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Sex-related growth differences are present but not enhanced in IVF pregnancies

Kathleen E. O'Neill, M.D.^a, Methodius Tuuli, M.D., M.P.H.^b, Anthony O. Odibo, M.D., M.S.C.E.^b, Randall R. Odem, M.D.^c, and Amber Cooper, M.D., M.S.C.I.^c

^aDivision of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Pennsylvania School of Medicine, Pennsylvania, PA

^bDivision of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Washington University in St. Louis, St. Louis, MO

^cDivision of Reproductive Endocrinology, Washington University in St. Louis, Department of Obstetrics and Gynecology, St. Louis, MO

Abstract

Objective—To determine whether in vitro fertilization (IVF) modifies the effect of fetal sex on growth.

Design—Retrospective cohort study

Setting—Tertiary care center and related facilities

Patients—Singleton live births without fetal/maternal comorbidities from fertile women who conceived without the use of assisted reproductive technologies (ART) and infertile women who conceived with IVF.

Interventions—None

Main Outcome Measures—The primary outcome was birth weight (BW). Secondary outcomes were fetal crown-rump length (CRL) in the first trimester, biparietal diameter (BPD) and estimated fetal weight (EFW) in the second trimester.

Results—There were no differences in baseline characteristics between women carrying male fetuses and those carrying female fetuses in either mode of conception. In unadjusted analyses, the male-female differentials in fetal BPD and BW were more pronounced in the IVF cohort than in the unassisted cohort. In multivariable regression analysis, male BPD exceeded female BPD by 0.12 cm, male EFW exceeded female EFW by 12 grams (gm), and male BW exceeded female BW by 172 gm. IVF did not have a significant effect on BPD but was associated with a 52 gm increase in EFW in the midgestation. IVF was associated with an 81 gm reduction in BW. IVF did not modify the magnitude of size differences between the sexes in the midgestation or at birth.

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Contact information: Kathleen E O'Neill, MD, University of Pennsylvania, 3701 Market Street, Suite 800, Philadelphia, PA 19104 (kathleen.o'Neill2@uphs.upenn.edu).

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Conclusions—Comparable sex-dependent differential growth occurs in unassisted and IVF pregnancies.

Keywords

fetal sex; IVF; birth weight; gestational age

Introduction

Over the last 30 years, the use of assisted reproductive technologies (ART) has become increasingly common; in 2009, *in vitro* fertilization (IVF) and related technologies played a role in 1.4% of births in the U.S. (1). Thus, it is extremely important that we fully understand any potential risks of the IVF process, which involves laboratory manipulation of sperm and eggs, culture of embryos, and uterine transfer. Many studies have suggested that infants born as a result of assisted reproduction have significantly lower birth weights than those who are conceived without ART (2-7). However, the mechanism behind this effect is not well understood, and debate remains as to whether the effect is related to IVF itself, the underlying infertility, or both (8).

In unassisted term births, male infants are approximately 150 grams heavier than female infants (9-11). Although this is often attributed to the higher concentrations of circulating androgens synthesized by the testes, it has been suggested that there are sex-associated differences in growth rate before differentiation of the fetal gonads. As a result, there has been disagreement about the point in development at which this difference begins and can be detected, with some arguing that the difference does not become apparent until the second trimester, and others contending that these differences are apparent at much earlier stages of development (12-21).

The differential XX-XY development rates have been studied extensively in animal models. Increased cell numbers were observed in XY embryos compared to XX embryos as early as 3.5 days post-coitus in mice (22) and bovine male embryos were found to have developed to more advanced stages than females during the first 8 days after insemination *in vitro* (23). Research in humans has shown that CRL and BPD in male fetuses were on average larger than female ones at the first measurement between the 8th to 12th week (13).

Given that *in vitro* culture of animal embryos has been shown to correlate with aberrant fetal and perinatal development (24, 25), and that IVF has been suggested to affect fetal growth (8), a key question about the safety of IVF is whether or not it enhances growth differential between male and female fetuses.

Several studies have addressed this question *in vitro*. For example, animal studies have suggested that *in vitro* culture conditions enhance sex-dependent growth rates during preimplantation embryonic development(16). The sex of the embryo may influence the embryo's response to environmental stress, such as exposure to transient elevated temperatures during the culture period (26). Additional micromanipulation, such as intracytoplasmic sperm injection (ICSI) has been postulated to further affect sex-related growth differences in human embryos. In one study, the mean log cell number of male blastocysts after ICSI was significantly greater than that of similarly treated female embryos, whereas no such difference was found among conventionally inseminated IVF-derived embryos (9). In an effort to ascertain if the sex-dependent growth differential was seen without micromanipulation and culture stressors, day 3 and 4 mouse embryos were recovered from reproductive tracts. In this study female embryos compacted earlier than males *in vivo*, however *in vitro* conditions supported the development of male embryos to the blastocyst stage(27). These data suggested that the increased cell proliferation observed in

male embryos was an artifact caused by the *in vitro* environment. Similarly no sex effect on size was seen in pig embryos flushed at 12 days gestation (28). No study has yet examined the sex-dependent growth differential throughout pregnancy in a cohort of infertile couples undergoing IVF in comparison to a cohort of unassisted conceptions in a fertile.

The objective of this study was to determine whether the effect of fetal sex on fetal growth is modified by IVF. We hypothesized that the differential growth observed between males and females in a population conceived without the assistance of ART would be present and enhanced in pregnancies conceived through the use of IVF.

Materials and Methods

The Institutional Review Board at Washington University in St. Louis approved this study. The Society for Assisted Reproductive Technologies (SART) database was used to identify women 18 to 45 years of age with singleton live births conceived as a result of IVF from our unit between January 1, 1999 and February 1, 2009. We only included those with complete pregnancy and birth data in the Washington University Prenatal Genetics Ultrasound Database. This database is comprised of all patients seen in our prenatal diagnosis center and is maintained by a dedicated nurse coordinator. Each patient is given a standardized form requesting pregnancy outcome to be returned following delivery and medical records are reviewed for accuracy. Demographic and health information is obtained prior to the visit through intake forms and the information is reviewed and confirmed with patients at the time of their ultrasound. When a follow-up form is not returned within 4 weeks of the expected date of delivery, the patient receives a phone call from the coordinator. In cases where the patient cannot be contacted, her referring physician is contacted for outcome information. For patients delivering in our healthcare system, outcome data were extracted from our perinatal computerized database. On average, survey return rate is over 90%.

A singleton live birth was defined as a viable infant delivered at 23 completed weeks or later in gestation with a fetal weight more than 500 grams. Precise gestational dating for conceptions from IVF was by the date of oocyte retrieval. The IVF pregnancies were all fresh embryo cycles. Frozen embryo, donor oocyte, and cycles using testicular sperm extraction were excluded. All IVF cycles were performed according to standard controlled ovarian hyperstimulation protocols with gonadotropins and gonadotropin releasing hormone agonist or antagonist pituitary suppression, ultrasound-guided transvaginal oocyte aspiration, and transcervical embryo transfer. The number and timing of the embryo transfers were individualized on the basis of clinical indications but were done on either day 3 or day 5.

A cohort of women 18 to 45 years of age with unassisted singleton live births between January 1, 1999 and February 1, 2009 was identified from the aforementioned prenatal ultrasound database. This cohort has been described previously (8).

Exclusion criteria for both the IVF and unassisted conceptions included pregnancies with selective reduction, fetal chromosomal or major congenital anomalies, maternal pregestational diabetes, preexisting hypertension, renal disease, sickle cell disease, other major medical conditions, and tobacco use. Patients with a first-trimester spontaneous reduction of a second gestational sac were included but were adjusted for in the multivariable analyses. Maternal age was recorded as age at the time of delivery. Race/ethnicity was self-reported information, with patients subdivided into white, black, and other for analyses.

The primary outcome was the difference in weight at birth between male and female fetuses stratified by mode of conception. The secondary outcomes were differences in *in utero* fetal size as measured by biparietal diameter (BPD), estimated fetal weight calculated by Modified Hadlock Model (EFW)(29) in the midgestation and crown-rump length (CRL) in the first trimester.

Statistical analyses were performed with STATA 11.0 SE software (College Station, TX). Baseline characteristics between women carrying male and female fetuses were compared separately in unassisted and IVF-conceived pregnancies.

Differences in birth weight, BPD, EFW, and CRL between male and female fetuses were estimated separately for the two modes of conception. The magnitude of the differences between male and female fetuses in IVF and unassisted pregnancies were then compared in a univariable analysis. Categorical variables were compared by using the chi-square test or Fischer's exact test as appropriate. The Kolmogorov-Smirnov test was used to assess normality of distribution of continuous variables. Normally distributed variables were compared by using the Student *t* test, and skewed variables were compared by using the Mann-Whitney U test. Multivariable regression analysis was used to estimate the independent effect of fetal sex on the differences on birth weight, BPD, EFW, and CRL in IVF and unassisted conceptions. To test for a relationship between IVF and fetal sex, an interaction term that controls for their effect together in the model was added. Variables were selected for inclusion based on biological plausibility, prior studies, and results of our baseline analyses. The number of variables was reduced by using backwards elimination. Interaction between variables was tested to assess the significance of coefficients associated with the included interaction terms.

Results

The cohort consisted of 1246 unassisted conceptions and 240 IVF assisted singleton live births from Jan 1, 1999 to Feb 1, 2009, all of whom had close follow up within our hospital system (See Figure 1). The initial query for IVF live births cross-referenced with our perinatal database resulted in 498 patients; 182 (37%) were excluded for multiple gestations, and 65 (13%) were excluded for other reasons (frozen embryos, oocyte donors, maternal/fetal exclusions listed above). Indications for the first-trimester ultrasound in the fertile cohort were for confirmation of viability or gestational age (63.1%), first-trimester bleeding (17.1%), advanced maternal age (8.8%), prior loss (8.7%), to rule out ectopic pregnancy (1.3%), or other (1%). There were no demographic differences between the fetal sexes within conception groups [Table 1].

A univariable analysis using the Student *t* test was performed between male and female conceptions with respect to CRL, BPD, EFW, and birth weight in each of the respective cohorts [Table 2]. This analysis showed a significant difference in BPD and birth weight between males and females in both the IVF and unassisted fertile cohorts. There were no significant differences seen between male and female fetuses with respect to CRL ($p=0.93$) or EFW ($p=.24$) in the IVF cohort or in CRL in the unassisted cohort ($p=.44$). In the IVF cohort, male fetuses had BPDs that were 0.28 ± 0.93 cm larger than female fetuses ($p=.03$). In the unassisted cohort, male fetuses had BPDs that were 0.12 ± 0.69 cm larger than their female counterparts ($p<.01$). Consistent with previous data, in the unassisted cohort, male fetal weight exceeded female fetal weight by 164 ± 584 grams at birth ($p<.01$). In the IVF cohort, male birth weight was 291 ± 640 grams greater than female birth weight ($p<.01$). The differences in these observed unadjusted absolute differences were compared and only the differences in BPD and BW were statistically significant [Supplementary Table 1].

After adjusting for gestational age, maternal age, maternal race, and presence of an early second gestational sac, male BPD exceeded female BPD by .12 cm (coefficient -0.120, 95% CI -0.173 to -0.067, $p < .001$) [Table 3]. IVF did not have a significant effect on BPD (coefficient 0.028, 95% CI -0.067 to 0.123, $p = .058$) and did not modulate the relationship between fetal sex and BPD (coefficient -0.029, 95% CI -.167 to 0.108, $p = .674$) [Table 3]. . IVF and fetal sex did not impact CRL in multivariable regression analysis.

Although univariable analysis did not reveal a significant difference in estimated fetal weight in the midgestation, after adjusting for the aforementioned clinically significant factors, male EFW exceeded female EFW by 12 gm (coefficient -12.29, 95% CI -21.027 to -3.560, $p = .006$) [Table 3]. Surprisingly, IVF was associated with an 52 gram increase in estimated fetal weight (coefficient 52.5195, 95% CI 36.79 to 68.25, $p < .001$). IVF did not modulate the relationship between fetal sex and size in the mid trimester (coefficient -19.24, 95% CI -42.00 to 3.52, $p = .098$)

With respect to size at the time of delivery, male weight exceeded female weight by 172 grams (coefficient -172.653, 95% CI -217.084 to -128.221, $p < .001$) [Table 3]. Consistent with previous literature, IVF was associated with an 81 gram decrease in birth weight (coefficient -81.35, 95% CI -158.59 to -128.22, $p < .001$). IVF was not a significant effect modifier on the relationship between fetal sex and birth weight (coefficient -67.004, 95% CI -177.870 to 43.862, $p = .236$) [Table 3].

Discussion

This study confirms sex differences in fetal size seen in previous studies with males generally larger than females exist in an IVF cohort. Although in our analysis EFW was not found to be significantly different between male and female fetuses in the IVF cohort on unadjusted univariable analysis, there was a statistically significant difference detected on adjusted multivariable regression analysis. Additionally, BPD is a reliable and consistent growth measure and was significantly different in male and female fetuses in the univariable analysis between an IVF and the unassisted cohort (35). One potential explanation for these findings is confounders were present and we were not adequately powered to detect small differences in EFW on our unadjusted univariable analysis, which is most likely given our adjusted multivariable regression findings and our previous research that did detect a significant difference in EFW in an IVF cohort.

Our multivariable analysis expanded upon the observation that males are larger than females in both unassisted and IVF conception and showed that the *in vitro* fertilization process does not further enhance the observed sex-dependent differences in growth. This suggests that mechanisms underlying growth differences are related to fetal sex and that those related to infertility and/or the IVF process are independent, or alternatively could be dependent but similar.

The mechanisms leading to sex-dependent differences in fetal and neonatal size have yet to be elucidated. Some have suggested that the growth differential is related to androgen action; however, differences between males and females in growth rate, body weight, and metabolism have been demonstrated before development of the gonads. One possible explanation is suggested by the findings that male and female fetuses and neonates employ different mechanisms to cope with adverse environments or events, such as maternal asthma and pre-eclampsia (30, 31). The process of IVF introduces a number of potential stressors including *in vitro* media, handling of embryos, temperature and light fluctuations, ICSI and prolonged culture. Previous research has demonstrated that the act of handling of embryos alone, without culture or additional micromanipulation results in epigenetic changes (32).

Moreover, optimized culture media and microfluidic environments designed to mimic the dynamic mechanical and biochemical setting of the oviduct and/or uterus continue to produce blastocysts with fewer cells than time-matched embryos *in vivo* (33, 34).

Several studies have shown that patients who require IVF to conceive have singletons that differ in fetal and neonatal size from those that are conceived without assistance. The mechanisms behind this altered growth have not been clearly explained; the decreased growth of IVF-conceived fetuses could be due to either the underlying subfertility or the *in vitro* gamete and embryo handling processes. Animal studies have suggested that *in vitro* culture conditions can enhance the sex-dependent growth rate during preimplantation embryonic development (16). By contrast, our data show that although both fetal sex and IVF affect growth, they do not synergize to further affect fetal size in humans. Or alternatively, although males and female embryos respond to some stressors differently, the specific stressors present as a result of IVF trigger similar responses in both male and female embryos and fetuses, and the sex-specific differences are not further enhanced in this process. This theory could explain why differences seen in culture are not seen after transfer and are then seen again in the mid-gestation; different stressors are introduced at the different time points and the ability of the female and male embryo to adapt to individual stressors varies. Furthermore, since first trimester CRL measurements in our study were often performed around 8 weeks gestation, this is the early end of where previous human studies found differences between male and females and measurement differences at this gestational age/size is subject to more human and technical error it is possible we were unable to detect small differences (13). The concept of exposures giving rise to altered responses to stress could also explain our finding that IVF was associated with a 52 gm increase in EFW in the midgestation and a 81 gm reduction in BW; the underlying infertility and/or IVF could resulting in developmental programming that may be advantageous to growth early in pregnancy, but hinder growth later in pregnancy.

Our study was mainly limited by the retrospective design, which impeded obtaining complete historical information from the patients. Several of the data we were unable to assess can significantly impact birth weight most notably sociodemographic factors, education, pre-pregnancy body mass index, weight gain in pregnancy, and indication for delivery.

In conclusion, in both an IVF and an unassisted population of singleton neonates, male fetuses were found to be larger than their female counterparts at birth and at least as early as the second trimester. Although IVF has been reported to be a potential stressor in the perimplantation period, we found that the IVF process did not further enhance the sex-dependent growth differential. This suggests that underlying mechanisms of altered fetal growth patterns due to both chromosomal sex and *in vitro* manipulation are independent. An alternative explanation could include a finding where female and male embryos have similar stress responses during IVF and therefore we would be unable to detect an increased difference in fetal size based on sex. We believe our research question is novel and though limited in its retrospective design will trigger further prospective basic and clinical research to further address these lingering questions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Flow chart outlining selection process for IVF cohort.

Table 1

Study patient characteristics

	IVF Conceptions (n=240)			Unassisted Conceptions (n=1246)		
N	Male (n=130)	Female (n=110)	P value	Male (n=635)	Female (n=611)	P value
Demographics						
Mean age (years)	34.57±4.26	34.87± 4.16	.58	30.57±5.22	30.67±5.20	.74
African American	5 (4%)	7 (6%)	.37	97 (15%)	103 (17%)	.45
Gestational age at 1 st Trimester US (wk)	6.91±0.69	6.98±0.68	.46	6.99±0.73	6.98±0.69	.80
Gestational age at 2 nd Trimester US (wk)	19.37±3.56 (n=129)	18.87±3.13 (n=106)	.26	19.49±1.41	19.49±1.40	.96
Gestational age at Delivery (wk)	38.62±2.06	38.37±2.12	.36	38.30±2.03	38.35±2.06	.66
Infertility Diagnosis						
PCOS	7 (5%)	4 (4%)	.52	NA		
Male Factor	48 (36%)	35 (31%)	.43			
IVF Technique						
ICSI	63 (49%) (n=129)	53 (49%) (n=109)	.97			
Assisted Hatching	59 (46%) (n=129)	49 (45%) (n=110)	.85			
Day 3 Embryo Transfer	82 (62%)	76 (68%)	.49			
Antenatal History						
Preeclampsia	11 (9%)	11 (10%)	.72	54 (9%)	47 (8%)	.57
Gestational Diabetes	7 (6%)	7 (6%)	.78	35 (6%)	28 (5%)	.44
Preterm Labor	12 (10%) (n=126)	11 (10%) (n=109)	.88	124 (20%)	101 (17%)	.15

Table 2

Differences between fetal sexes in IVF and unassisted conceptions. CRL=crown rump length in millimeters, BPD=biparietal diameter in centimeters, EFW=estimated fetal weight (calculated by Hadlock method) in grams. Birth weight is given in grams. Measurements displayed are unadjusted means $\pm$ standard deviations of the mean.

	IVF Conceptions (n=240)				Unassisted Conceptions (n=1246)			
	Male (n=130)	Female (n=110)	Absolute Difference	P value	Male (n=635)	Female (n=611)	Absolute Difference	P value
1 st Trimester CRL (mm)	8.18 $\pm$ 4.67 (n=129)	8.23 $\pm$ 4.62 (n=109)	.06 $\pm$ 9.33 (n=238)	.93	8.78 $\pm$ 4.93 (n=635)	8.56 $\pm$ 5.12 (n=611)	.22 $\pm$ 10.05 (n=1246)	.44
2 nd Trimester BPD (cm)	4.58 $\pm$ 0.94 (n=116)	4.30 $\pm$ 0.92 (n=98)	0.28 $\pm$.93 (n=214)	.03	4.54 $\pm$.71 (n=634)	4.42 $\pm$.6 (n=611)	0.12 $\pm$.69 (n=1245)	<.01
2 nd Trimester EFW (g)	393 $\pm$ 461 (n=119)	332 $\pm$ 254 (n=98)	61 $\pm$ 767 (n=217)	.24	340 $\pm$ 103 (n=634)	327 $\pm$ 98 (n=611)	13 $\pm$ 200 (n=1245)	.02
Birth weight (g)	3395 $\pm$ 632 (n=130)	3104 $\pm$ 616 (n=110)	291 $\pm$ 625 (n=240)	<.01	3356 $\pm$ 576 (n=635)	3192 $\pm$ 580 (n=611)	164 $\pm$ 578 (n=1246)	<.01

Table 3

Multiple regression models of the influence of predictive variables on biparietal diameter, estimated fetal weight (Hadlock method), and birth weight. CI indicates confidence interval. Model controlled for gestational age, maternal age, maternal race, and presence of an early second gestational sac.

Independent Variable	Coefficient	95% CI	P
Biparietal diameter (cm)			
Constant (β_0)	-1.50	-1.80 to 1.19	<.001
Fetal Sex (β_1)	-0.12	-0.17 to -0.07	<.001
IVF (β_2)	0.03	-0.07 to 0.12	.06
Gestational Age (β_3)	0.30	0.29 to 0.32	<.001
Maternal Age (β_4)	0.003	-0.002 to 0.008	.21
African American Race (β_5)	0.01	-0.06 to 0.08	.77
IVF & Sex Interaction Term (β_6)	-0.03	-0.17 to 0.11	.67
Estimated Fetal Weight (g)			
Constant (β_0)	-1335.85	-1387.10 to -1284.61	<.001
Fetal Sex (β_1)	-12.29	-21.027 to -3.56	.006
IVF (β_2)	52.52	36.79 to 68.25	<.001
Gestational Age (β_3)	84.97	82.75 to 87.18	<.001
Maternal Age (β_4)	.68	-.14 to 1.49	.10
African American Race (β_5)	-8.75	-20.64 to 3.15	.15
IVF & Sex Interaction Term (β_6)	-19.24	-42.00 to 3.52	.098
Birth Weight (g)			
Constant (β_0)	-4604.16	-5008.44 to -4199.87	<.001
Fetal Sex (β_1)	-172.65	-217.08 to -128.22	<.001
IVF (β_2)	-81.35	-158.59 to -4.11	.039
Gestational Age (β_3)	201.20	191.18 to 211.23	<.001
Maternal Age (β_4)	9.17	5.05 to 13.28	<.001
African American Race (β_5)	-163.46	-223.78 to 103.14	<.001
IVF & Sex Interaction Term (β_6)	-67.00	-177.87 to 43.86	.236