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Article

Increased Risk of High Birthweight in Singleton Newborns after Frozen-Thawed Embryo Transfer According to Endometrial Preparation, Ovulatory or Artificial Cycle

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Abstract:

Background: It is unknown whether prolonged artificial hormonal environment during early fetal development affects the birthweight of singletons born after frozen-thawed embryo transfer (FET).

Methods: A retrospective observational study included singleton births>22 weeks of gestation obtained after FET between 2013-2019, after endometrial preparation with ovulatory cycle (OC) or artificial cycle (AC). Our primary objective was to compare birthweight of singletons after FET between endometrial preparation by OC or AC. Secondary objectives included prolonged pregnancies, high birthweight, low birthweight, SGA and LGA rates. Multivariate analyses were performed considering potential confounding factors.

Results: Among 198 singleton live births after FET, 112 were obtained with OC and 86 with AC. Prolonged pregnancies rate was higher in AC (25.6% *vs.* 7.1%, respectively, p=0.001). Mean birthweight was higher (+219g) in AC (3386g *vs.* 3167g, p=0.01; *adjusted-p*=0.052), as well as the rate of babies exceeding 4000g (16.3% *vs.* 2.7%, p=0.03, *adjusted-p*=0.015). The rate of babies <2500g was lower in AC (3.5% *vs.* 11.6%, respectively, p=0.050, *adjusted-p*=0.049).

Conclusions: Since OC does not strain the chances of pregnancy and in the incomplete knowledge of the consequences of neonatal overweight on the future health of children, OC preparation could be advocated as first-line endometrial preparation in FET.

Keywords: Frozen-Thawed Embryo Transfer; Birthweight; Endometrial Preparation; Ovulatory Cycle; Artificial Cycle

1. Introduction

More than 35 years after the first successful pregnancy from a frozen-thawed embryo transfer (FET) [1], the practice of FET has increased. In French registers, FET represented

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37.4% of IVF attempts in 2017, versus 30.9% in 2014 [2,3]. Developments in cryopreservation methods, higher rates of elective single embryo transfers (e-SET) and "freeze-all" policies have also promoted embryo freezing [4–6].

Besides embryo survival and quality, successful FET relies on endometrial receptivity at the time of transfer. The most common protocols for endometrial preparation are: (1) the artificial cycle (AC), and (2) the ovulatory cycle (OC), either natural cycle (NC) or mild ovarian stimulated (OS) cycle. In OC, a luteal phase support using progesterone can be prescribed but a *corpus luteum* (CL) naturally secretes progesterone, enabling endometrial receptivity for implantation. In AC, estrogen and progesterone supplementation are mandatory since no CL exists, and is continued up to 12 weeks in case of pregnancy. The choice of the best protocol is still debated. While studies reported equally successful pregnancy rates (PR), ongoing pregnancy rates (OPR) and live birth rates (LBR) between protocols in women with regular cycles [7–9], a recent systematic review and meta-analysis [10] suggested mild OS as a promising option since PR and LBR were higher compared to AC. We recently published data showing that OC was associated to higher LBR compared to AC in multivariate analysis [11].

Hence, it seems that the choice of the best protocol should consider obstetric and neonatal outcomes [12]. Numerous studies have indicated that newborns conceived after IVF and immediate transfer (so-called "fresh") had lower average weights compared to natural conception, even for singletons. Subsequently, birthweight, macrosomia (birthweight >4000g), and Large for Gestational Age syndrome (LGA) (birthweight >90th percentile for gestational age) were reported to be higher in children born after FET compared to fresh transfer, regardless of techniques used [13–22]. However, the underlying mechanisms are still poorly understood [15,23–27]. Characteristics of the cellular process of fetal growth and epigenetic regulation during pre-implantation are still in question [28]. Intrauterine growth potential may be affected by epigenetic changes in the early embryonic stages during freezing and thawing [29]. Biological conditions (IVF technique, culture medium, embryonic stage) may play a role. A study led by *Pinborg et al.* [21] reported higher LGA and macrosomia rates in FET *vs.* fresh transfer, even after adjustment for birth order, suggesting that results could not only be explained by being the second born or by intrinsic maternal factors, but may also be related to freezing/thawing procedures per se. Altogether, whether the periconceptional hormonal environment induced by endometrial preparation protocols for FET has an impact on birthweight remains to be elucidated. A recent study suggested that the absence of CL in AC may play a role in the increased risk of hypertensive disorders during pregnancy [30]. Another study concluded that placental volume and other 1st trimester parameters were modified by IVF with fresh embryo transfer or FET compared to spontaneous conceptions, but with opposite trends, and that hormonal treatment *per se* may have a major effect on pregnancy outcomes through the modification of placental invasiveness [31]. LGA, as Small for Gestational Age (SGA) (birthweight <10th percentile for gestational age), are at increased risk of complications in subsequent developmental delay or mortality [32–34].

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Identifying and quantifying these events aims to anticipate the consequences at an individual level and try to identify their causes [15,23–27].

The main objective of this monocentric retrospective study was to compare the birthweight of singletons conceived after FET between endometrial preparation by OC or AC in daily clinical practice. The secondary objectives were to compare prolonged pregnancies high birthweight, low birthweight, SGA and LGA rates between these two groups and to evaluate whether endometrial preparation was predictive of these neonatal outcomes.

2. Materials and Methods

2.1. Endometrial preparation protocols

The choice of endometrial preparation protocol depended on the physician's decision. The 2 endometrial preparation protocols were:

- OC: gonadotropin stimulation (37.5 to 75 IU) was initiated between cycle Day 2 (if oligo-anovulatory) and 10, Day 5 for most ovulatory women. A GnRH antagonist could be used to program FET. Ultrasound and hormonal testing (estradiol, progesterone and LH) was performed Day 8 to 11 and repeated if needed. Once the dominant follicle reached 16-20 mm, ovulation was induced (6500 UI r-hCG: Ovitrelle®, Merck, Germany). Then, a luteal phase support with vaginal micronized progesterone (VMPg), 200 mg/day, was administered for maximum 6 weeks of gestation (WG) if pregnancy, since the major source of progesterone derived from the CL. FET was performed 5 days after r-hCG injection for cleaved embryos and 7 days after for blastocysts.
- AC: endometrial preparation started on Day 1 with estradiol (E2) administrated orally at 4-6 mg/day or transdermally (200 µg/3days).The few for which GnRH agonist was co-prescribed started E2 substitution 10 days after agonist introduction. Ultrasound and hormonal testing were performed Days 12- 14 and repeated if needed. When endometrial thickness reached 7 mm and serum progesterone level <1ng/ml, VMPg (600 mg daily) was administered (3 and 5 days before FET for cleaved embryos or blastocysts respectively) until the 12th WG if pregnancy.

2.2. Embryos

Embryos were obtained from conventional IVF or ICSI cycles. Egg donation cycles were not included. Embryos were mainly frozen at cleavage stage until April 2016, then at blastocyst stage after evolution of laboratory policy. Cleaved embryos were considered alive if at least 50% blastomeres were intact after thawing.

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2.3. Births

All live births > 22 WG were included. Medical terminations of pregnancies and antenatal deaths were excluded. No perinatal deaths were reported. Premature delivery was defined as < 37 WG. Prolonged pregnancies were considered for terms > 41WG [35].
Macrosomia was defined for any child born at term > 4000g. SGA and LGA were defined as birthweight < 10th or > 90th percentile for gestational age, respectively.

2.4. Statistics

Descriptive and regression analyses were performed using Stata for Windows (version 14; StataCorp). Paternal and maternal ages at freezing, maternal smoking status, body mass index, PCOS, parity, technique (IVF/ICSI), embryo stage and freezing technique were included in the multiple regression model as potential confounders.

3.5. Ethics

The Ethical committee gave its unrestricted approval for the study and all patients had previously given their consent to use their data (CEERB Paris Nord, IRB 00006467: ID 2018-013). All data were collected from Medifirst® software, meeting recognized medical and ethical specifications according to the French information protection commission (ID 2068638).

3. Results

Among the 198 singletons born after FET during the 5-year study period (2013- 2018), 112 were conceived with OC and 86 with AC, respectively. No difference was observed concerning parental characteristics, except for PCOS (more frequent in AC, p = 0.002). Regarding embryonic characteristics, higher rates of blastocysts and freezing by vitrification were observed for OC (Table 1).

Table 1. Parental and embryonic characteristics

Variable	**Protocol**				***p*-value**
	OC (n=112)		**AC (n=86)**		
Parental characteristics	mean (sd)	n (%)	mean (sd)	n (%)	

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Maternal age at freezing	32.8 (4.5)		33.1 (4.1)	0.62
Maternal age at transfer	34.0 (4.2)		33.6 (4.5)	0.54
Maternal age at birth	34.7 (4.2)		34.4 (4.5)	0.55
Paternal age at freezing	38.7 (7.7)		38.7 (4.5)	0.98
BMI (kg/m2)	25.0 (4.0)		25.3 (4.8)	0.59
Smoking		14 (12.8)	9 (10.7)	0.65
Primary infertility		55 (49.1)	40 (46.5)	0.72
≥ 2 miscarriages		17 (15.2)	7 (8.1)	0.14
IVF indication				
Endometriosis		14 (12.5)	15 (17.4)	0.33
Tubal infertility		42 (37.5)	24 (27.9)	0.16
PCOS		14 (12.5)	27 (31.4)	0.002
Male infertility		65 (58.0)	43 (50.0)	0.26
Delivery characteristics				
Primiparous		82 (73.2)	69 (80.2)	0.25
C-section		17 (15.2)	21 (24.4)	0.10
Embryonic characteristics				
Standard IVF technique		33 (29.5)	30 (34.9)	0.42
Embryo stage at freezing				
Blastocyst		62 (55.4)	34 (39.5)	0.028
Freezing technique				
Vitrification		98 (87.5)	64 (74.4)	0.020
Duration of storage (days)				
< 90		41 (36.6)	31 (36.1)	0.88
90-365		46 (41.1)	35 (40.7)	
365-1095		16 (14.3)	15 (17.4)	
>1095		9 (8.0)	5 (5.8)	
Single embryo transfer		62 (59.6)	49 (37.7)	0.79

Abbreviations: ovulatory cycle (OC); artificial cycle (AC); in vitro fertilisation (IVF); polycystic ovarian syndrome (PCOS); standard (Std)

Concerning birth outcomes, there was no difference in gender, mean gestational age or preterm birth. Prolonged pregnancies rate was higher in AC (25.6% *vs.* 7.1%, respectively, *p* and *adjusted-p* = 0.001). Mean birthweight was significantly higher in AC (3386g *vs.* 3167g, difference: +219g; *p* = 0.010; *adjusted-p* = 0.052) (Table 2).

Table 2: Neonatal outcomes in ovulatory and artificial cycles

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Variable	Protocol				*p*-value
	OC (n=112)		AC (n=86)		
	mean (SD)	n (%)	mean (SD)	n (%)	
Male sex		53 (47.3)		40 (46.5)	0.91
Gestational age, WG+D	39+1 (2+0)		39+5 (2+2)		0.064
Preterm birth					
<37 weeks		10 (8.9)		3 (3.5)	0.14
<32 weeks		2 (1.8)		2 (2.3)	0.79
Prolonged pregnancy					
>41 weeks		8 (7.1)		22 (25.6)	0.001
>42 weeks		1 (12,5)		5 (22.7)	0.54
Height, cm	49.7 (3.0)		49.0 (3.1)		0.20
Birthweight, g	3167 (557)		3386 (619)		0.010
<1500 g		1 (0.9)		2 (2.3)	0.43
<2500 g		13 (11.6)		3 (3.5)	0.050
>4000 g		3 (2.7)		14 (16.3)	0.003
>4500 g		0		3 (3.5)	NA
SGA		11 (9.8)		8 (9.3)	0.90
LGA		12 (10.7)		13 (15.1)	0.36

[a]Adjusted on paternal and maternal age at freezing, maternal smoking status, body mass index
Abbreviations: PCOS, parity and technique (IVF/ICSI), embryo stage and freezing technique, ovulatory cycle (OC); artificial cycle (AC); weeks of gestation+days (WG+D), small for gestational age (SGA); large for gestational age (LGA)

The rate of birthweights >4000g was significantly higher in AC (16.3% *vs.* 2.7%, $p = 0.003$; *adjusted-p* = 0.015) (not applicable for > 4500g, only 3 cases in AC, and none in OC). The rate of birthweights < 2500g was lower in AC (3.5% *vs.* 11.6%; $p = 0.050$; *adjusted-p* = 0.049).

These discrepancies are highlighted in the rugplot and shift in birthweight density curves according to OC or AC preparation (Figure 1). When representing birthweight distribution in relation to gestational age at birth, the increased incidence of > 4000g is objectified in extended terms > 41WG, standing out in the AC group (Figure 2). SGA and LGA rates were not different.

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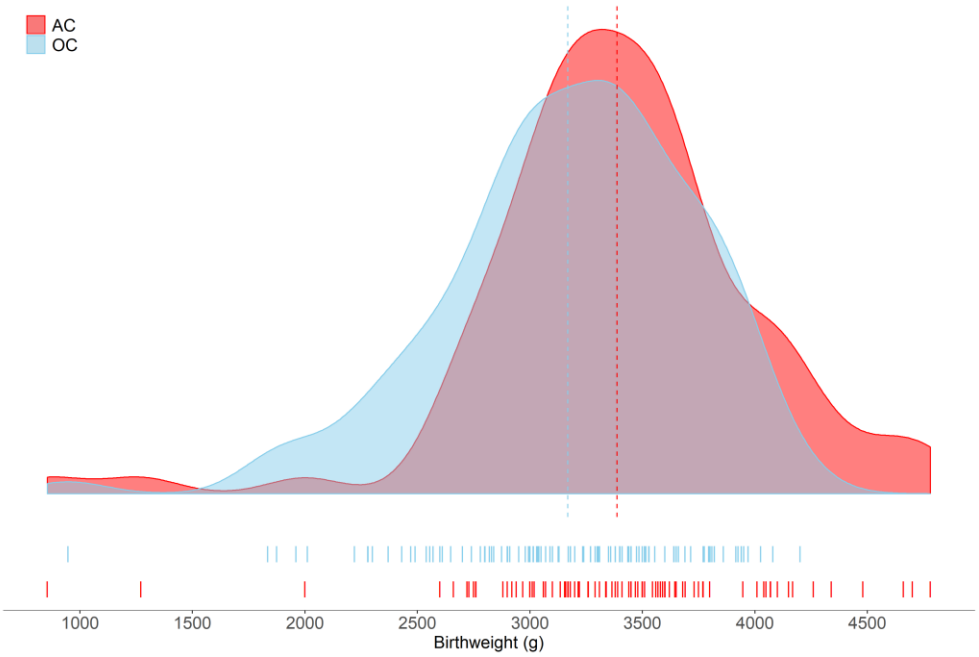

Figure1: Birthweight density curves and rugplot according to ovulatory (OC) or artificial (AC) endometrial preparation for FET (frozen embryo transfer)

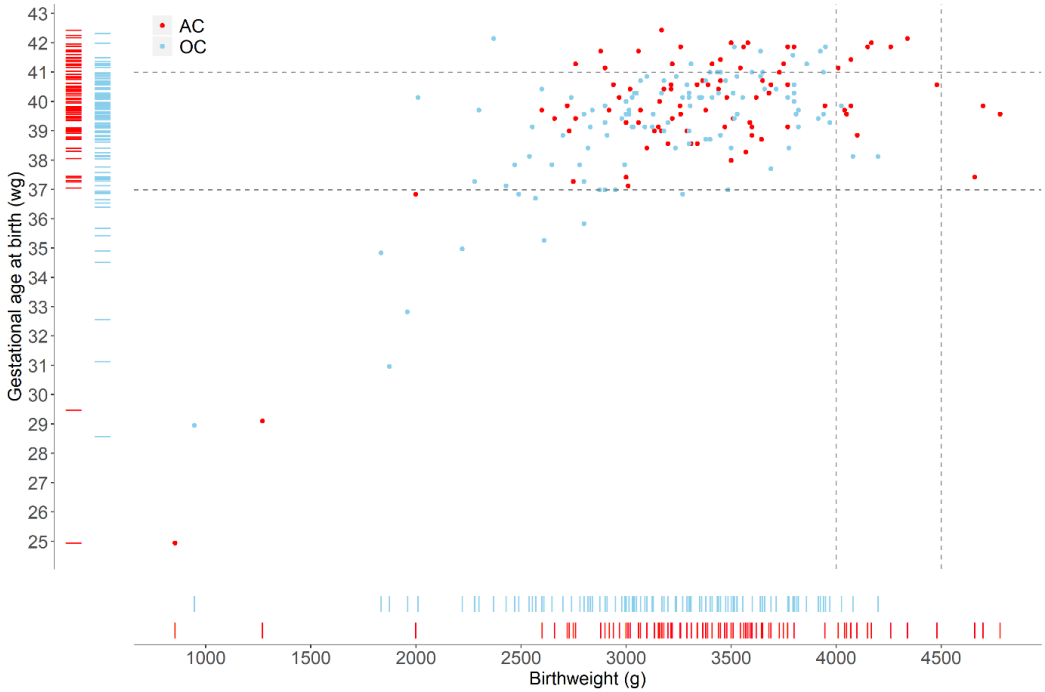

Figure 2: Birthweight and gestational age rugplots and scatterplot according to endometrial preparation for Frozen Embryo Transfer (FET) in ovulatory (OC) or artificial (AC) cycles and gestational age). Dotted lines represent 4000g and 4500g and 37WG and 41 WG.

4. Discussion

This retrospective analysis of neonatal outcomes of 198 singletons born after FET showed that endometrial preparation with AC was associated to higher birthweights and rates of

birthweight > 4000g compared to OC. These results remained significant after multivariate analysis. Among the maternal factors known to influence neonatal birth weight, BMI and parity were identical in both groups. PCOS, more frequent in the AC group, was included as an adjustment variable. Gestational age was not significantly different, nor were preterm or very pre-term birth rates. However, prolonged pregnancies > 41 WG were significantly higher in AC, which may impact the increased birthweight observed (+ 219g).

Our study focused on the difference in neonatal growth indicators according to treatments framing the early stages of embryo-fetal development. It is an exhaustive series comprising an all-round population over a 5-year period and mirroring daily clinical practice. Its monocentric character and limited period in time guarantees a stability and homogeneity of practitioners and protocols, enhancing comparability. Nevertheless, as a result of laboratory policy evolutions, different embryo stages and freezing protocols were included. Due to higher LBR observed with OC [11], clinical policies also evolved and endometrial preparation by OC became the privileged protocol for all patients with ovulatory cycles. AC was essentially prescribed in case of PCOS or history of failed OC. However, the 2 groups are comparable for most parental and embryonic characteristics (freezing technique, embryo stage, duration of conservation, e-SET). There was a concordance of significance without and with adjustment.

Unlike us, one of the first prospective randomized studies focusing on the influence of endometrial preparation on neonatal data by Cerillo *et al.'s* found no statistical difference in terms of weight and height of newborns in 570 FET cycles, of which n=280 AC and n=290 NC [36]. Nevertheless, the study comprised a very selected population, as it excluded women over 39, with severe endometriosis, or PCOS. Recently, Jing *et al*. [37] showed that AC was associated to higher birthweights compared to NC in multiple pregnancies (2550g *vs.* 2600g, respectively, $p = 0.023$). Consistently to our results, Ishii *et al*. [38] found significantly greater birthweights (+ 137g, $p < 0.01$) after AC (n = 403) compared to OC (n = 117). In blastocyst transfers, average birthweight was significantly higher in AC ($p < 0.01$). For cleaved stage embryos, although not significant, average BW was higher in AC. Average BW from the AC-blastocyst transfers was, as described in our study, higher compared to OC-cleaved stage embryos ($p < 0.01$). Putative factors affecting BW such as stimulation protocols, stage and quality of embryos could not explain the difference observed.

To date, multiple findings indicate that FET leads to heavier babies compared to fresh transfers [12–19 ,21,23,26,37]. However, the underlying mechanisms remain unclear. The first hypotheses evoked were maternal factors and biological conditions. Vidal *et al.'s* [40] analysis including 14262 singletons births > 24 WG suggested that maternal factors may play a role, as babies born from FET (n = 1158) had significantly higher birthweights (+ 190g), lower LBW and SGA rates ($p < 0.001$) compared to fresh transfer (n = 5560) in autologous cycles, while no difference was observed for egg donation transfers.

Conversely, the role of maternal factors was questioned by Pinborg *et al*. [21] in a study comparing FET (n = 896) *vs.* fresh transfer (n = 9480). Sibling pairs were analysed, where

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one singleton was born after FET and the consecutive sibling born after fresh transfer or vice versa. The adjusted Odds Ratio (AORs) of LGA and macrosomia in singletons were significant when conceived in FET *vs.* fresh transfer (1.34 [95%CI 0.98–1.80] and 1.91 [95%CI 1.40–2.62], respectively), and the increased risk was confirmed after adjustment on birth order. Therefore, results could not only be explained by being the second born, or by intrinsic maternal factors, but could also be related to freezing/thawing procedures per se.

Consistently, Anav *et al*. [41] suggested that cryopreservation in itself could be responsible of birthweight variations, independently of parity. Furthermore, animal studies such as the description of Big Calf Syndrome [42] and murine models focusing on the role of freezing techniques on genomic imprint showed that culture media constituents could affect birthweight, although no consensus exists concerning human data. Indeed, while Dumoulin *et al.* [43] and Nelissen *et al.* [28] showed a significant impact of embryo culture medium on early embryonic and fetal development and birthweight, no significant association was reported by Eaton *et al.* [44] and Vergouw *et al.* [45], even when adjusted for gestational age, gender and parity.

Concerning a possible influence of embryo stage on birthweight, Ishihara *et al.* [46] compared pregnancy outcomes between 4 groups: fresh Day 2 (n=10928); fresh Day 5 (n = 5981); vitrified-FET Day 2 (n = 3841); vitrified-FET Day 5 (n = 27408). FET was associated to lower LBW < 2500g and SGA, and to higher LGA rates (AOR 1.48). Lower rates of SGA and higher rates of LGA were observed for Day 5 *vs.* Day 2 embryos. Conversely, Belva *et al.* [47] found no influence of embryo stage on neonatal outcomes in live born singletons after fresh or FET cycles, although lower rates of SGA (p = 0.005), higher rates of birthweight standard deviation score (SDS) (p = 0.008), length at birth SDS (p = 0.001), and head circumference SDS (p = 0.005) in FET groups were reported. The impact of endometrial preparation was not considered. Our work comprised more D5 and vitrified FET in OC than in AC group, but no difference in birthweight and LGA according to embryo stage was observed.

Although no transnational report exists on the proportion of protocols used for endometrial preparation, it seems that FET is mostly conducted using AC worldwide. However, using AC implies the continuous administration of E2 and progesterone during the first trimester of pregnancy. Specific attention should be paid to potential foetal effects of extended hormonal treatments, especially because epigenetic modifications can induce specific pathologies revealed decades after exposure. For instance, one must consider the dramatic consequences of diethylstilbestrol (DES) exposure during early pregnancy and its persistent effect in subsequent generations [48–51]. Unlike oocyte donation in which AC is mandatory to prepare embryo implantation, the choice of protocol remains open for FET. In case of endometrial preparation with OC, a *corpus luteum* is active and progressively secretes progesterone until stabilisation of serum levels, leading to constant progesterone levels [52]. Moreover, ovulation triggering with hCG sustains the luteotropic effect in early luteal phase [53]. Finally, other factors are also secreted by the CL, leading to possibly more natural endometrial protein secretion profiles compared to AC [52], and recent findings

suggest increased obstetrics risks in the absence of CL [54]. Besides maternal complications, adverse fetal outcomes such as stillbirth, shoulder dystocia, hypoglycemia, respiratory distress and perinatal mortality are increased in macrosomic babies, and further concerns during child- and adulthood include metabolic, cardiovascular and endocrine complications [55–58].

5. Conclusions

In conclusion, this study demonstrates that endometrial preparation by AC and its prolonged artificial hormonal environment during early foetal development in FET was significantly associated to higher mean birthweight, macrosomia and prolonged pregnancy rates compared to OC. The precautionary principle implies that the prevention of short or long term potential consequences of these neonatal characteristics, since possible, seems coherent. Since results obtained by OC do not strain the chances of pregnancy, and in the incomplete knowledge of the consequences of neonatal overweight on the future health of children, OC preparation could be advocated as first-line endometrial preparation in FET. Randomized controlled trials should be undertaken to assess these preliminary results.

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All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethical Committee (CEERB Paris Nord, IRB 00006467: ID 2018-013).

Informed Consent Statement: According to French regulations, written informed consent from the patients was not required for this study. Patient consent was waived due to French regulations. All data were rendered anonymous before access and analysis.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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