Early maternal serum β -human chorionic gonadotropin (β -hCG) levels and sex-related growth difference of IVF embryos

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Abstract

Purpose Maternal serum β -human chorionic gonadotropin (β -hCG) represents the trophoblastic cell mass and is an indirect measurement of embryo development at early implantation stage. Studies in animals and human embryos detected sex-related growth differences (SRGD) in favour of male embryos during the pre-implantation period. The purpose of our study was to correlate SRGD and maternal serum β -hCG at 16 days after embryo transfer.

Methods We retrospectively analysed all (fresh and frozen) non-donor, single embryo transfers (SET), elective and not elective, that were performed between December 2008 and December 2013. We included β -hCG values from day 16 after oocyte collection of pregnancies resulting in live birth. Neonatal gender was retrieved from patient files. Male and female embryos were further grouped to cleavage and blastocyst stage transfers. Regression analysis for confounding variables included maternal age, maternal body mass index (BMI), use of micromanipulation (ICSI), embryo quality (grade), assisted hatching, day of transfer and fresh or frozen embryo transfer. **Results** Seven hundred eighty-six non-donor SETs resulted in live birth. After including only day 16 serum β -hCG results, 525 SETs were analysed. Neonatal gender was available for 522 cases. Mean maternal serum β -hCG levels were similar, 347 ± 191 IU/L in the male newborn group and 371 ± 200 IU/L

in the female group. The difference between β -hCG levels remained insignificant after adjusting for confounding variables.

Conclusions Early maternal β -hCG levels after embryo transfers did not represent SRGD in our study.

Keywords β -hCG · IVF · Embryo · Sex related · Sex-related growth difference

Introduction

Sex-related growth differences (SRGD) have been extensively studied in animals and human fetuses. More than 50 years ago, it was recognized that male offspring have a higher average birth weight [1]. This was thought to result from a more advanced developmental rate of male fetuses compared to females at different stages of pregnancy [2, 3]. The proposed mechanism of this phenomenon during pregnancy was suggested to be the effect of secreted androgens and male hormones.

The introduction of assisted reproductive technology (ART) enabled evaluation of early embryos. Data started to accumulate showing sex-related growth differences in the pre-implantation period even before the differentiation of the gonads. Early studies in animals detected SRGD in favour of male embryos during the pre-implantation period, mainly in mouse [4], and bovine blastocyst embryos [5, 6]. The limiting factor with human embryo studies was the ethical issue of confirming the gender prior to embryo transfer. Therefore, human studies were limited to either the use of surplus embryos that were not transferred or to correlation between pre-transfer embryo cell number and the gender detected after birth.

Pergament et al. [7] found that a higher cleavage stage embryo cell number increased the probability of a male

Capsule Early maternal β -hCG levels after embryo transfers did not represent SRGD in our study.

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offspring. Ray et al. [8] assessed surplus embryos' cell number and metabolic requirements at different embryo stages and correlated with embryo gender using karyotype. They reported higher average number of cells for cleavage stage male embryos compared to female. The most pronounced difference occurred at days 4–6, with a non-significant difference on day 6. Dumoulin et al. [9] used surplus embryos to correlate developmental rate with embryo gender determined by fluorescent in situ hybridization (FISH). They found a higher number of cells in male blastocysts fertilized by ICSI.

Another indirect evidence of a more rapid male embryo development is an altered sex ratio in favour of males observed at birth for the IVF population. There is no evidence of bias in spermatogenesis or fertilization in favour of Y sperm cells [10]. Therefore, a possible explanation is a rapid development of male embryos, leading to a bias in selection of male embryos for transfer [11].

It is unknown if SRGD at advanced stages of pregnancy is a continuation of that of the pre-implantation period or a completely separate phenomenon affected by a different mechanism. It is difficult to directly assess embryo developmental rate in the time frame between implantation and the sonographic detection of a fetal pole.

Maternal serum beta human chorionic gonadotropin (β -hCG) is a subunit of HCG dimer, secreted by the embryonic trophoblastic cells and represents the trophoblastic cell mass [12]. It is not a direct measurement of embryonic growth but is one of the earliest methods to assess pregnancy progression and correlates well with pregnancy outcome as early as 2 weeks after embryo transfer [13–15]. Previous studies found the influence of fetal gender on maternal serum β -hCG, and women carrying female fetus had higher β -hCG levels than those with male fetus in late first, second and third trimesters of pregnancy. This is most probably related to the differences in the activity of the fetal hypothalamic-hypophyseal-gonadal axis. [16–19]. Yaron et al. [20] found higher levels of maternal serum β -hCG in female fetus in pregnancies that resulted from the transfer of both single and multiple embryos in IVF patients.

In order to assess whether embryonic sex-related growth differences persist after the pre-implantation period stage, we evaluated maternal serum β -hCG from single embryo transfers resulting in male and female neonates.

Materials and methods

This retrospective study was done at the reproductive unit of the McGill University Health Center (MUHC) in Montreal, Canada. The study was approved by the Research and Ethics Board of McGill University Health Center (MUHC Study code 12-283-SDR).

All fresh and frozen non-donor oocyte single embryo transfer (SET) cycles that resulted in a singleton live birth, between December 2008 and 2013, were included. Patient demographic details, treatment parameters, embryos and transfer data were retrieved from patient computerized files. Only maternal serum β -hCG levels taken on day 16 after collection or corresponding day in frozen thawed cycles were included. Pregnancy outcome was recorded after delivery as part of routine follow-up. We obtained missing information (if any) by detailed telephone survey conducted by trained personnel at our centre. β -hCG levels were compared for pregnancies that resulted in a male or female neonate. Patients were then subgrouped by day of transfer to further evaluate if there was a difference between male and female pregnancies.

Patients underwent in vitro ovarian stimulation protocols as previously described, either mid-luteal long GnRH agonist, "microdose flare up" GnRH agonist, flexible or fixed GnRH antagonist or natural cycle protocols [21]. Frozen thawed embryos were transferred after hormonal priming of the endometrium or after natural cycle. Embryo transfer either on days 2, 3 (cleavage) or 5 (blastocyst) was determined based on the quantity and quality of embryos available. Cleavage embryos were graded as good quality if they had four cells on day 2 or seven to eight cells on day 3, <20 % fragmentation and exhibited no apparent morphological abnormalities [22].

All embryos were cultured in Cook medium (Cook Medical, Sidney, Australia) during all stages of embryo development. Assisted hatching with laser zona drilling (20 μ m) when indicated was first performed under an inverted microscope in a microdrop (40 μ L) using a ZILOS-tk Zona Infrared Laser Optical System (version 3.29, Hamilton Thorne Instruments Biosciences, Beverly, MA). Extended culture to blastocyst was performed according to the sequential media system. Blastocysts were graded according to the level of expansion, the presence and the quality of inner cell mass and quality of trophoctoderm. Good-quality blastocysts were defined as those $\geq$ 3BB based on the Gardner and Schoolcraft scoring system [23]. When there was more than one blastocyst, the best-quality embryo was transferred and surplus embryos were frozen using vitrification.

Embryo transfer was performed under ultrasound guidance and placed 1.5 cm from the uterine fundus. Luteal support included progesterone (either vaginal or intra-muscular) and estrogen until 8–10 weeks post embryo transfer for viable intrauterine pregnancy. Serum β -hCG level was measured using immunometric sandwich assay with Immulite 2000 system (Siemens Medical Solutions Diagnostics, Germany). The analytical sensitivity of the assay was 0.4 mIU/mL. All measurements included in the study were done by the same assay.

Statistical analysis was performed using SAS 9.2. Patient characteristics and β -hCG outcomes were tabulated by the gender of the neonate. Chi-square test was performed for categorical variables and Wilcoxon rank sum test for continuous

variables. Multivariable regression analysis was performed to determine the association of potential factors on β -hCG value for pregnancies resulting in a male versus female neonate. Covariates included maternal age, maternal body mass index (BMI), use of micromanipulation (ICSI), embryo quality (grade), assisted hatching (AH), day of transfer and fresh or frozen embryo transfer.

Results

During the study period, there were 786 live births resulting from single embryo transfers including 766 singleton deliveries. After excluding cases where β -hCG was not measured 16 days after oocyte collection or on the calculated equivalent day in frozen cycles, 525 cases were included in the study. Three cases with unknown neonatal gender were excluded (Fig. 1). Of the 522 live births, 271 were males and 251 were females. There was no significant difference in maternal and paternal age and maternal BMI between the two groups. Embryo quality, prevalence of AH and use of ICSI were also similar in the two groups (Table 1).

Mean maternal serum β -hCG levels were similar, 347 ± 191 IU/L in the male newborn group and 371 ± 200 IU/L in the female group. The difference between β -hCG levels remained insignificant (p value=0.159) after adjusting for the confounding factors (maternal age, BMI, use of micromanipulation (ICSI), embryo quality, assisted hatching and fresh or frozen transfer). No difference in maternal serum β -hCG levels was found for male and female neonates after stratifying for the day of transfer (cleavage versus blastocyst) (Table 2).

Fig. 1 Study population of early maternal serum β -human chorionic gonadotropin (β -hCG) levels and sex-related growth difference of IVF embryos

Table 1 Demographic characteristics, fertilization and embryo parameters of the study group

	Male (271)	Female (251)	<i>p</i> value
Maternal age (years)	33.4 $\pm$ 3.8	33.5 $\pm$ 3.8	0.63
Paternal age (years)	36.7 $\pm$ 5.9	37.6 $\pm$ 6.1	0.11
Maternal BMI	24.5 $\pm$ 5.8	24.4 $\pm$ 5.7	0.76
ICSI	175 (64)	174 (69)	0.45
<i>n</i> (%)			
Good-quality embryos	230 (85)	205 (81)	0.51
<i>n</i> (%)			
Assisted hatching	55 (20)	51 (20)	0.996
<i>n</i> (%)			

Maternal age, paternal age and maternal BMI are presented as mean $\pm$ SD. BMI body mass index at start of infertility treatment

Discussion

In our study, the initial maternal serum β -hCG values were not affected by embryo gender. These results remained after controlling for potential cofactors including maternal age, BMI, use of micromanipulation (ICSI), embryo quality, assisted hatching, day of transfer and fresh or frozen transfer.

There have been reports of different average β -hCG values for cleavage stage and blastocyst embryo transfer [24–27]. Accordingly, we subgrouped the study groups to day of transfer. The results revealed that β -hCG values for male and female embryos were comparable.

We included only single embryo transfers (elective and non-elective) to eliminate the possibility of the vanishing twin phenomenon that could be as high as 10 % of double embryo transfers resulting in a singleton live birth [28]. By including only β -hCG measurement taken 16 days after egg collection in fresh cycles or on the calculated equivalent day in frozen thawed cycles, we created a precise base for comparison. All the β -hCG measurements were performed at the same laboratory using the same equipment. This also contributed to the comparability of the results.

There have been reports that there is a difference in the developmental rate between male and female human IVF embryos during the pre-implantation stage in favour of male embryos [9, 7, 8]. This issue raises a concern about possible long-

Table 2 Mean maternal serum β -hCG levels for male and female newborns

	Male	Female	<i>p</i> value
Total	(<i>n</i> =271) 347 $\pm$ 191 IU/L	(<i>n</i> =251) 371 $\pm$ 200 IU/L	0.14
Day 3 embryo transfer	(<i>n</i> =45) 319 $\pm$ 170 IU/L	(<i>n</i> =41) 302 $\pm$ 136 IU/L	0.85
Day 5 embryo transfer	(<i>n</i> =226) 352 $\pm$ 195 IU/L	(<i>n</i> =210) 385 $\pm$ 208 IU/L	0.08

Data are presented as mean $\pm$ SD

term effects of IVF. Using animal models, a few authors have compared in vitro embryos developed under laboratory conditions to in vivo embryos washed out from the fallopian tubes at the same stage. They found SRGD among in vitro but not in vivo embryos [29, 30]. They proposed that ART-induced stress may cause epigenetic changes by imprinting specific genes on chromosome X that is responsible for this artificial phenomenon. Whether this phenomenon is unique to IVF remains unclear.

While our study does not advance the clinical utility of β -hCG in patient counselling for predicting the fetal gender, it may shed some light on the epigenetic processes occurring during the pre-implantation period. Finding a clear difference in β -hCG levels between male and female embryos could indicate measurable long-term IVF-induced epigenetic changes. While SRGD may still be induced by stress-related IVF epigenetic changes, our results do not support that SRGD is expressed by β -hCG. In fact, there was a trend for higher β -hCG levels in female embryos, the opposite of what we expected to find. This is in agreement with a previous study by Yaron et al. [20] that found a higher maternal serum β -hCG levels 3 weeks after fertilization in female fetus pregnancies. However, their study was not restricted to single embryo transfers. In any event, the authors suggested a different expression of placental proteins by female and male fetuses, possibly by escape from inactivation of X-linked genes responsible for hCG metabolism in the placenta. Possible explanations for not finding an effect of SRGD on maternal serum β -hCG at early implantation stage may be that the difference is not expressed by β -hCG levels or the pre-implantation SRGD phenomenon is limited to very early stages of implantation prior to the secretion and measurement of β -hCG. A self-correction mechanism was also suggested by some observation comparing SRGD in blastocyst embryos, suggesting that the phenomenon is more prominent on day 5 than on day 6 [11, 31, 8].

The limitations of our study include its retrospective nature and the ability to include only live births. Furthermore, the results of this study do not necessarily reflect the SRGD phenomenon since β -hCG, while is the best tool available, is an indirect measurement of embryo development. However, to the best of our knowledge, this was the first study evaluating β -hCG levels for male and female embryos.

In conclusion, our study suggests no difference in maternal serum β -hCG levels between male and female embryo pregnancies. Although indirect, this is the only method to evaluate a gender-related difference at the early stage of pregnancy.

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