, some features were less common and less severe in the ART patients’ group than in the naturally conceived patients. Patients with ART-BWSp tended to have less severe abdominal defects with a lower incidence of omphalocele, less commonly had macrosomia, and showed lower birth parameters, and fewer cases had ear signs. A milder phenotype in cases from ART with respect to the naturally conceived ones was consistent with previous observations.\textsuperscript{41} These differences were mostly evident in the IC2-LoM group, although a tendency was also observed in the UPD(11)pat one.

Although the milder phenotype observed in the ART-BWSp group could simply result from the smaller sample size, on the other hand, the lower incidence of major abdominal wall defect could be the result of probably higher rate of pregnancy termination in cases with severe malformation diagnosed at the prenatal ultrasound. The smaller fetal size (and the lower rate of overgrowth) could be attributable to an average more diseased pregnancy in ART, to an average higher parental age, or to the higher incidence of multiple pregnancies in this group. Accordingly, the ART group also had a lower mean gestational age at birth with a higher incidence of preterm births. The milder phenotype we observed in the IC2-LoM born from ART and naturally conceived, however, could also result from a different timing of onset of the methylation defect during the blastogenesis, resulting in a less represented mosaic in patients with ART-BWSp. However, we did not observe any correlation between the kind of technique used, nor with other variables as cause of infertility or parental age.

A higher rate of twin births was observed in our cohort, compared with that of naturally conceived patients with BWSp (14.9% vs. 3.3%) and no twins births were observed in the subgroup with UPD(11)pat. These observations further corroborate the hypothesis of the close interconnection between methylation abnormalities, maternal infertility, oocyte abnormalities, disruption of early embryo developmental stages, and twinning.\textsuperscript{42,43}

Finally, this study shows that the incidence of patients with BWSp conceived thought ART increase over time paralleling the trend of ART in Italy over the last decades and may therefore further change in the future consistent with ART usage. Although the ART-BWSp cohort we collected in this study is far from including all the Italian BWSp patients conceived thought ART, we used our data to estimate a minimum prevalence of BWSp in ART-conceived children, resulting in nearly 1:2500 live births. This estimate is less precise than that we previously calculated on the basis of regional data based on merged ART/BWSp patients’ registries (1:1126),\textsuperscript{6} but provides the first minimum prevalence appraisal on a national basis.

In conclusion, this study describes the clinical and molecular features of the largest cohort of patients with BWSp born though ART, making a comparison with previous literature, with naturally conceived patients with BWSp, and with the general population from ART. These results allow spotting some new insights into the connection between ART and BWSp. First, the breakdown of the various molecular subtypes of BWSp is not greatly different in the ART and the naturally conceived patients as previously thought, while UPD(11) pat fraction is similar in the two groups, in contrast with previous reports. Second, there is evidence that patients with BWSp born though ART might have a milder phenotype. Finally, our data first show a progressive increase in the prevalence of BWSp over time, paralleling that of ART usage in the last decades.

ACKNOWLEDGMENTS

The authors are grateful to the patients, their family members and the Italian Association of Patients with BWS (AIBWS.org) for their collaboration. Open Access Funding provided by Università degli Studi di Torino within the CRUI-CARE Agreement.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14193.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Carli D, Operti M, Russo S, et al. Clinical and molecular characterization of patients affected by Beckwith-Wiedemann spectrum conceived through assisted reproduction techniques. Clinical Genetics. 2022;102(4):314-323. doi:10.1111/cge.14193