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ARTICLE

Perinatal outcome of babies born after using a simplified IVF culture system versus ICSI with sibling oocytes: a prospective cohort study

BIOGRAPHY

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KEY MESSAGE

The study found no difference in perinatal outcomes in the same patient cohort for babies conceived following randomization of sibling oocytes allocated to a simplified IVF culture system (SCS) or ICSI followed by conventional culturing. This provides evidence that the SCS technology is effective and safe.

ABSTRACT

Research question: Is there a difference in perinatal outcome in the same patient cohort for babies conceived following randomization of sibling oocytes allocated to a simplified IVF culture system (SCS) or intracytoplasmic sperm injection (ICSI) followed by conventional culturing?

Design: The study compared the perinatal outcomes of 367 babies born from 1 January 2013 until 31 December 2020 after using split SCS and ICSI insemination of sibling oocytes in a selected group of normo-responsive women, excluding cases of severe male infertility. Primary outcome measures were preterm birth (PTB; <37 weeks' gestation), low birthweight (LBW; <2.5 kg) and small for gestational age (SGA) as a primary outcome parameter while secondary outcome measures included mean birthweight, mean gestational age, extreme prematurity (<32 weeks), very low birthweight (<1.5 kg), perinatal mortality, multiple pregnancy and Caesarean section rate.

Results: A total of 105 and 103 singleton babies were born after fresh embryo transfer (FRET) and 71 and 50 singletons after frozen embryo transfer (FET) in the SCS and ICSI groups, respectively. For babies born after FRET, the LBW rate was 2.9% (3/105) for SCS and 7.8% (8/103) for ICSI (P = 0.10). LBW occurred in 4.2% (3/71) and 0% (0/50) of babies born after the transfer of cryopreserved-thawed SCS and ICSI embryos, respectively (P = 0.14). The rate of PTB was 3.8% and 6.8% for SCS and ICSI in FRET cycles (P = 0.33), and 8.5% and 6.0% for SCS and ICSI in FET cycles (P = 0.62). One congenital malformation was found in the SCS FET group.

Conclusion: There was no difference in perinatal outcome for singleton and twin babies born after SCS and ICSI.

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KEY WORDS

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Low birthweight
Perinatal outcome
Prematurity
Simplified IVF

INTRODUCTION

One of the most important reasons behind the inequity of accessible and cost-effective fertility treatment within and between countries and continents is the high cost associated with assisted reproductive technology (ART; *Ombelet et al., 2008*). This cost will be substantially cut only by a simplification of diagnostic procedures, using low-cost, less intense ovarian stimulation protocols and/or simplified low-cost IVF culture methods (*Nargund et al., 2017*; *Ombelet and Campo, 2007*).

As part of the Walking Egg project (*Dhont, 2011*; *Ombelet, 2014a*), this study group developed a simplified IVF method called the Walking Egg lab system (tWE lab system) or SCS (simplified culture system) to reduce the high costs associated with regular high-tech IVF/intracytoplasmic sperm injection (ICSI) laboratories by avoiding the need for expensive medical gases and complex incubation equipment (*Van Blerkom et al., 2014*, *2019*). A connection is made between two tubes, the first containing citric acid and sodium bicarbonate in water and the second containing IVF culture medium. Due to the chemical reaction between the acid and the base enough CO_2 gas is produced to equilibrate the pH of the medium to approximately 7.30. A precise control and regulation of culture conditions such as pH, temperature and humidity, critical for successful embryo development and survival, are achieved with this 'closed system'. Fertilization and embryo scoring is performed by looking through the wall of the glass tube.

This SCS self-generates the specific culture conditions necessary for human IVF and progressive preimplantation embryogenesis such that development from insemination to embryo transfer is completely undisturbed within an enclosed system. Consequently, the typical pitfalls of regular IVF laboratories, such as unwanted temperature changes, pH and air quality issues, can be avoided, and fertilization and embryo development can take place in a controlled environment closely resembling natural conception.

Because the sperm concentration required for insemination using the SCS is only 1000–5000 motile washed spermatozoa, it has been shown to be highly successful even with moderate or severe male factor involvement (*Boshoff et al., 2018*; *Van Blerkom et al., 2014*). Comparing SCS with conventional IVF or conventional culturing after ICSI, major differences such can be discerned as (i) oocyte insemination with a lower number of progressively motile spermatozoa, (ii) culture with a higher volume of medium, (iii) continuous exposure of oocytes/embryos to culture media containing spermatozoa and cumulus cells for a longer incubation time, and (iv) the sealing of the culture environment from the ambient environment (*Van Blerkom et al., 2014*).

Unlike costly time-lapse instruments designed to document embryo morphokinetics during the preimplantation stages in microlitre volumes that require constant monitoring of culture conditions, the SCS is a self-contained, self-generating system that supports human preimplantation embryogenesis from fertilization though blastocyst hatching with no ongoing external support except maintenance of 37°C using inexpensive heating devices. While time-lapse systems generally involve ICSI, which requires the removal of cumulus and corona granulosa cells to permit continuous optical imaging of the entire embryo, the SCS can be used to culture embryos created by ICSI but is intended to be used primarily with conventional IVF (insemination) with the somatic support cells removed *in situ* as described (*Van Blerkom et al, 2014*). As reported, this allows the detection of pronuclei, occurring at intervals timed to when developmental landmarks are known to occur, and also allowing, for embryo selection based on morphology and morphokinetics, the normality of preimplantation embryogenesis through to the blastocyst stage.

When investigating a new IVF laboratory method for clinical use in human infertility, it is both a necessity and an obligation to be sure that the method is safe and effective. This group has already reported on the effectiveness of the SCS system when compared with ICSI followed by regular culturing (*Ombelet et al., 2017*, *2022*). Considering the safety, perinatal outcome data are crucial because it is well known that pregnancies following IVF/ICSI, when compared with spontaneously conceived pregnancies, are more likely to be affected by perinatal complications including preterm birth (PTB) and low birthweight (LBW). This is not only due to the higher multiple pregnancy rate: it has also been shown that singletons born after ART are also more likely to have adverse perinatal outcomes compared with spontaneously conceived singletons, depending on the ART method (*Ombelet et al., 2016*). Fresh embryo transfer (FRET) is associated with a higher risk of PTB, small-for-gestational-age (SGA) babies and LBW, while frozen embryo transfer (FET) is associated with large-for-gestational-age babies. ICSI may be associated with a higher risk of birth defects and transferral of the poor semen quality to any male progeny (*Belva et al., 2016*; *Berntsen et al., 2019*; *Davies et al., 2012*). As a result of a population-based cohort study including 135,051 children born after ART, Luke and colleagues (*Luke et al., 2021*) found that the use of ICSI was associated with an increased risk of major non-chromosomal birth defects, gastrointestinal defects and any defect when compared with other ART singleton births conceived from autologous oocytes and fresh embryos.

Beside an increased risk of SGA, PTB and LBW, obstetric complications such as operative delivery and hypertensive disorders in pregnancy are also seen more often in ART pregnancies (*Palomba et al., 2016*; *Pandey et al., 2012*; *Pinborg et al., 2013*; *Qin et al., 2017a*, *2017b*; *Sunderam et al., 2020*; *Wennerholm and Bergh, 2020*). According to a meta-analysis by Cavoretto and co-workers (*Cavoretto et al., 2018*) the risk of PTB was 8.4% for the IVF/ICSI cohorts compared with 5.9% after spontaneous conception. By excluding FET cycles, the PTB rate was 10.9% for IVF/ICSI, and 6.4% for babies conceived spontaneously.

In 2014 the current authors reported the birth of the first 11 healthy babies as a result of FRET and FET conceived using the SCS (*Ombelet et al., 2014b*; *Van Blerkom et al., 2014*). The present study is part of a prospective non-inferiority study comparing pregnancy outcomes after fertilization using sibling oocytes divided between SCS and ICSI in a selected population. The study examined the perinatal outcome of 329 singleton and 38 twin babies born after the transfer of SCS and ICSI embryos between 2013 and 2020.

MATERIALS AND METHODS

Participants

From January 2013 until December 2020, the authors prospectively examined the perinatal outcome of 176 singletons born after using the SCS, 105 after FRET, and 75 after FET. These results were compared with the perinatal outcomes of 153 singletons born after ICSI followed by regular culturing in the same patient cohort, 103 after FRET, and 50 after FET. During the same period the perinatal outcome data of 16 ICSI and 22 SCS twin babies were examined.

Selection of patients

The patient cohort studied is part of a larger non-inferiority prospective study performed at the ZOL Hospitals in Genk, Belgium (*Van Blerkom et al., 2014*). A diagnostic workup of the women included a medical history, a physical examination, pelvic ultrasonography, serum hormone assays between days 2 and 4 of the menstrual cycle and hysterosalpingography, hysterosalpingo-foam sonography or hysteroscopy. Laparoscopy was performed only when tubal pathology or endometriosis was suspected, or the presence of ovarian cyst(s) was shown on ultrasonography. Sperm examinations were categorized according to the guidelines from the World Health Organization (WHO; *Cooper et al., 2010*; *WHO, 2010*).

All the women included in this study were less than 43 years of age and a minimum of six oocytes were recovered at oocyte collection. Cases of mild to moderate male infertility were also included provided the number of motile spermatozoa after washing (the inseminating motile count) was above 1 million.

Only couples who had been trying to conceive for at least 1 year without success were eligible for treatment. In couples with unexplained or moderate male subfertility and open tubes at least three or four intrauterine inseminations were performed before starting IVF or ICSI, with the exception of woman above 40 years of age or with a duration of infertility of more than 3 years; in these circumstances immediate SCS/ICSI was undertaken.

Ovarian stimulation protocol, oocyte collection and semen processing

Recombinant FSH (Puregon; MSD, Belgium) or urinary FSH (Menopur; Ferring, Belgium) was used for ovarian stimulation. In most cases an antagonist protocol was started with recombinant FSH 150 IU or urinary FSH 150 IU. Depending on the size of the follicles the antagonist Ganirelix 0.25 mg daily (Orgalutran; MSD, Belgium) was added on day 6 or 7. When a diagnosis of moderate to severe endometriosis, adenomyosis or the presence of uterine myomata was made, a long-acting agonist scheme with nasal busereline 0.4 mg daily (Suprefact; Sanofi, Belgium) was used for 14 days starting in the luteal phase of the previous cycle; thereafter recombinant FSH or urinary FSH was added as described in the antagonist protocol. In some patients the short agonist protocol was used, with the agonist triptoreline 0.1 mg (Gonapeptyl; Ferring, Belgium) being administered intramuscularly for 7 days.

For final oocyte maturation 5000 IU human chorionic gonadotrophin (HCG; Pregnyl; MSD, Belgium) was given when three or more follicles measuring 17 mm were present. When a high risk of ovarian hyperstimulation syndrome was suspected Gonapeptyl 0.2 mg intramuscularly was used instead of HCG to trigger ovulation in the antagonist protocol. Oocyte retrieval was planned for 35–36 h after HCG or agonist administration. Spermatozoa for insemination were isolated by buoyant density gradient centrifugation with PureSperm 40/80 (Nidacon International, Sweden) according to the manufacturer's instructions.

Sibling cumulus–oocyte complexes were randomly equally divided for SCS fertilization or ICSI followed by conventional IVF culturing, following the usual Genk protocol (*Van Blerkom et al., 2014*). In the event of an odd number of retrieved oocytes the extra egg was treated with ICSI. For practical reasons and because SCS is more time consuming for the laboratory, it was decided that, for women presenting with more than 16 eggs at retrieval, a maximum of eight eggs would be treated with SCS and the surplus embryos with ICSI. After follicular aspiration of cumulus–oocyte complexes, the meiotic status of the cumulus- and corona-cell-enclosed oocytes was unknown and, owing to the randomization of the allocation process, differences in meiotic status were equally distributed between the two insemination groups, with a slight numerical bias towards ICSI based on the patient-centric protocol noted above.

The choice of the embryo(s) to be transferred was made by an independent embryologist using the digital images but whether these embryos were the result of SCS or ICSI was not disclosed at the time of selection. According to the Belgian reimbursement law, a single embryo transfer was carried out in the majority of cases (*Ombelet et al., 2005b*). All surplus embryos were vitrified (RapidVit Omni; Vitrolife, Sweden) according to the manufacturer's protocol.

When only one or two embryos were available on day 2, a day 2 or day 3 transfer was planned, while in all other cases blastocyst transfer on day 5 was preferred. Micronized progesterone (Utrogestan; Besins, Belgium) starting on the day of oocyte retrieval was used for luteal phase supplementation (600 mg in three separate dosages per day) until 18 days after ovum retrieval. When the pregnancy test was positive, progesterone was continued until 5–6 weeks after oocyte retrieval.

Outcome measures

Perinatal data for all babies born after the transfer of SCS or ICSI fresh and cryopreserved-thawed embryos were prospectively studied. The collection of different patient- and treatment-specific factors was checked by a third person on a monthly basis for a possible lack of data (M.J.). Three months after the expected day of delivery patients received a phone call from a dedicated midwife (P.J.) to enquire about the obstetric and perinatal outcomes. The following data were collected: date of delivery, sex of the baby, birthweight, mode of delivery and presence of congenital malformations. In the majority of cases the data obtained were compared with the information received from the obstetric unit where the delivery had taken place.

The primary end-points for comparison were PTB, SGA and LBW. According to the WHO a PTB is defined as a birth before 37 weeks of completed gestation (*WHO, 1977*). LBW is defined as a birthweight below 2.5 kg, caused by PTB and/or intrauterine growth restriction. SGA is most commonly defined as a weight below the 10th percentile for the gestational age. Secondary end-points included mean birthweight, mean gestational age, extreme prematurity

(<32 weeks), very low birthweight (<1.5 kg), perinatal mortality, prevalence of congenital malformations, multiple pregnancy and Caesarean section rate.

Ethical committee approval

This study was approved by the ethical committees of the ZOL hospitals in Genk and the ethical committee of the Free University of Brussels (reference no. 2011/011) and registered as B.U.N. 143201110348 on 19 May 2011. Participation in this clinical trial required informed consent documentation.

Statistics

To analyse the significance of the differences in perinatal outcome parameters between the SCS and ICSI singletons, the MedCalc statistical software package Comparison of Proportions Calculator was used (www.medcalc.org/calc/comparison_of_proportions.ph). MedCalc uses the $n - 1$ chi-squared test for the comparison of two proportions expressed as a percentage.

When comparing female age, birthweight and duration of pregnancy the MedCalc Comparison of Means Calculator (www.medcalc.org/calc/comparison_of_means.php) was used. With this procedure the difference between the observed means in two independent samples could be calculated. A *P*-value of <0.05 was considered statistically significant.

RESULTS

From January 2013 until December 2020 this study prospectively examined the difference in perinatal outcome of 208 singleton babies born after using SCS or ICSI with conventional culturing in a fresh cycle in the same patient cohort. General data relating to both groups are shown in TABLE 1. There was no difference in female age, primary or secondary infertility, body mass index (BMI), smoking habit, cycle rank, ovarian stimulation protocol, day of embryo transfer or number of embryos transferred. Considering the singleton babies born after FRET, prematurity (<37 weeks) occurred in 6.8% (7/103) of the ICSI group compared with 3.8% (4/105) of the SCS group, a non-significant difference ($P = 0.33$), while LBW (<2.5 kg) was present in 7.8% (8/103) of the ICSI group compared with 2.9% (3/105) of the SCS group, which was also not significantly different ($P = 0.10$) (TABLE 2).

The study also investigated the perinatal outcome of 121 singleton babies born after FET in the SCS and ICSI group. General data are shown in TABLE 1. For babies born after FET the prematurity rate was 8.5% (6/71) in the SCS group compared with 6.0% (3/50) in the ICSI group ($P = 0.62$). LBW was found in 4.2% (3/71) and 0% (0/50) of babies in the SCS and ICSI groups, respectively ($P = 0.14$) (TABLE 2).

A birthweight of more than 4.2 kg was observed in 6.7% (SCS) and 2.9% (ICSI) of babies in fresh cycles compared with 8.4% (SCS) and 10.0% (ICSI) after FET; the differences were not statistically significant (TABLE 2). After FRET, SGA was observed in 14.3% (SCS) and 20.4% (ICSI) of the newborns ($P = 0.24$) whereas after FET the SGA prevalence was 4% and 9.9% in the SCS and ICSI groups, respectively ($P = 0.22$) (TABLE 2).

After FRET the Caesarean section rate was 21% in the SCS group and 17.5% in the ICSI group ($P = 0.53$). The Caesarean section rate after the transfer of FET embryos was 23.9% and 16.0% in the SCS and ICSI groups, respectively ($P = 0.29$).

TABLE 1 GENERAL CHARACTERISTICS FOR WOMEN DELIVERING SINGLETONS AFTER SCS OR ICSI IN FRET AND FET CYCLES

Parameter	ICSI-FRET (*n* = 103)	SCS-FRET (*n* =105)	Statistics		ICSI-FET (*n*=50)	SCS-FET (*n*=71)	Statistics
Female age (years)	32 ± 3.5	33 ± 5.0	0.09		31 ± 3.9	31.2 ± 4.2	0.09
BMI (kg/m^2)	24.2 ± 2.9	24.7 ± 3.0	0.22		24.3 ± 2.8	24.9 ± 3.0	0.26
Primary infertility	66 (64.1)	62 (59.1)	0.45		25 (50.0)	37 (52.1)	0.45
Secondary infertility	37 (35.9)	43 (41.0)	0.45		25 (50.0)	34 (47.9)	0.45
Smokers	11 (10.7	12 (11.4)	0.86		5 (10.0)	8 (11.3)	0.82
Rank (cycle)							
1	58 (56.3)	49 (46.7)	0.16		19 (38.0)	36 (50.7)	0.16
2 or 3	40 (38.8)	48 (45.7)	0.31		18 (36.0)	18 (25.4)	0.31
>3	5 (4.9)	8 (7.6)	0.40		13 (26.0)	17 (23.9)	0.40
Ovarian stimulation							
Antagonist	89 (86.4)	92 (87.6)	0.79	Natural cycle	35 (70.0)	54 (76.1)	0.79
Short agonist	8 (7.8)	10 (9.5)	0.62	Substitution	15 (30.0)	17 (23.9)	0.62
Long agonist	6 (5.8)	3 (2.9)	0.28				
Day of embryo transfer							
Day 2–3	35 (34)	33 (31.4)	0.70		–	–	
Day 4–5	68 (66)	72 (68.6)	0.69		–	–	
Embryos transferred					–	–	
Single-embryo transfer	93 (90.3)	92 (87.6)	0.53		41 (82)	62 (87.3)	0.53
Double-embryo transfer	10 (9.7)	13 (12.4)	0.53		9 (18)	9 (12.7)	0.53

Data are *n* (%) or mean ± SD.

FET, frozen embryo transfer; FRET, fresh embryo transfer; ICSI, intracytoplasmic sperm injection; SCS, simplified culture system.

TABLE 2 PERINATAL AND OBSTETRIC OUTCOME RESULTS FOR PATIENTS DELIVERING SINGLETONS AFTER SCS OR ICSI IN FRET AND FET EMBRYO TRANSFER CYCLES

Parameter	ICSI-FRET (*n* = 103)	SCS-FRET (*n* = 105)	*P*-value	ICSI-FET (*n* = 50)	SCS-FET (*n* = 71)	*P*-value
Birthweight (g)	3230 ± 536	3360 ± 554	0.08	3637 ± 449	3478 ± 560	0.09
Duration of pregnancy (weeks)	39.1 ± 1.6	39.4 ± 2.5	30	39.3 ± 1.3	39.3 ± 1.7	1.00
<32 weeks	1 (1.0)	2 (1.9)	0.54	0 (0.0)	1 (1.4)	0.40
<37 weeks	7 (6.8)	4 (3.8)	0.33	3 (6.0)	6 (8.5)	0.62
Birthweight cut-off						
<1.5 kg	0 (0.0)	1 (1.0)	0.33	0 (0.0)	1 (1.4)	0.40
<2.5 kg	8 (7.8)	3 (2.9)	0.10	0 (0.0)	3 (4.2)	0.14
>4.2 kg	3 (2.9)	7 (6.7)	0.20	5 (10.0)	6 (8.4)	0.76
SGA (<10th percentile)	21 (20.4)	15 (14.3)	0.24	2 (4.0)	7 (9.9)	0.22
LGA (>90th percentile)	7 (6.8)	11 (10.5)	0.37	9 (18.0)	11 (15.5)	0.71
Perinatal mortality	0 (0.0)	1 (1.0)	0.33	0 (0.0)	0 (0.0)	–
Congenital malformation	0 (0.0)	0 (0.0)	–	0 (0.0)	1 (1.4)	0.40
Delivery						
Vaginal	80 (77.7)	78 (74.3)	0.57	40 (80.0)	49 (69.0)	0.17
Vacuum extraction	5 (4.9)	5 (4.8)	0.97	2 (4.0)	5 (7.0)	0.48
Caesarean section	18 (17.5)	22 (21)	0.53	8 (16.0)	17 (23.9)	0.29

Data are *n* (%) or mean ± SD.

FET, frozen embryo transfer; FRET, fresh embryo transfer; ICSI, intracytoplasmic sperm injection; LGA, large for gestational age; SCS, simplified culture system; SGA, small for gestational age.

One perinatal mortality was observed in the fresh SCS transfer group, caused by an abruptio placentae at 28 weeks (birthweight 800 g) without obvious reason. After FET one congenital malformation, a clubfoot, was noted in the SCS group (TABLE 2).

For all singletons (FRET and FET) born after using the SCS the prematurity rate was 5.7% (10/176) compared with 6.5% (10/153) in the ICSI group. LBW was found in 3.4% (6/176) and 5.2% (8/153) of babies after SCS and ICSI respectively (TABLE 3). All the differences between the SCS and ICSI groups, whether FRET or FET, were non-significant.

A total of 32 twin babies were born after FRET: 16 after SCS and 16 after ICSI. Although a higher mean birthweight and pregnancy duration were observed in the SCS-inseminated group, these differences were non-significant (TABLE 4). Six twin babies were born after SCS FET, with four babies born before 32 weeks and four with a birthweight below 1.5 kg.

DISCUSSION

Considering the fact that, worldwide, more and more babies are being born after ART, safety is an essential issue in general, and in particular when IVF is the only means likely to achieve pregnancy. Although some studies claim that the adverse outcomes in babies born after IVF/ICSI are mainly attributable to the factors leading to infertility (*Romundstad et al., 2008*; *Seggers et al., 2016*),

TABLE 3 PERINATAL AND OBSTETRIC OUTCOME RESULTS FOR PATIENTS DELIVERING SINGLETONS AFTER SCS OR ICSI IN COMBINED FRET AND FET CYCLES

Parameter	ICSI (*n* = 153)	SCS (*n* = 176)	*P*-value
Female age (years)	32 ± 3.65	32 ± 4.46	1.00
Birthweight (g)	3363 ± 542	3411 ± 558	0.70
Duration of pregnancy (weeks)	39.25 ± 1.52	39.33 ± 2.23	0.43
<32 weeks	1 (0.7)	3 (1.7)	0.35
<37 weeks	10 (6.5)	10 (5.7)	0.76
Birthweight cut-off			
<1.5 kg	0 (0.0)	2 (1.1)	0.19
<2.5 kg	8 (5.2)	6 (3.4)	0.41
>4.2 kg	8 (5.2)	13 (7.4)	0.41
SGA (<10th percentile)	23 (15.0)	22 (12.5)	0.51
LGA (>90th percentile)	16 (10.5)	22 (12.5)	0.57
Perinatal mortality	0 (0.0)	0 (0.0)	
Congenital malformation	0 (0.0)	1 (0.6)	0.38
Delivery			
Vaginal	120 (78.4)	127 (72.2)	0.18
Vacuum extraction	7 (4.6)	10 (5.7)	0.65
Caesarean section	26 (17.0)	39 (22.2)	0.23

Data are *n* (%) or mean ± SD.

FET, frozen embryo transfer; FRET, fresh embryo transfer; ICSI, intracytoplasmic sperm injection; LGA, large for gestational age; SCS, simplified culture system; SGA, small for gestational age.

TABLE 4 PERINATAL AND OBSTETRIC OUTCOME RESULTS FOR PARTICIPANTS DELIVERING TWINS AFTER SCS OR ICSI IN FRESH EMBRYO TRANSFER CYCLES

Parameter	ICSI fresh *n* = 16 (8 pregnacies)	SCS fresh *n* = 16 (8 pregnacies)	*P*-value
Female age (years)	32.8 ± 2.29	35.0 ± 4.23	0.07
Birthweight (g)	2164 ± 588	2473 ± 515	0.12
Duration of pregnancy (weeks)	34.2 ± 2.5	35.8 ± 2.2	0.06
<32 weeks	4 (25.0)	0 (0.0)	
<37 weeks	14 (87.5)	8 (50.0)	
Birthweight cut-off			
<1.5 kg	3 (18.8)	1 (6.3)	
<2.5 kg	12 (75.0)	7 (43.8)	
Perinatal mortality	0 (0)	0 (0)	
Congenital malformation	0 (0)	0 (0)	
Sex of newborn			
Male	11 (68.8)	12 (75.0)	
Female	5 (31.3)	4 (25.0	
Delivery			
Vaginal	8 (50.0)	9 (56.3)	
Vacuum extraction	0 (0.0)	1 (6.3)	
Caesarean section	8 (50.0)	6 (37.5)	

Data are *n* (%) or mean ± SD.

ICSI, intracytoplasmic sperm injection; SCS, simplified culture system.

most studies still show a slightly poorer outcome for ART singletons when compared with spontaneously conceived babies, even in sibling studies where the mother gave birth to both an ART and a spontaneously conceived baby (*Berntsen et al., 2019*; *Henningsen et al., 2011*; *Luke et al., 2016*; *Pinborg et al., 2013*). The present study described and compared the perinatal outcome of 176 singletons and 22 twin babies born after using the SCS and 153 singletons and 16 twins born after ICSI followed by conventional culturing in the same patient cohort.

The reasons why the SCS system was compared with ICSI followed by regular culturing (Genk standard protocol) and not with regular IVF was the following. First, in Belgium reimbursement for a maximum of six IVF/ICSI cycles is offered by the government for all infertile patients below 43 years of age regardless of the technique applied (*Ombelet et al., 2005b*). Therefore, to examine the effectiveness of a new simplified IVF technique under these conditions, it was necessary to include ICSI for sibling oocytes in order to avoid as far as possible the possibility that no embryos would be unexpectedly unavailable for transfer if IVF alone was used. It is worth noting that IVF, whether in the SCS or another culture system used in IVF programmes, involves an interaction between male and female gametes and, as such, there is an increased risk of fertilization failure compared with ICSI, mostly due to spermatozoa–zona binding problems (*Liu and Baker, 2000*). With the adoption of this strategy, a substantial number of patients agreed to participate in this study because of the popular belief that ICSI is the most successful ART technique and, for this study, the guarantee that at least three oocytes or more would be subjected to ICSI.

The main outcome parameters examined were PTB, SGA and LBW. According to the WHO a PTB is defined as a birth before 37 weeks of completed gestation (*WHO, 1977*), a duration that is easy to calculate when ART is performed. PTB is the leading cause of mortality in children under 5 years of age and is also associated with long hospital admissions due to serious morbidity (*Chawanpaiboon et al., 2019*; *Lee et al., 2019*). LBW is defined as a birthweight below 2.5 kg caused by PTB and/or intrauterine growth restriction. SGA is most commonly defined as a weight below the 10th percentile for the gestational age. This original classification was developed by a 1995 WHO expert committee, and the definition is based on a birthweight-for-gestational-age measure compared with a gender-specific reference population (*de Onis and Habicht, 1996*). All three parameters are associated with important short-term and long-term consequences for the child, the family and society (*Rüdiger et al., 2019*; *Thanh et al., 2015*).

Considering singletons born in FRET cycles very low PTB (3.8%) and LBW (2.9%) rates were found after using SCS, compared with 6.8% and 7.8% after ICSI followed by conventional culturing. Although these differences are not significant these figures are unexpectedly low for the SCS group when compared with outcome data reported in the literature using other IVF or IVF-related procedures (*Liu et al., 2020*; *Nouri et al., 2013*; *Ombelet et al., 2005a*).

Most studies have reported a better perinatal outcome after ICSI than regular IVF, but these reports are still controversial (*Ombelet et al., 2005a*; *Nouri et al., 2013*; *Liu et al., 2020*). US data including almost 200,000 procedures report an LBW of 8.1% LBW and a PTB of 14% (*Sunderam et al., 2020*), while according to the 2017 data of the Latin American Registry, preterm deliveries reached 9.5% in IVF/ICSI singletons (*Zegers-Hochschild et al., 2020*). The 21st European Society for Human Reproduction and Embryology report describing ART data in 2017 from 39 European countries showed a 13.4% PTB rate, with large differences between countries (*Wyns et al., 2021*). According to the 2013–2018 data of the Belgian Register for Assisted Procreation the prevalence of PTB and LBW was 7.7% and 7.0%, respectively, after fresh transfer in more than 19,000 IVF/ICSI cycles (*BELRAP*). In a systematic review and meta-analysis where Qin and colleagues (*Qin et al., 2017b*) reported on the worldwide prevalence of adverse pregnancy outcomes among singletons after IVF and/or ICSI, the prevalence of PTB and LBW was 10.9% and 8.7%, respectively, compared with 6.4% (PTB) and 5.8% (LBW) for spontaneously conceived singletons.

The current study's excellent outcome results showing low values for PTB, LBW and SGA, especially in the FRET SCS

group, might be explained by the fact that the study included a selected group of normo-responsive patients and cases of severe male infertility were excluded; however, this hypothesis is unlikely as a higher prevalence of adverse perinatal outcome has never been associated with severe male infertility, with the exception of a slightly higher incidence of congenital malformations (*Davies et al., 2012*).

Another possible explanation is related to the fact that there were no cases of a vanishing twin in this series. It is well known that vanishing twin pregnancies are associated with a higher risk of PTB, LBW, SGA and perinatal mortality for the surviving singletons, suggesting the presence of harmful intrauterine factors (*Magnus et al., 2017*; *Zhu et al., 2020*). Because a single-embryo transfer was performed for most of the women in this study, the adverse effect of vanishing twins on perinatal outcome was avoided.

As the SCS is an enclosed system, temperature and pH control being critical elements that can affect the normality of preimplantation development *in vitro*, it can be assumed that the stable SCS environment may closely parallel natural conception, so epigenetic modifications of the embryos are possibly less pronounced compared with regular IVF or ICSI. This is especially important in the FRET group, which excludes FET babies that are possibly more prone to epigenetic modification as a result of cryopreservation (*Pinborg et al., 2016*).

No statistically significant difference was found in terms of the vaginal birth rate in the SCS group (FRET and FET) compared with the ICSI group. The Caesarean section rate in the two groups was also not significantly different, and was comparable with the 20.1% Caesarean section rate reported for all 430,543 singletons born in Flanders between 2013 and 2019 (*Devlieger et al., 2019*).

In the current study, when comparing the perinatal outcome of the singletons after FET the figures for PTB and LBW are very similar in the SCS and ICSI groups. The mean birthweight and the prevalence of singletons with a birthweight of more than 4.2 kg was higher in the FET group compared with FRET cycles, although the differences were not significant, probably due to the small number of cases (**TABLE 2**). This finding is consistent with several reports showing that singletons born after FET are heavier, with a higher risk of being large for their gestational age, compared with FRET (*Berntsen et al., 2018*; *Conforti et al., 2021*; *Terho et al., 2021*; *Wennerholm et al., 2013*; *Westvik-Johari et al., 2021*).

Here, the following factors provide some potentially important insights into outcome using the SCS approach versus ICSI. First, a major strength of the design is the elimination of bias due to maternal and paternal factors. Perinatal outcome data were compared within the same patient cohort, the only difference being the culture method (SCS versus ICSI followed by conventional culturing). Second, many factors associated with a poorer perinatal outcome, such as female age, duration of infertility, indication for performing ART, cryopreservation method, lifestyle factors including BMI and smoking, were similar in the two groups. Third, the different patient and treatment-specific factors were collected prospectively, and the follow-up of the obstetric and perinatal outcomes was performed on a regular basis by phone call 3 months after the expected day of delivery. Finally, in the majority of cases, i.e. between 82% and 90%, a single embryo transfer was performed, and because an ultrasound scan was always performed between 7 and 9 weeks of gestation, vanishing twins could be excluded as a possible reason for a worse perinatal outcome.

Considering the limitation of this study, the study was performed on a small series of patients at a single centre and is limited by including only normo-responsive participants; therefore the results cannot be extrapolated to other centres or types of patient. In addition, because the study was not powered to determine a difference in perinatal outcomes, the results should be interpreted with caution and the conclusion drawn from the outcomes be equally as conservative. A multicentre prospective trial is necessary to confirm the results.

Nevertheless, the multi-year outcome results reported here support the group's initial findings (*Ombelet et al, 2014b*; *Van Blerkom et al, 2014*, *2019*) that the SCS is a safe and effective low-cost method for undertaking IVF. The very low PTB and LBW rates in the fresh SCS group indicate the need to gain more information and biological understanding of the reasons why infertility in general, and certain parameters associated with ART in particular, can be accompanied by adverse perinatal and long-term outcomes, even for singleton births. With respect to the low PTB and LBW rates recorded, more human data are required to provide important insights into the molecular, genetic and physiological mechanisms that may contribute to such outcomes so that conditions for fertilization and early embryogenesis *in vitro*, including the SCS method, can be improved. Animal models show convincing evidence that epigenetic changes in genes involved in growth and development occur during the IVF process, subsequently altering the fetal phenotype and long-term health (*Sullivan-Pyke et al., 2017*).

Considering these findings, special attention should be given to the extended culture of human embryos in different environments in by different culture media and culture conditions with a special emphasis on enclosed systems such as the SCS. More studies are needed to explore the possibility of minimizing the risks of genetic conditions, epigenetic disorders, malformations and microdeletion syndromes by exposing oocytes and embryos to culture medium containing spermatozoa and cumulus cells for a longer incubation time in a culture environment that is sealed from the ambient environment.

CONCLUSION

In the selected normo-responsive participants in this study, which excluded only cases of severe male infertility, the data suggest no difference in perinatal outcome for singleton and twin babies born after SCS compared with ICSI, in both FRET and FET cycles. This study provides evidence that the SCS technology is an effective and safe method for fertilization and embryo culture, although trials in different centres will ultimately determine the general applicability of this method, especially when increasing access to and the affordability of complex fertility treatments is desired goals.

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