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ASSISTED REPRODUCTION TECHNOLOGIES

Independent factors influencing large-for-gestation birth weight in singletons born after in vitro fertilization

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Abstract

Purpose Higher risk for birth of singletons being large for gestational age (LGA) has been revealed after in vitro fertilization (IVF) frozen–thawed embryo-transfer (FET). This phenomenon is now being investigated, since there is a speculation that these neonates could suffer from underlying epigenetic disturbances. The aim of the study was to expose independent LGA risk factors and to identify those connected to the IVF techniques.

Methods Altogether, 4508 singleton pregnancies and births were included in the cohort case-matched study. Two hundred eleven singleton pregnancies and births after FET and 916 after fresh embryo transfer (ET) were included into two study groups. The IVF procedures were performed at the University Medical Centre Ljubljana between 2004 and 2011. For each IVF pregnancy, three matched consecutive controls after natural conception were included. Using logistic regression models, we observed LGA connection to maternal parameters (smoking, hypertension, parity, BMI, gestational diabetes, IVF conception, FET, double ET, and ICSI procedure).

Results Singletons born after FET had a significantly higher risk for being LGA ($p=0.032$; OR 1.697; 95 % CI 1.047–2.752). BMI 25–30 was a significant independent risk factor for LGA in the IVF groups (FET $p=0.041$, OR 2.460, 95 % CI 1.030–5.857 and fresh ET $p=0.003$; OR 2.188, 95 % CI 1.297–3.691). ICSI and double ET had no significant effect on LGA occurrence.

Conclusions Besides maternal BMI, FET is a significant independent LGA risk factor in IVF patients. Other observed factors (smoking, hypertension, multiparity, GDM, ICSI procedure, or number of embryos transferred) do not influence LGA risk significantly.

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Introduction

The proportion of children born after frozen–thawed embryo transfer (FET) after the in vitro fertilization–embryo transfer (IVF–ET) procedure grows worldwide [1]. Several studies have shown that singletons born after FET have a perinatal outcome more comparable to those born after natural conception than to those born after fresh embryo transfer (fresh ET), having higher birth weight and less pregnancy complications [2–10]. However, higher risk for birth of a singleton being large for gestational age (LGA) has been revealed after FET [2, 3, 7, 8, 11–14].

The reasons for these differences are now being sought. The LGA child has an increased risk for stillbirth, asphyxia, distress, trauma, caesarian section, and metabolic disturbances at birth [15–17]. Moreover, another concern in LGA neonates born after assisted reproductive techniques (ART) is the possible underlying epigenetic pathology, since certain rare

epigenetic disorders in fact occur more often after IVF–ET than after spontaneous conception [18]. For example, higher "large offspring syndrome" incidence has been noticed following ART in the cattle and sheep. This syndrome has been proven to be epigenetically and phenotypically similar to epigenetic Beckwith–Wiedemann's syndrome in humans, which also seems to occur more often after IVF conception [19]. Besides, placental expression of certain imprinted genes has already been connected to altered LGA risk in term pregnancies [20].

There are different hypothetical mechanisms for higher LGA rate after FET to explore:

– An average woman involved in FET procedure has supernumerary embryos and is therefore considered a good prognosis patient. More embryos of higher quality are indicating that a patient is more likely to conceive in an ongoing IVF cycle than in a patient without them.
– The techniques of freezing and thawing procedures enable positive selection of those high-quality embryos, which survive the cryopreservation procedure. It seems reasonable to speculate that they could be more successful also in other selective events, such as implantation, invasion, and placentation or nutrition supplementation.
– The FET performed in a natural cycle provides different embryo implantation and growth conditions due to a different hormonal environment in comparison to the stimulated cycle.
– The cryopreservation technique itself could induce changes in the embryo on an epigenetic level.

Pinborg et al. have recently shown that siblings conceived after FET have an increased risk for being LGA when compared to the siblings born after fresh ET from the same mother [8]. The influences of the culture media [21–23], culture period [24, 25], hormonal protocols [26], and infertility [12, 14, 27] on the IVF singleton birth weight have already been considered. There are several studies confirming that IVF placentas differ from spontaneous placentas in their size, shape, weight, and abnormality rate [3, 6, 7, 28–31], and that placentation following FET differ from the placentation following fresh ET [3, 6, 7, 13]

In this study, we analyzed the connection of IVF procedure type and maternal background factors to LGA risk. The factors observed were the fertilization technique, ET type, number of embryos transferred, maternal age, smoking, hypertension, parity, BMI, and gestational diabetes mellitus (GDM).

Materials and methods

We analyzed the LGA rates in neonates, born after FET and fresh ET, in comparison to case-matched population of neonates, born after spontaneous conception. Furthermore, we analyzed the correlation of LGA rate with specific maternal and IVF factors.

Patients

We included 4507 singleton pregnancies and births in this retrospective, case-matched cohort study.

Two hundred eleven singleton pregnancies and births after FET and 916 after fresh ET were included into two study groups: the "FET group" and the "fresh ET group". For each of the IVF pregnant women included in the two study groups, we included three consecutive controls who gave birth after natural conception and were matched by age, parity, and maternity hospital. Altogether, 3381 singleton pregnancies and births after natural conception were included in the study: 633 in the group of "FET controls" and 2748 in the group of "fresh ET controls".

IVF–ET procedure

The IVF–ET procedures were performed at the Department of Human Reproduction, University Medical Centre Ljubljana between January 2004 and December 2011.

Short antagonist cetrorelix protocol (Cetrotide: Serono, London, UK or AstaMedica AG, Frankfurt, Germany) or long agonist buserelin protocol (Suprefact: Hoechst AG, Frankfurt/Main, Germany) was used for ovarian stimulation. The short protocol consisted of GnRH antagonist cetrorelix acetate in a dose of 0.25 mg, which was administered when the dominant follicle measured ≥14 mm in diameter, after daily stimulation with 225 IU of follicle stimulating hormone (FSH) (Gonal F: Serono, Switzerland, Puregon), from day 2 of the menstrual cycle. In the long protocol, buserelin was used from day 22 of the menstrual cycle, at a daily dose of 0.6 ml (600 pg) subcutaneously until ovarian desensitization. Afterward, 125 to 300 IU of FSH was administered daily. All patients were administered 10,000 IU of human chorionic gonadotrophin (hCG) (Pregnyl; N.V. Organon) when at least three follicles measured ≥17 mm in diameter.

Transvaginal ultrasound-guided oocyte retrieval was performed 34 h after hCG administration. Fertilization of oocytes was performed using classical in vitro fertilization or intracytoplasmic sperm injection (ICSI).

Embryos were cultured to the blastocyst stage in the sequential media M1 and M2 (Origio/MediCult, Jyllinge, Denmark) until day 5. One or two best quality blastocysts were transferred in the uterus on day 5. Blastocyst quality was evaluated according to the blastocyst grading system introduced by Gardner et al. [32]. Supernumerary blastocysts were cryopreserved on day 5 or day 6 using our original, modified two-step Menezo's method, described by Virant-Klun et al. [33]. Blastocysts were stored in liquid nitrogen at −196 °C. Day 2 or 3 ET was performed only in patients with

previous blastocyst development failure and patients with only one or two embryos developed.

Coordination between the endometrium and blastocysts in the frozen–thawed cycles was performed as described in Virant-Klun et al. [33]. Three different protocols for the FET endometrial synchronization have been used: the spontaneous cycle in 103 patients, minimal stimulation protocol using 75 IE of gonadotrophins from day 5 of the menstrual cycle in 85 patients and hormonal supplementation in 8 patients. For 15 patients, the type of protocol was unknown. The estradiol serum measurements were performed only in patients having FET performed in the spontaneous cycles for more accurate monitoring. Luteal phase support consisted of 3×200 mg of daily progesterone (Utrogestan: ViatrisPharma, Paris, France) until a gestational sac with a fetal heart activity was visible on day 30 after ET .

Data collection

Data on IVF procedures were collected in the IVF laboratory of our department on a daily basis (Table 1). All 14 Slovenian maternity hospitals systematically collect data on maternal demographic characteristics, medical, gynecological and reproductive history, prenatal care, pregnancy, delivery, postpartum period, and neonates for each mother–infant pair using the same definitions of variables and the same form of medical record.

The research was performed according to the Personal Data Protection Act (UL). The data obtained from National Perinatal Information System (NPIS) were depersonalized in order to ensure the anonymity of women and neonates. The research was performed according to the Personal Data Protection Act.

Definitions

Slovene reference standard curves for weight, length, and head circumference at birth for a given gestational age were used for fetal and neonatal growth estimation [34]. LGA was defined as growth above the 90th percentile (or 95th percentile when specifically mentioned in the text, see Table 4) according to the aforementioned population growth curves. Diagnosis of GDM was made following screening test with oral 50 g glucose challenge test at 24 to 28 weeks of pregnancy and confirmed by diagnostic 100 g oral glucose tolerance test [35]. Preterm delivery was defined as the delivery before completed 37 weeks of gestation.

Statistics

Statistical analysis was performed using IBM SPSS Statistics, version 19 (IBM Corp, Armonk, NY). For assessment of normality, we used normal Q–Q plot, along with the Shapiro–Wilk normality test. Student's t test was used to compare normally distributed parametrical variables, Mann–Whitney's test was used to compare non-normally distributed variables, and chi-squared test was used to compare categorical variables. A multivariate logistic regression was used to identify independent risk factors. p value of <0.050 was considered significant.

Results

Among all patients having IVF, 653 (58 %) women were treated for female factor infertility, 202 (18 %) for male factor infertility, and 271 (24 %) for both female and male factor infertility. In 95 % of ET cases, a blastocyst transfer was performed.

The characteristics of FET and fresh ET cycles are shown in Table 1.

There were no significant differences in infertility cause between patients conceiving after FET and patients conceiving after fresh ET. Women conceiving after FET had significantly less previous IVF attempts and less double ET in comparison to women conceiving after fresh ET.

Mean maternal age, multiparity, smoking, irregular menstrual cycle rate, mean birth weight, hypertension rate, GDM rate, impaired glucose tolerance rate, mean gestation, preterm birth rate, fetal distress rate, cesarean section rate, and congenital malformation rate of FET and fresh ET with both control groups are shown in Table 2.

Significantly fewer women had hypertension in the FET group, while there were no other significant differences between the observed maternal factors and pregnancy outcome between FET group and their controls. In the fresh ET group, however, the birth weight and mean gestation were

Table 1　Characteristics of IVF treatment cycles of patients conceiving after FET and fresh ET

	FET No. (%)	Fresh ET No. (%)	p value
No. of patients	211	916	
Infertility cause			
Tubal	52 (24.6)	253 (27.7)	0.511
Endometriosis	43 (20.3)	187 (20.4)	0.982
Endocrine	44 (20.9)	198 (21.6)	0.931
Male	39 (18.5)	166(18.1)	0.277
Others[b]	33 (15.6)	111 (12.1)	
No. of IVF attempts (Mean±SD)	1.89±1.2	2.19±2.1	[a]0.03
Double ET N (%)	160 (75.8)	751 (82.1)	[a]0.041

FET frozen–thawed embryo transfer, *ET* embryo transfer, *SD* standard deviation

[a] Statistically significant (p<0.05), as revealed by chi-squared or Mann–Whitney test

[b] Other causes include oncologic patients and idiopathic infertility

Table 2 Maternal factors and pregnancy outcome following FET and fresh ET and comparison to control groups

	FET N=211	FET controls N=633	p value[a]	Fresh ET N=916	Fresh controls N=2748	p value[a]
Mean age (years±SD)	33.5±3.7	33.5±3.7	1.000[b]	33.4±4.1	33.4±4.1	1.000[b]
Multiparous N (%)	80 (37.9)	240 (37.9)	1.000[b]	232 (25.3)	696 (25.3)	1.000[b]
Irregular menstrual cycle N (%)	2(0.9)	8(1.3)	1.000	11(1.2)	39 (1.4)	0.743
Smoking N (%)	19 (9.0)	48 (7.6)	0.556	69 (7.5)	224 (8.2)	0.574
Hypertension N (%)	3 (1.4)	30 (4.7)	0.036[a]	43 (4.7)	147 (5.3)	0.491
Gestational diabetes N (%)	8(3.8)	25(3.9)	1.000	35(3.8)	104(3.8)	1.000
Impaired glucose tolerance N (%)	2(0.9)	9(1.4)	0.740	10 (1.1)	22(0.8)	0.414
Birth weight (grams±SD)	3355±626	3290±621	0.191	3200±633	3314±579	<0.001[a]
Mean gestation (weeks±SD)	38.6±2.5	38.8±2.3	0.545	38.5±2.5	38.9±2.2	<0.001[a]
Preterm birth N (%)	19 (9.0)	60 (9.5)	0.838	111 (12.1)	199 (7.2)	<0.001[a]
Fetal distress N (%)	7 (3.3)	25 (3.9)	0.677	34 (3.7)	127 (4.6)	0.245
Caesarean section N (%)	53 (25.1)	152 (24)	0.746	236 (25.8)	588 (21.4)	0.006[a]
Congenital malformation N (%)	13 (6.2)	37 (5.8)	0.866	68 (7.4)	180 (6.6)	0.362

FET frozen–thawed embryo transfer, *fresh ET* fresh embryo transfer

[a] Statistically significant (p<0.05) as revealed by chi-squared, Mann–Whitney test or *t* test

[b] Mothers were matched according to age and parity

significantly lower in comparison to their control group. There were also significantly higher rates of preterm birth, fetal distress, cesarean section, and malformations in the fresh ET group, compared to their controls.

The distribution of BMI is shown in Table 3. There was a significant shift among FET and fresh ET patients from normal BMI toward overweight BMI.

The LGA rate (gestational weight above 90th or 95th centile) was significantly higher in the FET group in comparison to their controls. The LGA rate in the fresh ET group of singletons was similar to the LGA rate of their control group. The results are shown in Table 4.

The LGA rate did not differ significantly among the subgroups of patients having FET after different endometrial preparation: there were 17 LGA neonates born after FET in spontaneous cycle (18 %), 10 LGA neonates born after FET using minimal stimulation (12 %), and 2 LGA neonates born after hormonal replacement therapy (25 %) (*p*=0.386).

We included smoking, hypertension, multiparity, BMI, GDM, and IVF–ET procedure into an LGA (above 90th centile) logistic regression model. We analyzed the influence of the aforementioned factors on the LGA rate (above 90th centile) in four groups of patients: FET with controls, fresh ET with controls (Fig. 1, graph "All cases"), FET only, and fresh ET only (Fig. 1, graph "IVF only").

The LGA odds for independent factors are shown in Fig. 1.

In FET with controls, higher BMI and the IVF (FET) procedure increase the risk for birth of an LGA singleton. In the

Table 3 Distribution of BMI in IVF and control groups

	BMI <18.5 Underweight	BMI 18.5–25 Normal	BMI 25–30 Overweight	Bmi >30 Obese	p value
FET group N (%)	2 (0.9)	145 (68.7)	51 (24.2)	13 (6.2)	**0.014**
FET controls N (%)	26 (4.1)	453 (71.6)	104 (16.4)	50 (7.9)	
Fresh ET group N (%)	36 (3.9)	612 (66.8)	181 (19.8)	87 (9.5)	**0.002**
Fresh ET controls N (%)	145(5.3)	1983(72.2)	435(15.8)	185(6.7)	

Numbers in bold are statistically significant (*p*<0.05), as revealed by chi-squared test

FET frozen-thawed embryo transfer, *fresh ET* fresh embryo transfer

Table 4 Comparison of LGA rates (gestational weight above 90th and 95th centile) in FET and fresh ET group to their control groups

	FET N=211	FET controls N=633	Fresh ET N=916	Fresh controls N=2748
LGA (above 90th centile) N (%)	31 (14.7)	55 (8.7)	86 (9.4)	249 (9.1)
p value	0.017[a]		0.791	
LGA (above 95th centile) N (%)	21 (10.0)	31 (4.9)	45 (4.9)	141 (5.1)
p value	0.012[a]		0.862	

FET frozen–thawed embryo transfer, *fresh ET* fresh embryo transfer, *LGA* large for gestation birthweight

[a] Statistically significant ($p<0.05$), as revealed by chi-squared test

fresh ET with controls, the hypertension reduces the LGA risk, whereas higher BMI increases the LGA risk significantly. In fresh ET patients with controls, the IVF procedure has no significant LGA risk effect.

Smoking and diagnosis of GDM have no significant effect on LGA risk.

In both the FET and fresh ET group, higher BMI (25–30) increases the LGA risk significantly. Other extreme BMI classes have insignificant effect on LGA risk. Smoking, hypertension, multiparity, GDM, ICSI, and double ET have no significant effect on LGA risk in IVF groups either.

These results are shown in Table 5.

FET—frozen–thawed embryo transfer, fresh ET—fresh embryo transfer, ICSI—intracytoplasmic sperm injection,, BMI—body mass index, double ET—embryo transfer of 2

embryos, OR—odds ratio, C.I—confidence interval statistically significant when $p<0.050$, as revealed by logistic regression.

Discussion

This study investigates the connections of maternal and IVF factors in singleton pregnancies to the LGA rate.

Since both maternal age and parity correlate with higher birth weight, LGA rate, and other maternal pathology, such as hypertension or placenta previa [3, 36–39] we matched the controls to the study groups in order to exclude the possible bias.

Fig. 1 Graph "*All cases*" shows a comparison of two logistic models for independent factors effecting LGA (above 90th centile) odds ratio (with 95 % C.I) *light line* shows FET with controls, *black line* shows fresh ET with controls. Graph "*IVF only*" is showing LGA (above 90th centile) risks in IVF singletons only, following FET (*light line*) or fresh ET (*black line*)
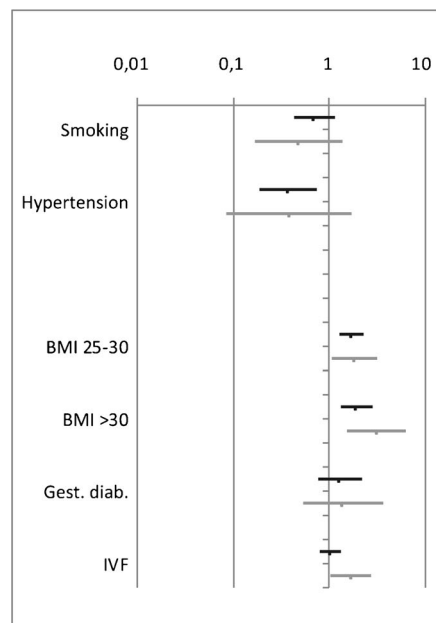
"All cases"comparison is showing 2 logistic models for independent factors influencing LGA (above 90th centile). Odds ratio (with 95% C.I.) shown. Black lines represents fresh ET, light lines FET.
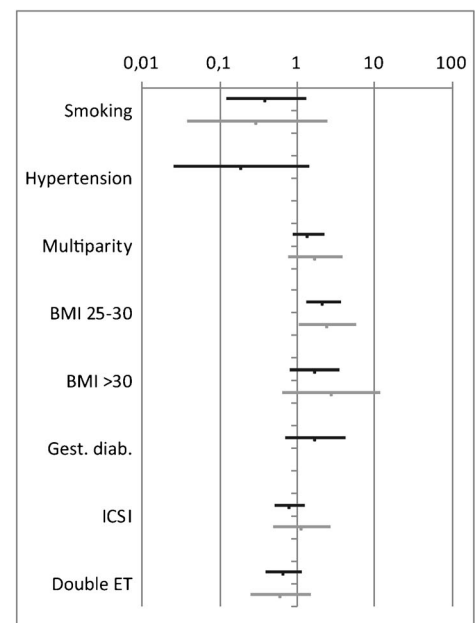
"IVF only" comparison is showing LGA (above 90th centile) risks in IVF singletons only. Black lines represents fresh ET, light lines FET.

Table 5 Independent factors influencing LGA odds ratio (as shown in Fig. 1, graph "All cases" and graph "IVF only")

	Factor	*p* value	OR (95 % CI)
All cases—fresh ET with controls (*black line*)	Smoking	0.143	0.7 (0.43–1.13)
	Hypertension	0.006	0.38 (0.19–0.75)
	BMI (ref=18.5–25)	<0.001	
	BMI <18.5	0.099	0.54 (0.26–1.12)
	BMI 25–30	<0.001	1.71 (1.29–2.26)
	BMI >30	0.001	1.93 (1.32–2.83)
	Gestational diabetes	0.322	1.3 (0.77–2.19)
	FET procedure (IVF)	0.882	0.98 (0.76–1.27)
All cases—FET with controls (*light line*)	Smoking	0.171	0.48 (0.17–1.37)
	Hypertension	0.209	0.38 (0.09–1.71)
	BMI (ref=18.5–25)	0.008	
	BMI <18.5	0.999	0.00
	BMI 25–30	0.026	1.85 (1.08–3.18)
	BMI >30	0.002	3.12 (1.52–6.42)
	Gestational diabetes	0.508	1.38 (0.53–3.61)
	FET procedure (IVF)	0.032	1.7 (1.05–2.75)
IVF only—fresh ET (*black line*)	Smoking	0.124	0.39 (0.12–1.29)
	Hypertension	0.108	0.19 (0.03–1.44)
	Multiparity	0.178	1.4 (0.86–2.28)
	BMI (ref=18.5–25)	0.029	
	BMI <18.5	0.915	1.07 (0.31–3.66)
	BMI 25–30	0.003	2.19 (1.3–3.69)
	BMI >30	0.175	1.68 (0.79–3.54)
	Gestational diabetes	0.233	1.73 (0.7–4.23)
	ICSI	0.331	0.8 (0.5–1.26)
	Double ET	0.158	0.67 (0.39–1.17)
IVF only—FET (*light line*)	Smoking	0.257	0.3 (0.04–2.4)
	Hypertension	0.999	0.00
	Multiparity	0.182	1.73 (0.77–3.88)
	BMI (ref=18.5–25)	0.167	
	BMI <18.5	0.999	0.00
	BMI 25–30	0.043	2.46 (1.03–5.86)
	BMI >30	0.175	2.76 (0.64–11.91)
	Gestational diabetes	0.999	0.00
	ICSI	0.731	1.16 (0.5–2.71)
	Double ET	0.292	0.62 (0.25–1.51)

According to Lintsen et al., the impact of smoking on the live birth rate in IVF treatment is comparable with an increase in female age of >10 years in young women [40]. In our population, the rate of patients reporting smoking was similar in all groups and did not reduce LGA rate significantly.

Both our IVF groups had higher BMI than what the control groups had, and BMI was a significant independent factor connected to LGA in all regression models. There was a significant shift from normal BMI toward overweight BMI (25–30) in the IVF patients. Data on BMI effect on IVF treatment are conflicting [41–43]. Higher BMI, in overweight as well as in obese patients, was related to lower success and live birth rates in women undergoing ART and has negative consequences for pregnancy including preterm birth and lower birth weight [43, 44]. Can the patients in the FET group differ from other patients in their lifestyle or other health parameters? It was previously shown by Pinborg et al. that FET acts as an independent risk factor for an LGA child from the same mother; however, in that study, they did not include factors such as BMI or smoking [8].

Patients having FET represent a "good prognosis" group having more quality embryos. Are there some other fertility factors that put them in the "good prognosis" group? For example, it has been shown that chances to obtain a pregnancy

and live birth are threefold higher in obese infertile patients who are regularly physically active in comparison to those who are not, irrespective to their body weight loss [45]. We investigated irregular menstrual cycle rate in order to discover whether PCO syndrome represents a risk for an LGA singleton, and we found no connection to LGA. On the other hand, although GDM is a known risk factor for the birth of a macrosomic neonate, it was not significantly connected to the birth of an LGA singleton in our study population. The reason is probably in the adequate screening of the whole population of pregnant women for GDM and the active observation of those with the diagnosis of GDM.

ICSI procedure did not influence the LGA rate in our population. ICSI has mostly been used for treating male and, rarely, unexplained infertility during the study period. Some studies have already shown that ICSI does not significantly influence birth weight in comparison to IVF, and the differences between fresh and frozen ICSI birth weight are similar to those following classical IVF procedure [9]. In an Austrian study, ICSI singletons were more prone to neonatal complications lower gestation and birth weight, whereas maternal pregnancy pathology was more related to classical IVF [46].

Double ET was a factor that was insignificant for lower LGA rate. Nevertheless, it has been shown, that double ET, in comparison to single ET, results in lower birth weight of IVF singletons [11, 47]. The effect of embryo culture length on birth weight still remains a matter of debate. Prolonged embryo culture (days 5 and 6) has been linked to a higher LGA rate in comparison to day 2 or 3 ET in fresh ET singletons [3, 25, 37]. In contrast, others report an increased risk of low birth weight and preterm birth after blastocyst transfer [24, 39]. The policy of blastocyst transfer is prevailing at our center because of a presumed benefit. During the study period, over 95 % of FET and fresh blastocyst transfers have been performed; therefore, the blastocyst transfer was not included into a linear regression model. It was suggested that a protein source in human embryo culture media has significant effect on the singleton birth weight and LGA rate [21, 23]. The influence of culture media on LGA rate was not analyzed in our study, since all embryos were cultured equally, regardless of ET type or fertilization procedure used.

Several studies have confirmed that there are important differences in placental abnormality rates between FET and fresh ET singleton pregnancies [3, 6, 7, 13] and that placentas after IVF are larger and heavier [28]. In a recent study, the researchers speculated that increased placental size could promote glucose delivery to LGA fetuses [48]. The cryopreservation is known to damage some of the embryo's cells [49], leading to a conclusion that this is a mechanism for a positive selection of "more aggressive" blastocysts. According to Veleva et al. [50], the processes of freezing and thawing cause damage to the embryos, but the extent of the damage depends on the embryo's quality. Top quality embryos are more viable than embryos of a poorer quality, and the transfer of a frozen–thawed top quality embryo may lead to better results.

In this study, we found no explanation for FET neonates having higher LGA risk than fresh ET neonates or neonates born after spontaneous conception. Therefore, the epigenetic cause cannot be excluded. The study, however, had some limitations. One of them is the lack of the serum estradiol measurements. According to the literature, there were birth weight differences observed after fresh IVF–ET cycles according to peak estradiol serum levels [51]. However, there were no significant differences among the LGA rates in patients having FET performed in different endometrial preparation protocol. Although, the difference might become significant on a larger FET population of neonates. Another limitation of our study was the lack of the infertile population as a control group, which could strengthen the results of the study.

Conclusion

Besides maternal BMI, FET is a significant independent LGA risk factor in IVF patients. Other observed factors (smoking, hypertension, multiparity, GDM, ICSI procedure, or number of embryos transferred) do not influence LGA risk significantly.

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