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Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients

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Abstract

Objective—An association between assisted reproductive technique (ART) and specific imprinting mutations, such as Beckwith-Wiedemann syndrome (BWS), has recently been documented. Based on experiments in farm animals that demonstrated an association between alterations in culture media during ART and large offspring syndrome, we hypothesized that the culture media could be implicated as a common factor among the children with BWS conceived after ART.

Design—Retrospective case series.

Setting—Registry from Academic Medical Center.

Patient(s)—Nineteen children born after ART were identified within the registry.

Main Outcome Measure(s)—Demographics of patients, type of ART, culture media, IVF parameters.

Result(s)—Twelve of the 19 medical records from the reproductive endocrine centers were successfully obtained. Ten of 12 mothers of children with BWS had IVF, but no single, consistent culture media was used in this group. Half of the patients underwent IVF with intracytoplasmic sperm injection (ICSI; n = 5), whereas the other half had routine IVF. One child was conceived through clomiphene citrate (CC) stimulation and artificial insemination, whereas another patient conceived through gonadotropin stimulation with intrauterine insemination (IUI). The gonadotropin dosage and quantity of embryos transferred also varied significantly. The only consistent finding was that all 12 women received some type of ovarian stimulation medication.

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Conclusion(s)—Large epidemiologic studies are needed to further study the association between BWS and ART.

Keywords

Beckwith-Wiedemann syndrome; assisted reproductive technology; in vitro fertilization

Beckwith-Wiedemann syndrome (BWS) is a congenital disorder associated with a constellation of findings including macrosomia, macroglossia, midline abdominal defects, and a predisposition to embryonal cancer (MIM 130650). The molecular etiology of BWS includes both genetic and epigenetic mutations (1). More specifically, BWS has been shown to involve a loss of imprinting (LOI) of a group of imprinted genes on 11p15.

Approximately 42% of patients with BWS have aberrant methylation and imprinting of LIT1 (MIM 604115), an untranslated RNA (1, 2). Approximately 11% of patients with BWS have aberrant methylation of H19 (MIM 103280) (1), an untranslated RNA coupled with abnormal expression of IGF2. Approximately 13% of patients with BWS have altered methylation of both H19 and LIT1, and of these 75% have uniparental disomy (UPD) of 11p15, and the remaining 25% have an extensive imprinting disturbance throughout 11p15 (1). Finally, 5% of the patients with BWS have a mutation in P57^{K1P2} (MIM 600856) (3).

An association between assisted reproductive technology (ART) and congenital malformation syndromes associated with imprinting defects has recently been documented (4, 5). Three groups have reported an increased rate of ART conceptions among children with BWS (5–7). Only one of these studies prospectively determined the incidence of ART in children with BWS (5). Combined data from two studies suggest that approximately 4% of the children with BWS were born after ART (5, 7).

Angelman syndrome, a congenital malformation syndrome characterized by mental retardation, motor defects, and lack of speech is associated with imprinting abnormalities and ART (MIM 105830) (4). Angelman syndrome occurs in children with imprinting abnormalities of a gene cluster on chromosome 15 resulting in a loss of function of the maternal allele of ubiquitin-protein ligase E3A (UBE3A) (MIM 601623) (8). Three children with Angelman syndrome have been reported who were conceived after IVF with intracytoplasmic sperm injection (ICSI) lending further support for the association between congenital malformation syndromes with imprinting mutations and ART (4, 9).

Results from two embryo experiments conducted in cows and mice led to this proposed study. First, Thompson et al. (10) demonstrated that they could decrease the frequency of large offspring syndrome (LOS) in farm animals after ART by changing the protein supplementation in the culture medium from sera to albumin and amino acids. The LOS refers to a syndrome that occurs after ART in sheep and cows and is associated with macrosomia, polyhydramnios, hypoglycemia, and organomegaly (11). Second, Doherty et al. (12) showed that in preimplantation mouse embryos, the type of culture medium could alter the expression of H19. Based on these studies, we hypothesized that the culture media used for the embryos or blastocysts could be a common factor among children with BWS born after IVF. To test this hypothesis, we reviewed the laboratory data from the reproductive endocrine centers of mothers who gave birth to children with BWS after ART.

MATERIALS AND METHODS

We initially established the BWS Registry in 1994 at the Genetic Epidemiology Branch of the National Cancer Institute (NCI) to understand the natural history of this syndrome and to determine whether a genotype–phenotype relationship exists (13). For those families who agreed to participate, a comprehensive questionnaire was completed. Informed consent and medical information release documents were obtained. These documents were approved by the Institutional Review Board. For the purposes of the study, we defined a patient as having BWS if a clinical diagnosis of BWS had been made by a physician and the patient had at least two of the five most common features associated with BWS (14): [1] macroglossia, [2] birth weight greater than 90 percentile (15, 16), [3] hypoglycemia in the first month of life, [4] ear creases or ear pits, and [5] midline abdominal wall defects (e.g., omphalocele, diastasis recti, and umbilical hernia). All positive phenotypic features were diagnosed by a physician and were documented in the questionnaire.

In June 2001, the Registry was moved to the Washington University School of Medicine where the Human Studies Committee approved the new questionnaire. We used the same research definition for the Washington University BWS registry and the NCI BWS Registry. The only difference between the two questionnaires is that the new questions were added that pertained to the method of conception (natural vs. ART) and we omitted questions with regard to parental occupation at the time of conception. Assisted reproductive technology was broadly defined to include any artificial attempts at improving fertility including artificial insemination, ovulation induction (CC or gonadotropins), and IVF. The type of ART was ascertained, specifically, artificial insemination using biological father's sperm or donor sperm, ovulation induction with natural intercourse or artificial insemination, IVF using biological mother's egg and donor sperm, and IVF using donor egg and donor sperm. If IVF was used, we asked what type of ICSI was used: ICSI using ejaculated sperm, ICSI using testicular sperm extraction (TESE), or ICSI using microsurgical epididymal sperm aspiration. Between 1994 and October 2003, a total of 341 patients with BWS from 291 families were recruited to the Registry.

To better define the cause, if any, for the association between ART and BWS, we did the following: first, we reviewed all written comments made by parents who completed the original BWS registry started at the NCI ($n = 262$). We specifically determined whether the mothers received ART. The new BWS questionnaire asked the method of conception or if drugs were used for ovarian stimulation. For those women who had possibly undergone ovarian stimulation or some other form of ART, we attempted to secure the reproductive endocrine medical records from their treatment centers. Data on parental occupations was not obtained for the Washington University Registry but was available for the NCI registry. Specific occupations were assigned approximate income values using the Occupational Information Network online database and selecting median incomes for the United States for the year 2002.

Comparisons between children with BWS who were conceived with and without ART were performed in SPSS 10.0 (Statistical Package for the Social Sciences, Chicago, IL). Continuous variables were compared using independent t tests. A χ^2 statistic and two-sided

Fisher's exact test were used to test for significance. When the expected value in any cell of the tabulation was <5 , Fisher's exact test was used. P values $<.05$ were considered significant for all the statistical tests.

RESULTS

Patient Demographics

Nineteen children from the BWS Registry ($n = 341$) were born after ART. Only parental age and household income differed when comparing the demographics features in children with BWS born after ART to other children born after natural conception in the Registry. Fathers of children with BWS in the ART group were an average of 36.9 years old (range 32.0 – 45.0 years) at the time of delivery, which was significantly older than the fathers of children with BWS conceived without ART who were an average of 32.1 years old (range 17.9 – 64.3 years) ($P=.005$). Mothers of children with BWS born after ART were older when compared to those conceived without ART, 35.2 years (range 30.6 – 43.7 years) and 29.4 years (range 15.5– 41.7 years), respectively ($P .001$). The children conceived after ART were not significantly different than the rest of the patients in the BWS registry with respect to age and gender. The approximate annual household income for families participating in the NCI Registry was \$70,584 (range 0 –\$278,900). Household income differed between the families with a child with BWS born after ART ($n = 5$) when compared to the children born after natural conception ($n = 207$). We did not obtain household occupation from the remaining 14 patients. The mean annual income for the households with a child with BWS born after ART was \$120,720 (range \$66,700 –\$278,900), whereas the households with a child with BWS born after natural conception had a mean annual income of \$69,373 (range 0 – \$216,600).

No Phenotypic Differences Occurred Between Children Born After ART vs. Natural Conception

Children with BWS conceived after ART in the Registry had similar clinical features as children conceived naturally. Children with BWS who were born after ART had rates of hypoglycemia (21.0%, 4 of 19), macroglossia (89.5%, 17 of 19), ear defects (72.2%, 13 of 18), abdominal wall defects (68.4%, 13 of 19), macrosomia (61.1%, 11 of 18), and embryonal tumors (11.1%, 2 of 18), which did not differ significantly from the rates of those features in the children with BWS conceived without ART (data not shown). The clinical features of the children with BWS born after ART are shown in Table 1.

Type of ART Was Not Associated with BWS

We were able to obtain the reproductive endocrine medical records on 12 of 19 mothers. Records from five mothers were not sent from their respective centers despite at least a dozen requests from the treating reproductive endocrine physicians. The mothers of two children could not be located. Each medical record contained extensive information on ART treatment and management course. All 12 mothers were treated at different reproductive endocrine and infertility centers. Ten of 12 of the patients underwent IVF. Of the 10 mothers that received IVF, ICSI was performed in 5. One of the five mothers had a partial ICSI procedure with half of the embryos inseminated by traditional insemination and the other

half inseminated by ICSI. Of the 10 patients, there were five sets of dizygotic twins in which only one twin was affected.

Two children were conceived without IVF. One child was conceived through CC stimulation and artificial insemination of donor sperm, whereas another patient was conceived through gonadotropin stimulation with IUI. Table 2 describes the method of ART in the 12 children.

Use of Ovarian Stimulation Medication in Conception of a Child with BWS

All 12 women received medication to stimulate the ovaries. Two mothers received medications alone (CC or gonadotropins), and 10 mothers received ovarian stimulation as part of their IVF treatment. For the 10 receiving IVF, the quantity of gonadotropins administered for stimulation ranged from 16 to 102 ampules.

Diverse Etiologies of Infertility Among Parents of Children with BWS

No cause for infertility was common among the parents of children with BWS. The primary reasons for infertility for these parents ranged from endometriosis ($n = 4$), primary male infertility ($n = 4$), ovulatory dysfunction ($n = 2$), tubal factor ($n = 3$), or advanced maternal age ($n = 3$). Six of these patients had a combination of two or more of these factors.

Diverse Timing of Blastocyst Transfer and Media Type Used in Conception of Children with BWS

The number and timing of embryos transferred was variable (Table 2). The number of embryos/blastocysts that were transferred varied from one to eight in number. Half of the patients had embryo transfers 3 days after fertilization ($n = 5$), whereas the other half had blastocysts transferred on day 5 ($n = 5$).

The media used varied considerably and was not a common factor. Five different types of media were used for embryo culture. The media included sequential media from G1 to G2 (In Vitro Life) for blastocysts ($n = 3$), human tubal fluid (HTF; Irvine media) + serum substitute supplement (SSS) ($n = 2$), P1 ($n = 1$), P1 + SSS ($n = 2$), Cook's sequential media ($n = 1$), sequential media of P1 and then HTF + SSS ($n = 1$), sequential culturing in HTF, then P1 media, and then blastocyst media + SSS ($n = 1$) (Table 2). G1, G2, blastocyst medium, and Cook's sequential medium contained a number of amino acids, whereas HTF and P1 have no amino acid constituents. Each of the media combinations had a human sera albumin component either as a supplement (SSS) or within the media (G1, G2, and Cook's sequential media) except for one case of P1 alone.

DISCUSSION

In our case series, no specific ART method, specific in vitro media, or timing of embryo transfer was associated with BWS. Our original hypothesis was not supported by this case series, namely that the type of in vitro culture medium was the root cause for BWS after ART. One specific type of media did not predominate among the cases. Instead, the types of media varied, some glucose-rich (G2, blastocyst media), glucose-poor (HTF), or glucose-free (P1). The amino acid content in the medias also differed significantly, and no trend was

noted. The majority of the media combinations contained some form of human serum albumin but not sera that has been implicated as a causative factor in LOS in animal ART studies.

The cause of infertility also varied significantly from primary male infertility to female disorders such as ovulatory dysfunction, endometriosis, tubal factor, or uterine factor. The age of the mothers varied from 30.6 – 43.7 years. In addition, among the 19 patients with BWS, five were twins. We have no formal genetics study determining whether they were monozygotic or dizygotic; however, each set of these embryos appeared to be exposed to similar laboratory conditions and yet in three cases only one of the two twins had obvious stigmata associated with BWS. One set of dizygotic twins did result from a partial ICSI insemination where one-third of the embryos were fertilized through ICSI.

The observation that there were no common types of in vitro culture media or blastocyst transfer schedules among the patients with BWS was consistent with recent findings in studies failing to identify a single cause for LOS in farm animals conceived after IVF (11). Multiple factors have been implicated in LOS including high P levels, asynchrony between the uterus and embryo, and altered culture medium (11). Experiments conducted using conditions previously described as being associated with LOS have not resulted in consistent findings. The only common feature among the group who had children with BWS is the use of ovarian stimulation medication. We postulate that perhaps any manipulation of oocyte maturation or folliculogenesis can result in altered timing of the imprinted genes and lead to congenital malformations in humans. A larger study along with insight into the molecular mechanisms from farm animal models of overgrowth and IVF and newer mouse models for BWS (17) will be able to confirm or refute this postulate.

The epigenetic mutations have been previously reported for 6 of the 12 patients in this study. The molecular data were not available in the remaining patients. In the original study done by our group describing the association between BWS and IVF, imprinting abnormalities of both LIT1 and H19 were identified (5).

As with any case series, our study had inherent limitations. Given our small sample size, we did not have a large enough sample size to find a common factor. Due to the low incidence of BWS, between 1 in 12,000 and 1 in 15,000, only a substantially larger study with more children with BWS born after ART would allow us to exclude other factors. Based on the rare combination of both BWS and infants with BWS born after ART, an international effort would have to be undertaken with collaborations of parental support groups, obstetrician–gynecologists, and geneticists.

We could not exclude the possibility that parents of children with BWS after ART are infertile because they have a genetic or epigenetic mutation that may result in decreased fertility. Although plausible, we do not believe this to be the case because infertility is not a feature associated with BWS and none of the mothers and only one father had phenotypic features associated with BWS. Another possibility is that wealthy families are more likely to volunteer to be a part of the BWS registry and those individuals are more likely to have access to ART. We cannot directly exclude this possibility. Our estimates of income suggest

that the households with children with BWS had higher income than the U.S. Census Bureau's estimates for the population as a whole in 2002, with annual incomes of \$70,584 and \$42,409, respectively. These findings suggest that wealthier families joined the registry when compared to the general U.S. population. The well-documented high cost of ART suggests that household income may greatly influence who receives ART (18, 19). Given the demographics in our sample, we do not believe that the difference in family income is likely to account for the high rate of BWS after ART.

In summary, with the exception of taking ovarian stimulation medication either as part of IVF or to facilitate conception, no common factor was identified among the reproductive endocrine medical records of women who gave birth to children with BWS. Larger prospective studies are needed to systematically assess the potential risk factors associated with BWS after ART.

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APPENDIX

Electronic Database Information

Ascension numbers and URLs for data presented herein are as follows:

Occupational Information Network O*NET online, <http://online.onetcenter.org>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

U.S. Census Bureau Current Population Reports: Income in the United States 2002, www.census.gov/hhes/income/income02.sa.pdf

BWS REGISTRY DATABASE EMAIL, bwsregistry@kids.wustl.edu

TABLE 1
Clinical characteristics of patients with BWS who were conceived with assisted reproduction.

Patient	Hypoglycemia	Macroglossia	Ear anomaly	Midline abdominal wall defect	Macroemia	Hemihypertrophy	Embryonal tumor
Patient 1	No	Yes	Yes	Yes	No	No	No
Patient 2	No	Yes	Yes	No	Yes	Yes	No
Patient 3	No	Yes	Yes	No	Yes	No	No
Patient 4	Yes	Yes	Yes	Yes	Yes (twin)	No	No
Patient 5	Yes	Yes	No	Yes	Yes	No	No
Patient 6	No	Yes	Yes	Yes	Yes	No	Wilms
Patient 7	No	Yes	Yes	Yes	No	Yes	No
Patient 8	No	Yes	Yes	Yes	No	Yes	No
Patient 9	Yes	Yes	Yes	Yes	No (twin)	No	No
Patient 10	No	Yes	No	Yes	No	No	Hepatoblastoma
Patient 11	No	No	Yes	Yes	Yes	Yes	No
Patient 12	No	Yes	Yes	Yes	Yes (twin)	No	No
Patient 13	No	Yes	Yes	Yes	Yes	No	No
Patient 14	No	Yes	No	Yes	No	No	No
Patient 15	No	Yes	Yes	Yes	Yes	No	No
Patient 16	No	No	Yes	Yes	No (twin)	No	No
Patient 17	No	Yes	No	No	No (twin)	No	No
Patient 18	No	Yes	Yes	Yes	Yes	No	No
Patient 19	Yes	Yes	No	Yes	Yes	Yes	No

Note: BWS = Beckwith-Wiedemann syndrome.

TABLE 2

Assisted reproduction techniques used before conception of patients with BWS.

Patient	Day of transfer	Use of ICSI	Culture media
Patient 1	3	No	P1 + SSS
Patient 2	5	Yes	G1, G2
Patient 5	5	Yes	G1, G2
Patient 7	5	No	HTF + SSS
Patient 8	5	No	P1 blastocyst media
Patient 9	3	Partial	HTF + SSS
Patient 12	3	Yes	P1 + SSS, HTF
Patient 13	3	No	P1
Patient 14	3	Yes	Cook's sequential
Patient 19	5	No	G1, G2
Assisted reproduction without IVF			
Patient 3	Clomiphene citrate and intrauterine insemination		
Patient 10	Ovulation induction and intrauterine insemination		

Note: Abbreviations as in Table 1 and text.