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ASSISTED REPRODUCTION TECHNOLOGIES

A case series to examine the perinatal outcomes of infants conceived by intravaginal culture (IVC)

Madeline Kaye[1] · Elizabeth Williams[2] · Anthony Anderson[3] · Francisco Arredondo[4] · Jordyn Pike[1,5] · Winifred Mak[1,5]

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Abstract

Purpose In vitro fertilization (IVF) has been a well-established method for treating infertility for over four decades. The mainstay method of culture of oocytes and embryos has been in gas incubators. More recently, the novel use of a gas-permeable closed vessel to culture oocytes and embryos in the vagina, intravaginal culture (IVC), has been introduced as a viable lower-cost option for infertility patients. Several studies have studied the efficacy of IVC; however, there is no data on the perinatal outcomes of the babies born using this newer technology.

Methods Our study is a retrospective case series ($n=66$) from a single center, uniquely examining the perinatal outcomes of infants born after IVC.

Results There were 50 singleton and 16 twin gestations in this case series. For singleton infants conceived via IVC ($n=50$), the mean gestational age at delivery was 38 weeks and 4 days, and the mean birth weight was 3159.1 + / − 501.5 g. Four infants were born with low birth weight, three were born preterm, and one was born macrosomic. The twin pregnancies had a mean gestational age at delivery of 33 weeks 4 days and a mean birth weight of 1992.9 + / − 620.7 g. Twenty-seven infants met the criteria for low birthweight, and twenty-four infants delivered preterm. No twin infants met the criteria for macrosomia.

Conclusion This case series provides an initial description of the perinatal outcomes of IVC conceived infants, which shows no concerning trends in adverse birth outcomes for singleton infants. As expected, IVC twin gestations had a high rate of low birth weight and preterm delivery. Continued larger studies are essential to provide more comprehensive data on perinatal outcomes of infants conceived by this new technology.

Keywords Intravaginal culture · In vitro fertilization · Perinatal outcomes

Introduction

Intravaginal culture of oocytes (IVC) was first described by Ranoux et al. in 1988 with the goal of developing a simplified alternative to conventional in vitro fertilization using gas incubators (IVF) [1]. Ranoux introduced the concept of using a gas-permeable closed device containing the culture medium for oocyte culture with insertion into the vagina to maintain a stable temperature and pH, thus creating an environment conducive to fertilization. This method offers advantages including decreased oocyte manipulation, decreased need for lab materials and personnel, elimination of the need for a gas incubator, and ultimately significantly decreased cost of assisted reproduction. Sterzik also points out that this method allows the patient to be intimately involved with the conception process, which can have a profound psychological impact [2]. With increasing numbers of patients with infertility, access to more affordable assisted reproductive technology is paramount.

In 2015, the FDA approved the use of the INVOcell™ intravaginal culture system for the culture of human gametes and embryos for up to 72 h. Several studies have demonstrated the efficacy of the IVC procedure for 72 h and beyond, with clinical pregnancy rates between 20 and 59%

✉ Winifred Mak
winifred.mak@austin.utexas.edu

1 Department of Women's Health, Dell Medical School, University of Texas at Austin, Austin, TX, USA

2 HavenCryo, San Antonio, TX, USA

3 Embryodirector.Com, San Antonio, TX, USA

4 Positib Fertility, Monterrey, Mexico

5 Texas Advance Computing Center, Austin, TX, USA

[3–5]. However, at this time, there are no published studies investigating birth outcomes from infants conceived via IVC. There is a plethora of literature demonstrating differences in perinatal outcomes of infants conceived via assisted reproductive technology (ART) compared to spontaneous live births and birth outcomes amongst differing ART techniques. Therefore, for IVC to be considered as a viable alternative to conventional IVF, it is vital to ensure that it does not adversely affect the birth outcomes of infants born using this new technology. The study clinic was one of the early adopters of this technology and has had 74 live births using IVC since 2016. The aim of our study was to examine the perinatal outcomes of infants born after IVC.

Materials and methods

This study was a retrospective case series of patients receiving care at a single private clinic. The study period started in 2016, when IVC was introduced into the practice until 2019. Patients who meet the following inclusion criteria were included: (1) embryo culture using INVOcell™ device; (2) at least one blastocyst for transfer on day 5; (3) positive pregnancy test; (4) the first cycle of IVC. Exclusion criteria are pregnancies with incomplete data. Ninety-six pregnancies were included.

Deidentified data collected for SART (society for assisted reproductive technology) cycle submission was used for this study. Data obtained included demographic information including age, patient, and cycle characteristics such as infertility diagnosis, anti-mullerian hormone (AMH), estradiol (E2) at the time of trigger, number of oocytes retrieved, number of blastocysts transferred, gestational age, and birth weight. Spontaneous abortion is defined as pregnancy loss prior to 20 weeks gestation. Stillbirth is defined as pregnancy loss after 20 weeks gestation. Vanishing twin is defined as the presence of two gestation sacs at the first ultrasound and subsequent singleton live birth. Chart review was conducted only if there was missing data. The data were deidentified and deemed exempt by the University of Texas, Austin Institutional Review Board committee.

Included patients underwent ovarian stimulation either with clomiphene citrate ($n=45$), letrozole ($n=16$), or leuprolide acetate flare ($n=5$) and received 100 IU of FSH starting on day 3 after oral medication if BMI < 30 or 150 IU of FSH if BMI > 30. An antagonist for ovulation prevention was used when at least two follicles reached 16 mm. Oocyte retrieval was performed 36 h after 10,000 IU HCG (Novarel) trigger. Clomiphene and letrozole minimal stimulation protocols were used primarily to reduce medication costs for patients. Oocytes were collected, dissected from the coronal cells, and inseminated with 20,000 sperm per droplet. This mixture was incubated for 5 min before loading into the INVOcell™ device, which was preloaded with 1 ml of equilibrated culture media (CooperSurgical SAGE 1-Step). The loaded INVOcell™ device was placed in the patient's upper vagina for 5 days with the retainer. The intravaginal culture for 5 days was an off-label use of the INVOcell™ device with patient consent. The device was removed, and the embryos were evaluated using standard morphological parameters for suitability for embryo transfer. Depending on the grade of embryos and physician preference, one or two blastocysts were selected for transfer immediately, while any additional viable embryos were frozen for future use. Intramuscular progesterone in oil (50 mg) was used for luteal support until 9 weeks gestation.

Results

During the study period of 2016–2019, 233 IVC cycles were carried out at this single clinic. Ninety-six pregnancies resulted from these IVC cycles. Nine were biochemical pregnancies (9.4%), and 12 were spontaneous abortions (12.5%). There was one stillbirth. There were a total of 74 live births. There was incomplete data for 8 live births, which were excluded. The perinatal outcomes of 66 live births (82 infants) were investigated, including 50 singletons and 16 twin gestations. Twenty-two pregnancies had two gestational sacs present at the initial ultrasound, 16 of who went on to deliver twins, and 5 of whom had vanishing twins.

The baseline characteristics of the IVC patients are summarized in Table 1. The mean maternal age was 31.8 ± 3.8 years old, and the mean AMH was 4.7 ± 3.8 ng/mL. In the study clinic, IVC cycles were offered primarily to good prognosis patients with no or mild male factor, same-sex couples, and/or tubal factor. This is reflected in the breakdown of infertility diagnoses, with most patients

Table 1 Baseline maternal characteristics

Characteristic	IVC ($n=66$)
Age (years)[a]	31.8 +/− 3.8
AMH (ng/mL) [a]	4.7 +/− 3.8
Infertility diagnosis	20 (30.3)
Tubal[b]	11 (16.7)
PCO/anovulatory[b]	2 (3.0)
Male[b]	8 (12.1)
Same-sex[b]	3 (4.5)
Diminished ovarian reserve (DOR)[b]	4 (6.1)
Endometriosis[b]	16 (24.2)
Idiopathic[b]	2 (3.0)
Other[b]	
Use of donor sperm[a]	13 (19.7)

IVC intravaginal culture

[a]Mean±SD; [b](n) (%)

having tubal factor, polycystic ovary (PCO)/anovulatory, idiopathic infertility, or in a same-sex relationship.

The IVF cycle characteristics of each group are shown in Table 2. Estradiol level prior to transfer was 1855.0 ± 963.1 pg/mL. On average, approximately 12 oocytes were retrieved. Between 1 and 2 blastocysts were transferred, all on day 5.

The birth outcomes for singleton infants conceived via IVC are summarized in Table 3. Singleton IVC infants, on average, delivered at 270.5 days, or 38 weeks and 4 days gestation. The birth outcomes did not change when patients with vanishing twins were included. The average birth weight for singletons was 3194.9 g, or 7 pounds, 1 oz. When vanishing twins were included, this decreased slightly to 3159.1 g, and there was one additional low birth weight infant. There were no additional preterm deliveries when including pregnancies with a vanishing twin.

Birth outcomes for twin gestations conceived via IVC are summarized in Table 4. From 16 deliveries, 32 infants were born. IVC twin infants delivered earlier at 234.7 days, or 33 weeks and 4 days gestation, and their average birthweight was 1992.9 g, or 4 pounds 6 oz. Twenty-seven infants met the criteria for low birthweight, and twenty-four infants delivered preterm. No twin infants met the criteria for macrosomia.

Table 2 IVC cycle characteristics

Cycle characteristic	IVC ($n=66$)
Estradiol level prior to transfer (pg/mL) [a]	1855.0 ± 963.1 (414–4429)
Number of oocytes retrieved [a]	12.0 ± 6.1 (4–37)
Number of blastocysts transferred [a]	1.8 ± 0.4 (1–2)
Day of embryo transfer [a]	5 ± 0

[a]Mean±SD (range)

Table 4 Perinatal outcomes for twin deliveries

Birth outcome	IVC ($n=16$ deliveries/32 infants)
Gestational age (days) [a]	234.7 +/− 25.3
Birthweight (g) [a]	1992.9 +/− 620.7
Low birth weight (< 2500 g) [b]	27 (84.4)
Preterm delivery (< 37 weeks/256 days) [b]	24 (75.0)
Macrosomia (> 4000 g) [b]	0

[a]Mean±SD; [b] (*n*) (%)

Discussion

Our study uniquely examines the birth outcomes of infants conceived via an innovative ART technique, an intravaginal culture system. Though several studies have reported promising statistics on implantation and clinical pregnancy rates, data on infants born after this innovative technology has not been published. Furthermore, this technology has been touted as a method of decreasing the cost of ART significantly, and therefore, there will be an increase in utilization in the coming years. Therefore, data on the health outcomes of these infants is of the utmost importance.

Several studies have shown an increased risk of adverse perinatal outcomes such as the increased risk of preterm delivery, low birth weight, and macrosomia with ART, even after adjusting for multiple gestations, compared to

Table 3 Perinatal outcomes for singleton deliveries

Birth outcome	IVC ($n=45$ excluding vanishing twins, $n=50$ including vanishing twins)
Gestational age (days)[a]	270.5 +/− 11.4
Excluding vanishing twins	270.5 +/− 11.1
Including vanishing twins	
Birthweight (grams)[a]	3194.9 +/− 503.0
Excluding vanishing twins	3159.1 +/− 501.5
Including vanishing twins	
Low birth weight (< 2500 g)[b]	3 (6.7)
Excluding vanishing twins	4 (8.0)
Including vanishing twins	
Preterm delivery (< 37 weeks/256 days)[b]	3 (6.7)
Excluding vanishing twins	3 (6.0)
Including vanishing twins	
Macrosomia (> 4000 g)[b]	1 (2.2)
Excluding vanishing twins	1 (2.0)
Including vanishing twins	

[a]Mean±SD; [b](*n*) (%)

spontaneously conceived infants [6]. These outcomes are important as both low birth weight and macrosomia are associated with long-term adverse health outcomes, including increased risk of diabetes and cardiovascular disease for the former and obesity and kidney disease for the latter. Furthermore, these risks vary by type of ART, stimulation protocol, culture type, number of embryos transferred, time to culture, use of ICSI (intracytoplasmic sperm injection), and use of cryopreservation.

For example, a recent study investigated the perinatal outcomes of singleton births resulting from fresh and frozen transfers in a single US-based academic center over a 24-year period [7]. Their study included 9280 singletons from fresh transfers, including vanishing twins, and reported the unadjusted mean (SD) birthweight 3275 ± 605 g, mean gestational age 38.4 ± 2.1 weeks, 15.6% preterm delivery (PTD) rate, 7.3% small for gestational age, and 10.5% large for gestational age. Similarly, in the most recent 2019, CDC Assisted Reproductive Technology Surveillance which reported the birth outcomes of 65,332 singleton ART birth (fresh and frozen), 8.3% were born with low birth weight (LBW), 7% small for gestational age, and 15.4% were born preterm. Furthermore, the risk of low birth weight among singleton births with a vanishing twin is estimated to be significantly higher than that of singleton gestations from single embryo transfers [8]. In 2019 CDC Assisted Reproductive Technology Surveillance, 23.7% of singleton births with a vanishing twin were born preterm, and 24.5% of these infants were born with low birthweight. Therefore, in our case series, the IVC singleton infants, including vanishing twins, showed comparable perinatal outcomes (birthweight 3159.1 + / − 501.5 g, gestational age 38 + 4 weeks, 8% LBW) to reported outcomes of traditional IVF/ICSI conceived infants. However, interestingly, only 6% of IVC infants were born preterm.

In contrast, the IVC twin pregnancies had lower mean birthweight and gestational age at birth as well as a greater number were born preterm and with low birth weight. All these observations were predicted, given that they are multifetal gestations compared to singletons. It is well documented that the ART singletons are more likely to have adverse perinatal outcomes than spontaneously conceived singletons, whereas ART twins do not significantly differ from spontaneously conceived twins [9]. A UK study showed that IVF/ICSI twin pregnancies ($n = 171$) had similar perinatal outcomes to spontaneously conceived twins ($n = 368$) and reported IVF/ICSI twins had a mean birthweight for twin one 2471.8 ± 557 g and twin two 2451.1 ± 559.6 g, 41.6% were born LBW, 48.8% were born preterm [9]. Moreover, the national data from the 2019 CDC Assisted Reproductive Technology Surveillance shows 59.8% of twins conceived by ART were born preterm and 56.5% with low birthweight. Of note, the proportion of IVC twins born preterm and having low birth weight is higher than these prior reports. This could be due to our small sample size; therefore, larger cohorts are needed. Another explanation could be that DET were more likely to be performed in the IVC patients if there was suboptimal embryo quality; therefore, this could translate to more pregnancy-related complications leading to adverse perinatal outcomes.

Several differences in the characteristics of our IVC cohort could have influenced their perinatal outcomes:

1) Patient characteristics: The IVC patients were primarily good prognosis patients who are younger with a normal ovarian reserve and usually with tubal factor/idiopathic couples therefore, this could have influenced the perinatal outcomes. A prior study investigating the perinatal outcomes of couples with differing infertility diagnoses showed that preterm birth and low birthweight rates were the lowest in male factor and endometriosis patients [10].
2) Stimulation protocol: The majority of our IVC patients underwent clomiphene citrate or letrozole-based with low dose gonadotropin stimulation protocols to lower the medication cost and thus had lower estrogen exposure compared to other conventional ovarian hyperstimulation protocols. Prior studies have shown that hyperestrogenism leads to endometrial angiogenesis, impaired implantation, and abnormal placentation, thus leading to growth restriction of the fetus [11, 12]. This is thought to be one of the main contributors to the increased risk of low birth weight and preterm infants in ART pregnancies. Interestingly, this increased risk of low birth weight, and preterm delivery is significantly lessened with infants conceived after FET, which is thought primarily to be due to the lower E2 levels at the time of transfer in FET cycles compared to fresh transfers [13–15]. Therefore, the lower estrogen exposure during the IVC cycles could have affected the perinatal outcomes.
3) Intravaginal culture: IVC provides a unique environment for oocytes, sperm, and embryos entirely different from traditional incubators. The effects of intravaginal culture on early embryonic development have been studied and show that quality blastocyst development (> 2BB grade) with IVC (31%) is significantly lower than found using gas incubators (51% $p < 0.0007$ [5]. This suggests that the intravaginal culture may not be the most optimal for blastocyst culture. Therefore, the physicians performing these IVC cycles had a lower threshold for performing DET, resulting in a higher number of twin deliveries.

One significant limitation of this study is the small sample size. While our descriptive results appear promising, particularly for singleton infants, to detect true differences in perinatal outcomes, a much larger study would need to

be conducted. A future well-designed study would compare IVC patients to conventional IVF with similar diagnoses having fresh embryo transfers, with attention paid to differences in stimulation protocols to isolate a treatment effect of the culture modality. Based on our power calculation from a prior study investigating perinatal outcomes of natural IVF-conceived infants compared to conventional IVF [16], the minimum sample size would be at least 123 infants in the study and control group. Finally, another limitation of this study is that other confounders such as maternal BMI, maternal medications, pregnancy comorbidities, indications for delivery, and neonatal complications such as intensive care admission were not examined.

Despite the limitations of our study, the data presented is novel as this is the first case series reporting the perinatal outcomes of infants born from IVC. It provides crucial data on how extended intravaginal culture influences the longer-term outcomes of the embryos. This data is highly informative for physicians considering the use of IVC in their clinical practice. Importantly, further studies with larger sample sizes are still needed to confirm our initial findings and make direct comparisons to conventional IVF.

Declarations

Conflict of interest F.A. is a member of the medical advisory board for INVO Biosciences.

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