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ARTICLE

Multiyear outcomes using sibling oocytes demonstrates safety and efficacy of a simplified culture system consistent with use in a low-cost IVF setting

BIOGRAPHY

Willem Ombelet obtained his PhD degree at the University of Leuven in 1998 and from 2001 until 2004 he was the President of the Flemish Society of Obstetrics and Gynaecology. He was the founder of the Genk Institute for Fertility Technology and the non-profit organization The Walking Egg.

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KEY MESSAGE

No differences were found in ongoing pregnancy rate, implantation rate and miscarriage rate between SCS and ICSI in a selected patient cohort. Considering the economic advantage of SCS, this simplified method represents a real promise in countries where regular IVF procedures are too costly for a majority of the population.

ABSTRACT

Research question: Can a novel closed simplified IVF culture system be used to achieve outcomes comparable to those obtained with intracytoplasmic sperm injection (ICSI) followed by conventional culturing?

Design: This analysis is part of a non-inferiority prospective study comparing ICSI and a simplified culture system (SCS) for gamete fertilization in a selected group of patients. According to protocol, sibling oocytes in intact cumulus–oocyte complexes were randomly distributed between ICSI and conventional insemination in the SCS. For women, selection criteria included being under 43 years of age and at least six eggs at retrieval. An inseminating motile sperm count ≥1 million was required. The primary outcome measure was ongoing pregnancy rate (>12 weeks) per cycle; secondary outcome measures included fertilization rate, miscarriage rate and implantation rate (ongoing pregnancy rate per embryo).

Results: From January 2016 until December 2019, 653 SCS/ICSI cycles were performed yielding a total of 7915 oocytes. The fertilization rate was 61.1% and 50.4% for SCS and ICSI ($P < 0.0001$), respectively. The ongoing pregnancy rate was 32.0% for SCS and 36.7% for ICSI ($P = 0.27$). Implantation rate was 30.6% for SCS and 34.4% for ICSI ($P = 0.35$). The miscarriage rate was 7.5% and 6.5% for SCS and ICSI, respectively ($P = 0.75$).

Conclusion: No difference was found in ongoing pregnancy rate, implantation rate and the miscarriage rate between SCS and ICSI in this selected patient cohort.

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KEYWORDS

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INTRODUCTION

Infertility is a universal health issue that has been estimated to affect 8–12% of couples worldwide (*Boivin et al., 2007*). This suggests that 48 million couples and 186 million individuals live with infertility globally, most of them being residents of low- and middle-income countries (LMIC) (*Mascarenhas et al., 2012*; *Rutstein and Iqbal, 2004*). Despite the severe sociocultural, psychological and economical consequences associated with infertility and involuntary childlessness in LMIC, the availability of accessible and affordable infertility care to most affected couples remains very low or non-existent (*Chiware et al., 2021*; *Dyer and Patel, 2012*; *Dyer et al., 2004*; *Gerrits and Shaw, 2010*; *Inhorn and Patrizio, 2015*).

With bilateral tubal occlusion related to sexually transmitted diseases (STD), pregnancy-related infections (post-partum, post-abortion) and male infertility due to STD being the most common cause of infertility in these settings, the application of 'expensive' assisted reproductive technologies (ART) to treat infertility is often required. Yet in most LMIC up to now, these procedures have been absent; when they are available, it is mostly in private settings and only within reach of the fortunate few who can afford them (*Dyer and Patel, 2012*; *Ombelet and Onofre, 2019*; *Ombelet et al., 2008*).

In order to meet a population's need for ART, it has been estimated that at least 1500 IVF/intracytoplasmic sperm injection (ICSI) cycles per million inhabitants should be performed (*Fauser et al., 2002*). This is only possible if enough ART centres are available, and the costs associated with ART treatment are within reasonable limits. According to most recent reports (http://www.ivf-worldwide.com; *Chiware et al., 2021*; *Dyer et al., 2020*), this not the case in LMIC.

The high cost and complex know-how associated with the implementation of ART services is certainly the substantive reason behind the inequity of accessible and cost-effective treatment between LMIC and continents. Because high-end ART techniques (IVF/ICSI) have proven to be the best treatment option in the majority of infertility cases, optimization of infertility care in terms of availability, affordability and effectiveness are urgently required; this includes the simplification of diagnostic procedures, ovarian stimulation (i.e. mild ovarian stimulation), fertilization and subsequent embryo culture procedures to be safe, affordable and with respect to outcome, effective (*Datta et al., 2021*; *Nargund et al., 2017*; *Ombelet and Campo, 2007*).

As part of The Walking Egg project (*Dhont, 2011*; *Ombelet, 2014*) a simplified IVF method was developed called The Walking Egg lab system (^{t}WE), also called the simplified culture system (SCS), which significantly reduces the high costs of medical gases, complex incubation equipment, consumables and infrastructure typically used in high-end IVF laboratories (*Van Blerkom et al., 2014*).

Importantly, the sperm concentration required is based on the number of cumulus–oocyte complexes (COC) to be inseminated simultaneously, which in this system is only 1000–5000 motile washed spermatozoa; the latter has been shown to be highly successful with moderate or severe male factor involvement (*Boshoff et al., 2018*; *Van Blerkom et al., 2014*). Because development from insemination to embryo transfer is undisturbed using an enclosed system, common pitfalls of regular IVF laboratories, such as unwanted temperature changes, pH and air quality problems, can be avoided while providing additional physical protection to the gametes and developing embryos.

The first publication reporting the effectiveness of SCS and the birth of the first seven healthy babies was followed by the report of the birth of another four healthy babies upon cryo-thawing and embryo transfer from embryos conceived with the SCS (*Ombelet et al., 2014*; *Van Blerkom et al., 2014*). These findings provided a basis for the acceptance of this technique at the Ziekenhuis Oost Limburg in Genk, Belgium, and the protocol described below to continue with the study.

The outcome results are described here of 4 years of an ongoing prospective study in a selected group of normo-responder patients comparing laboratory and clinical outcomes of split IVF cycles in which insemination involved ICSI or SCS, with the former cultured under a mixed gas atmosphere produced within a conventional cell culture incubator. For partners or donor spermatozoa, the exclusion criterion was severe male factor, defined as <1 million inseminated motile sperm count (IMC) upon processing.

The protocol for this particular study of the effectiveness of SCS used ICSI rather than conventional IVF because: (i) in the first publication SCS was compared with conventional or 'regular' IVF (*Van Blerkom et al., 2014*), with similar results; (ii) according to the hospital's study protocol and upon the recommendation of the ethical committee, IVF and ICSI (50/50) is always performed during the first attempt if no severe male factor is involved (IMC >1 million) both to avoid the possibility of 'total fertilization failure' and to detect potential sperm–zona binding problems (*Hershlag et al., 2002*; *Johnson et al., 2013*; *Liu and Baker, 2000*; *van der Westerlaken et al., 2005*, *2006*); (iii) many patients in Belgium have been led to believe that ICSI is more successful than regular IVF even if no severe male factor is involved. In this instance, it was easier to enrol patients in this study if ICSI was performed in at least half of the eggs, also knowing that as a result of the reimbursement policy of the Belgian Government, there is no cost difference between IVF and ICSI; and (iv) it has been shown that ICSI is overused for non-male infertility indications with no convincing evidence of a significant difference despite a lack of evidence of or improvement in live birth rate per cycle (*Glenn et al., 2021*; *Hodes-Wertz et al., 2012*; *Li et al., 2018*).

MATERIALS AND METHODS

From January 2016 to December 2019, this study prospectively investigated the patient and cycle characteristics and the outcome results of 653 consecutive SCS/ICSI cycles at the Genk Institute for Fertility Technology, a tertiary infertility centre.

Selection of patients

The patient cohort described here is part of a larger non-inferiority prospective study performed at the ZOL Hospitals in Genk, Belgium, following the SCS technique of *Van Blerkom et al. (2014)* with few modifications. Briefly, couples failing to conceive following cessation of contraception for at least 12 months were eligible for treatment. Prior to treatment and according to Belgian

law, all couples were tested for HIV, syphilis, hepatitis B and hepatitis C virus. Female patients were subjected to a diagnostic work-up including medical history, physical examination, pelvic ultrasound, serum hormone assays between day 2 and 4 of the menstrual cycle and a hysterosalpingography (HSG), hysterosalpingo-foam sonography (HyFoSy) or hysteroscopy. Laparoscopy was performed only in cases of suspected tubal pathology, endometriosis or where ovarian cyst(s) were present. For men, analysis of anti-sperm antibodies (ASA) and at least two sperm examinations were performed prior to treatment. All samples were examined following the guidelines of the World Health Organization (WHO) (*Cooper et al., 2010*; *World Health Organization, 2010*).

All women were less than 43 years of age, had a minimum of six oocytes at collection and suffered from tubal occlusion, mild-to-moderate endometriosis or unexplained infertility. Couples with mild-to-moderate male infertility were also included provided the number of motile spermatozoa after processing (IMC) was above 1 million. According to the hospital's protocol, at least three or four intrauterine inseminations (IUI) were always performed before starting IVF or ICSI for patients with unexplained or moderate male infertility and open Fallopian tubes.

Ovarian stimulation protocol, oocyte collection and semen processing

Recombinant (Puregon®, MSD, Belgium) or urinary FSH (Menopur®, Ferring, Belgium) was used for ovarian stimulation. Stimulation with 150 IU FSH was initiated on cycle day 2 or 3. A gonadotrophin-releasing hormone (GnRH) agonist (long or flare-up protocol) or antagonist co-treatment protocol was used for LH peak suppression. The antagonist protocol was used in patients with a normal or high anti-Müllerian hormone (AMH >1.0 ng/ml) and antral follicular count (AFC) >8 follicles on ultrasound. The antagonist protocol started with recombinant FSH (rFSH) 150 IU for patients ≤35 years old or urinary FSH 150 IU for patients over 35 years old. The antagonist ganirelix 0.25 mg daily (Orgalutran®, MSD) was added on day 6 or 7 depending on the size of the follicles. In cases of known moderate to severe endometriosis, adenomyosis or the presence of uterine myomata, the long-acting agonist protocol was used. This protocol made use of nasal busereline 0.4 mg daily (Suprefact®, Sanofi, Belgium) for 14 days starting in the luteal phase of the previous cycle, thereafter rFSH or urinary FSH was added as described in the antagonist protocol and the agonist was continued until the day of human chorionic gonadotrophin (HCG) trigger. In cases of known normal ovarian response in previous IVF attempt(s) the short agonist protocol was used by starting injections on day 2 or 3 with the agonist triptoreline 0.1 mg i.m. for 7 days (Gonapeptyl®, Ferring). In the meantime, rFSH or urinary FSH was administered daily until the day of HCG trigger.

Final oocyte maturation was achieved by administration of 5000 IU HCG (Pregnyl®, MSD) when three or more follicles of 17 mm were present. If there was a high risk for ovarian hyperstimulation syndrome, Gonapeptyl® 0.2 mg i.m. instead of HCG was used to trigger ovulation in the antagonist protocol.

Oocyte retrieval was always carried out 35–36 h after HCG or agonist administration.

On the day of oocyte aspiration, the semen sample was obtained through masturbation after a 2–4-day abstinence period and collected in a sterile cup. Within 1 h of production and after liquefaction at room temperature, the specimen was examined according to WHO guidelines (*World Health Organization, 2010*). For sperm processing, density gradient centrifugation with PureSperm® 40/80 (Nidacon International AB, Mölndal, Sweden) was used following the manufacturer's instructions.

Embryo transfer was performed 3–5 days after ovum retrieval. Although embryo selection could be made through the glass culture tube, digital images of individual embryos from the SCS and ICSI group were taken 3 h prior to transfer. The selection of the embryo(s) for transfer was made by an independent embryologist who was unaware as to whether SCS or ICSI was used, nor could it be identified from the images. Criteria for single embryo transfer (SET) used recognized morphological and performance characteristics for stage- and time-appropriate development (*ALPHA Scientists in Reproductive Medicine; ESHRE Special Interest Group Embryology, 2011*). Regardless of the system used (SCS or ICSI), in most cases one top-quality embryo was transferred. If two or more top-quality embryos were available from both systems, the transferred embryo(s) was selected by randomization using a computerized system (www.randomizer.org). Digital images of embryos were coded to identify the culture system but what the identifier signified was unknown to the embryologist performing the selection (*Van Blerkom et al., 2014*).

Luteal phase supplementation consisted of 600 mg micronized progesterone (Utrogestan®, Besins, Belgium) in three separate dosages starting the day of oocyte retrieval and continuing until 18 days after ovum retrieval. The progesterone was continued when the pregnancy test was positive until the day of ultrasound 5–6 weeks after oocyte retrieval.

Surplus embryos were vitrified using a commercially available vitrification kit (RapidVit™, Omni, Vitrolife, Frölunda, Sweden) according to the manufacturer's protocol.

ICSI versus conventional fertilization in the SCS

In all treatment cycles sibling COC were randomly equally divided for SCS fertilization or ICSI. In case of an odd number of retrieved oocytes the extra COC were inseminated by ICSI. However, when more than 16 eggs were retrieved, a maximum of eight COC were allocated to conventional insemination in the SCS with the rest inseminated by ICSI. After follicular aspiration of COC, 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 would be equally distributed between the two insemination groups, with a slight numerical bias to ICSI based on the patient-centric protocol noted above.

Simplified culture system

Simplified IVF was performed as described by *Van Blerkom et al. (2014)* (FIGURE 1). In brief, a simplified incubation system including two glass tubes (Test Tube, 141118, Zhejiang Gongdong Medical Technology Co., Ltd, China), a catheter (CodanSet, 71.4590, Medizinische Geräte GmbH, Lensahn, Germany), two needles (301500, Microlance™, Becton

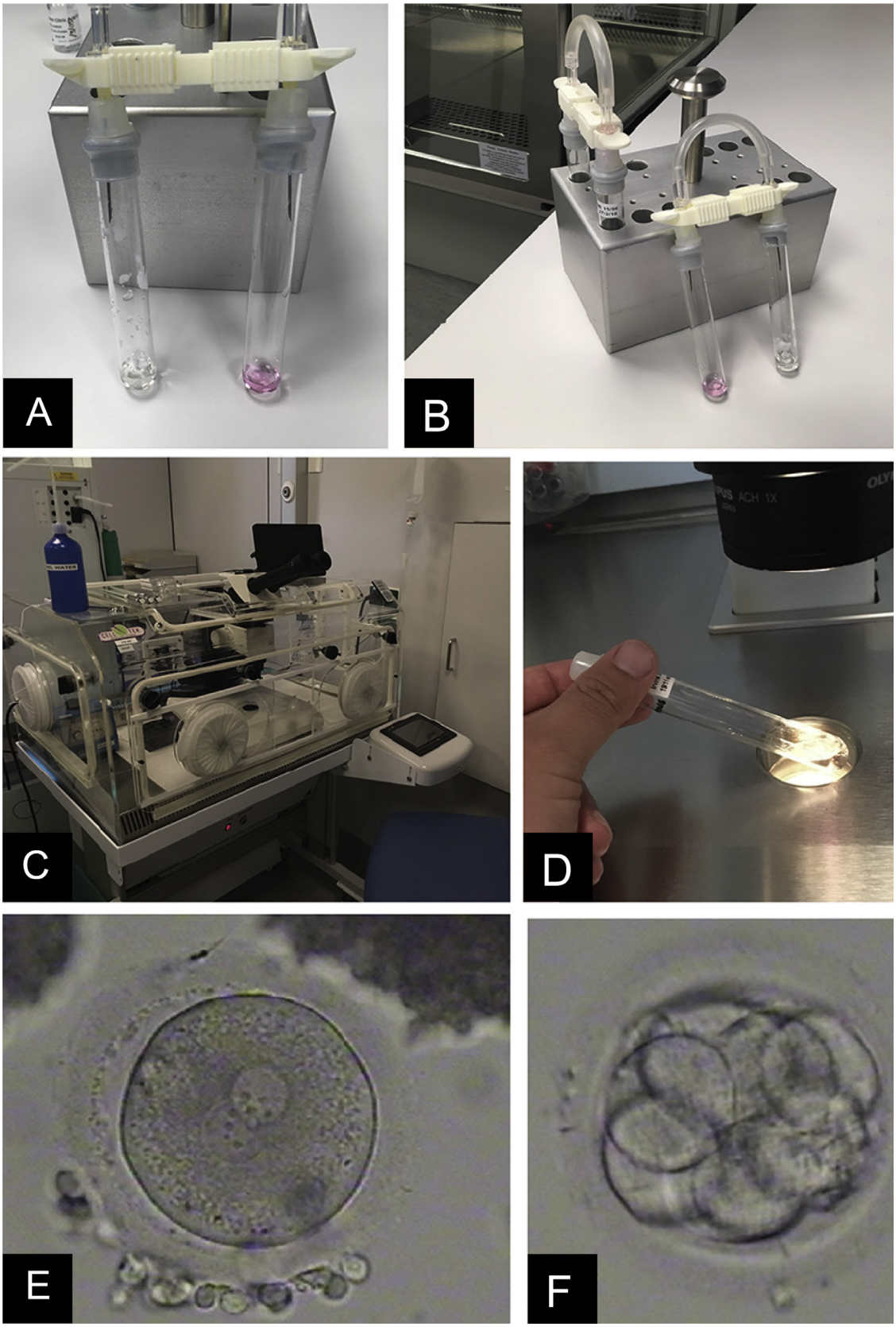

FIGURE 1 The tWE lab or SCS system: to achieve media equilibration a connection is made between tube 1 with citric acid and sodium bicarbonate in water and tube 2 with IVF culture medium (A), incubated overnight at 37°C in a heating block (B). It transfers enough CO_2 gas produced by the chemical reaction between the acid and the base to equilibrate the pH of the medium to approximately 7.30. All procedures are performed in an IVF chamber under stable temperature (37°C) (C). Fertilization and embryo scoring by looking through the glass tube (D, E, F). Precise control and regulation of the culture conditions such as pH, temperature and humidity, critical for successful embryo development and survival, are achieved with this 'closed system'.

Dickinson, Louth, Ireland) and a heating block (10719, BlockThermostat, Labotect, Gottingen, Germany) was assembled and used for culture of gametes and embryos. Prior to the IVF procedure, 1 ml of single-step IVF medium (Irvine, 90165, Fujifilm Irvine Scientific, CA, USA) was included in one Vacutainer tube and closed with a stopper (4432/50, Lyo NovaPure, WestPharma, Exton, USA) overlapped with a silicon sleeve (467013-50, Saint-Gobain, Courbevoie, France) for airtightness. In a different tube, the chemical generation of *de novo* CO_2 was facilitated by the dilution of a weak base (sodium bicarbonate) and a weak acid (citric acid) (CAS010, FertiPro NV, Beernem, Belgium) in water. Produced CO_2 was kept in the tube closed with a stopper (4432/50, Lyo NovaPure) overlapped by a silicon sleeve (467013-50, Saint-Gobain). To achieve media equilibration, both tubes were connected using a catheter (CodanSet, 71.4590, Medizinische Geräte GmbH) with two needles (301500, Microlance™, Becton Dickinson) and incubated overnight at 37°C in a heating block (10719, BlockThermostat, Labotect) (FIGURE 1). During this period the air space over the IVF medium inside the tube was supplemented with *de novo* CO_2 resulting in the equilibration of the IVF medium to a desired pH at 37°C. Thereafter, the connection between the two tubes was removed and the equilibrated media tubes used for embryo culture. Consequently, the possibility of changes in atmosphere or pH, adverse to the developmental potential of resulting embryos, are eliminated as previously shown (*Van Blerkom et al., 2014*, *2019*). A constant 37°C temperature (optimal for fertilization and preimplantation embryogenesis) was readily maintained with a conventional budget-friendly dry heating block. Sperm insemination was performed 2–3 h after oocyte retrieval using 2000 motile spermatozoa/ml. According to protocol, when sperm morphology was <4% (*World Health Organization, 2010*), approximately 5000 motile spermatozoa were used for insemination.

Direct injection of 2000–5000 processed spermatozoa and the COC in 1 ml of pre-equilibrated warm IVF medium was performed using single-use needles (301500, Microlance™, Becton Dickinson) and syringes (382903031764, BD Plastipak, Becton Dickinson, Madrid, Spain). During the pronuclear check, if the oocyte was tightly covered by cumulus cells, the tubes were vortexed for up to 30 s in a standard vortexing instrument set at approximately 50–60% of the maximum speed to dislodge the oocyte from the cumulus cells. This almost always removed most residual cumulus and coronal cells insofar that the number of pronuclei were identifiable, even if small fragments of the cumulus oophorus and corona radiate remained attached to the zona pellucida, as described previously (*Van Blerkom et al., 2014*). Fertilization check and daily embryo development were assessed under a stereomicroscope (SZX7, Olympus, Hamburg, Germany) until the day of embryo transfer.

All procedures were performed in an IVF chamber (Cell-Tek 3000, Tek-Event Pty Ltd, Sydney, Australia) under stable temperature (37°C). Pre-selection of the embryo(s) to be used for transfer was easily done without opening the test tubes until the very moment of embryo transfer.

ICSI followed by conventional culturing

For the application of ICSI, upon collection of oocytes, 80 IU/ml hyaluronidase HEPES-buffered (90017, HYASE™-10X, Vitrolife) was used to denude oocytes from surrounding cumulus cells. Metaphase II oocytes were then transferred to an ICSI dish (16006, Vitrolife) in 20 µl droplets HEPES-buffered medium (G-MOPS™ PLUS, 10130, Vitrolife) covered with mineral oil (OVOIL™, 10029, Vitrolife). Processed spermatozoa were also contained in HEPES-buffered medium in the same dish and later immobilized in polyvinylpyrrolidone (PVP) immobilization medium (ICSI™, 10111, Vitrolife) before micro-injection. Oocytes were micro-injected following a standard ICSI protocol at least 3 h after retrieval (day 0) as described elsewhere (*Palermo et al., 1992*; *Van Steirteghem et al., 1993*). In brief, the micro-injection was performed setting the first polar body at the 6 or 12 o'clock position to minimize the risk of meiotic spindle damage. Then, the oocyte was fixed with an aspiration pipette (MPH-LG-20, ORIGIO Inc., Charlottesville, USA) and micro-injected with an immobilized sperm cell previously loaded to an injection pipette (MIC-SLM-20, ORIGIO Inc.) according to standard protocol (*De Los Santos et al., 2016*). Upon injection, each oocyte was washed in IVF medium (G-1™, 10128, Vitrolife) and transferred to a multi-well dish (micro-droplet Culture Dish, 16003, Vitrolife) containing 30 µl droplets of pre-equilibrated IVF culture medium (G-1™, 10128, Vitrolife). Finally, the dishes were incubated in a desktop incubator (MIRI®, ESCO Medical, Egå, Denmark) at 37°C and in at atmosphere of 6% CO_2 and 5% O_2 overnight. Sixteen to 18 h post micro-injection a fertilization check was performed followed by daily embryo development assessment until embryo transfer was performed under an inverted microscope (Olympus, IX71, Hamburg, Germany).

Outcome measures

The primary outcome measure was an ongoing pregnancy (>12 weeks of gestation with positive heartbeat). Secondary end-points included fertilization rate, implantation rate and miscarriage rate (gestational age below 12 weeks).

Ethical committee approval

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

Statistics

The MedCalc statistical software package for biomedical research, comparison of proportions calculator (https://www.medcalc.org/calc/comparison_of_proportions.ph) was used to analyse the significance of differences between the SCS and ICSI groups.

As recommended by *Campbell (2007)*, MedCalc uses the N-1 chi-squared test for the comparison of two proportions (from independent samples), expressed as a percentage. A *P*-value of <0.05 was considered statistically significant. Multivariate analysis was not necessary because of the sibling protocol in which oocytes of both groups are recovered from the same woman.

RESULTS

From January 2016 until December 2020, this study prospectively examined a total of 653 SCS/ICSI cycles. The main characteristics of the study population

TABLE 1 PATIENT AND CYCLE CHARACTERISTICS (*N* = 653 CYCLES)

SCS/ICSI cycles 2016–19	*n*	%
Indication for treatment		
Female factor	222	34.0
Mild or moderate male factor	262	40.1
Female and male factor	98	15.0
Unexplained	71	10.9
Primary infertility	421	64.5
Secondary infertility	232	35.5
Cycle rank		
1	384	58.8
2	154	23.6
3 or 4	93	14.2
5 or 6	22	3.4
Ovarian stimulation protocol		
Antagonist	504	77.2
Short agonist	69	10.6
Long agonist	80	12.2
Number of eggs retrieved (range 6–42)		
6 to 10	294	45.0
11 to 15	195	29.9
16 to 20	98	15.0
>20	66	10.1
Number of cycles with embryo transfer	506	77.5
Number of cycles without embryo transfer	147	22.5
No viable embryos	13	2.0
Risk for OHSS (freeze all)	134	20.5

Female factor includes a tubal factor, mild to severe endometriosis and ovulatory disorders with or without PCOS. The diagnosis of mild or moderate male infertility was based on the threshold levels published by the (*WHO, 2010*). Patients were excluded if the IMC after processing was less than 1 million.

ICSI = intracytoplasmic sperm injection; IMC = inseminating motile count; OHSS = ovarian hyperstimulation syndrome; SCS = simplified culture system.

are shown in TABLE 1. A female factor was present in 34.0%, a male factor in 40.1%, combined female and male factors in 15.0% and unexplained factors in 10.9% of cases.

In the majority of cycles, the antagonist protocol was used for ovarian stimulation (77.2%). No embryo transfer could be performed in 147 cycles (22.5%). In 13 of these cycles there was no embryo available for transfer and in the remaining 134 cycles a freeze-all procedure was opted for due to an increased risk of ovarian stimulation (TABLE 1).

A total of 7915 oocytes were retrieved; 3548 oocytes were available for SCS (44.8%) and 4367 oocytes were selected for ICSI (55.2%). The number of fertilized oocytes was 61.1% (2168/3548) for SCS and 50.4% (2199/4367) for ICSI ($P < 0.0001$). Considering only metaphase II oocytes for ICSI the fertilization rate was 64.6% (2199/3402) (FIGURE 2, TABLE 2).

A total of 534 embryos were transferred, 235 SCS and 299 ICSI embryos accounting for 44.0% and 55.9% of transfers, respectively. In 72.9% and 76.9% of cases a day 5 transfer was performed for SCS and ICSI, respectively (TABLE 2).

The ongoing pregnancy rate per transfer was 32.0% for SCS and 36.7% for ICSI ($P = 0.27$); the implantation rate per embryo was 30.6% for SCS and 34.4% for ICSI (P = 0.35).

Embryos were available for cryopreservation in 50.2% and 51.6% of cycles after SCS and ICSI, respectively, presenting no statistical difference (P = 0.75). SET was performed in 95.5% and 93.6% of SCS and ICSI cycles, respectively ($P = 0.33$).

The miscarriage rate was 7.5% and 6.5% for SCS and ICSI, respectively (P = 0.75). An ectopic pregnancy was observed in two patients after SCS (2.2%) and in three patients after ICSI (2.4%) (P = 0.89) (TABLE 2, FIGURE 2).

DISCUSSION

Infertility is a universal health issue and the large majority of childless couples reside in LMIC. This silent population of more than 50 million couples face the struggles and consequences of involuntary childlessness with a poor outlook. Despite a high prevalence of infertility and the cultural values associated with childbearing in LMIC, infertility care remains a low priority for local healthcare providers and community leaders (*Chiware et al., 2021*; *Dyer et al., 2020*; *Ombelet et al., 2008*; *Van Blerkom et al., 2019*).

To increase access to infertility care and specifically to ART in these countries, lowering the limiting costs of IVF laboratory procedures including the retrieval, fertilization and culture of eggs and embryos is crucial (*Botha et al., 2018*; *Ombelet, 2011*). This awareness remains one of the main objectives of The Walking Egg project (*Dhont, 2011*; *Ombelet, 2014*). As such, it has previously been described that the simplification of IVF with the 'WE lab system or SCS was a safe and cost-effective method to perform IVF with good clinical pregnancy rates per cycle and the birth of healthy babies comparable to standard IVF outcomes (*Ombelet et al., 2014*; *Van Blerkom et al., 2014*, *2019*). The SCS is a closed culture system enabling fertilization and further embryo development to occur in undisturbed culture conditions for up to 3 or 5 days (FIGURE 1). Awareness that prolonged, albeit minor, temperature changes have the potential to negatively influence the outcome, from 2016 on, SCS occurred in an IVF chamber (Cell-Tek 3000, Tek-Event Pty Ltd), which was also suggested from initial experience that examinations for fertilization and embryo development through the glass tubes might be more time-consuming when compared with regular IVF or ICSI.

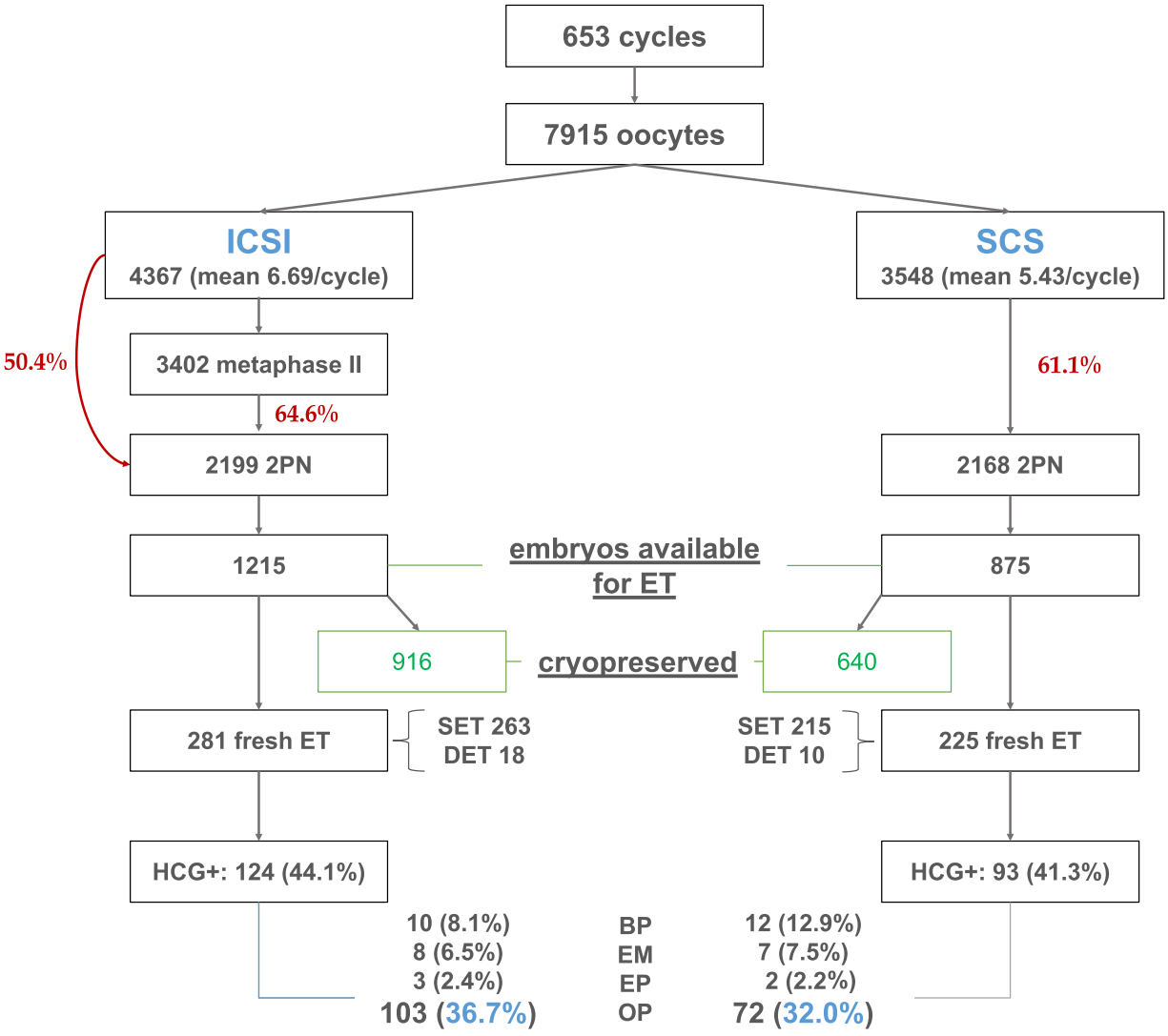

FIGURE 2 Flow chart of the study including 653 cycles. For BP, EM and EP the denominator was HCG+. 2PN = normal fertilization with two pronuclei; BP = biochemical pregnancy; DET = double embryo transfer; EM = early miscarriage (<12 weeks of gestation); EP = ectopic pregnancy; ET = embryo transfer; ICSI = intracytoplasmic sperm injection; OP = ongoing pregnancy per fresh ET (>12 weeks of gestation); SCS = simplified culture system; SET = single embryo transfer.

However, it is worth noting that inexpensive yet effective alternative methods to maintain a constant 37°C temperature when the SCS culture tubes are removed from the dry heating block for brief examination on a dissecting microscope stage to detect fertilization and assess preimplantation embryogenesis have been described, thus eliminating the need for a specific commercially produced enclosure (*Van Blerkom et al., 2019*).

In this prospective observational cohort study, SCS was compared with ICSI followed by conventional culture, but not with so-called 'regular IVF' using a plastic culture dish or chamber and a microprocessor-controlled cell culture incubator delivering a predetermined atmosphere.

The use of ICSI has increased dramatically in IVF treatment and is increasingly applied for indications other than male factor infertility. Recent data from the European Registry indicate that ICSI was used in more than 70% of cycles in Europe in 2017 (*Wyns et al., 2021*), although many studies have reported that the routine use of ICSI shows no benefit over conventional IVF and should only be applied in cases of moderate to severe male infertility and/or a history of total fertilization failure (*Glenn et al., 2021*; *Li et al., 2018*) and even in case of advanced maternal age (*Tannus et al., 2017*) and a low ovarian response (*Isikoglu et al., 2022*). To prove the effectiveness of SCS it was necessary to compare SCS with ICSI in ART cycles where a severe male factor was ruled out. It has been shown that ICSI in the absence of severe male infertility may be detrimental compared with IVF and this may have had a negative impact on outcomes in the control ICSI arm (*Li et al., 2018*; *McPherson et al., 2021*).

It was also necessary to properly inform patients that there was no financial incentive to participate in this trial because as a result of the Belgian reimbursement policy (*Ombelet et al., 2005*), reimbursement was already offered by the government for all patients regardless of the technique applied. For

TABLE 2 LABORATORY AND CLINICAL OUTCOME RESULTS OF THE STUDIED POPULATION (653 CYCLES WITH >5 OOCYTES, 506 CYCLES WITH FRESH EMBRYO TRANSFER)

	ICSI		SCS (^{t}WE)		*P*-value
	n	**%**	***n***	**%**	
Number of eggs available (7915)	4367	55.2	3548	44.8	
Fertilization rate (all oocytes)	2199/4367	50.4	2168/3548	61.1	**<0.0001**
Fertilization rate (metaphase II oocytes)	2199/3402	64.6			
No fertilization	27/653	4.1	55/653	8.4	**0.0014**
Number of cycles with embryo transfer	281/506	55.5	225/506	44.5	
Single embryo transfer	263	93.6	215	95.6	0.33
Double embryo transfer	18	6.4	10	4.4	0.33
Day 3 transfer	65	23.1	61	27.1	0.30
Day 5 transfer	216	76.9	164	72.9	0.30
HCG positive/embryo transfer	124/281	44.1	93/225	41.3	0.52
Biochemical pregnancy	10/124	8.1	12/93	12.9	0.24
Early miscarriage (<12 weeks)	8/124	6.5	7/93	7.5	0.75
Ectopic pregnancy	3/124	2.4	2/93	2.2	*P* = 0.89
Ongoing pregnancy rate per cycle (>12 weeks)	103/281	36.7	72/225	32.0	*P* = 0.27
Ongoing pregnancy rate per embryo (>12 weeks)	103/299	34.4	72/235	30.6	*P* = 0.35
Twin ongoing pregnancy rate per cycle	5/103	4.9	3/72	4.2	*P* = 0.83

this study and according to protocol, ICSI was performed on sibling oocytes to avoid the possibility that there might be no embryos to transfer because of a small risk of total fertilization failure, as has been observed in both regular IVF and the SCS when compared with ICSI, which has been shown to be mostly due to sperm–zona binding problems (*Hershlag et al., 2002*; *Johnson et al., 2013*; *Liu and Baker, 2000*; *van der Westerlaken et al., 2005*, *2006*).

Facilitated by the popular notion that ICSI is the most successful ART technique currently performed, it was possible to assure a substantial number of patients that participation in this study would not have been possible if only SCS and regular IVF were offered.

Considering pregnancy rates per cycle, the current results confirmed the findings described in the original preliminary report (*Van Blerkom et al., 2014*). There was no difference in ongoing pregnancy rate per transfer, implantation rate per embryo and miscarriage rate between the cases treated by SCS and ICSI. According to Belgian law, SET was performed in most cases: 97.9% and 93.6% of SCS and ICSI cycles, respectively (*Ombelet et al., 2005*). Consequently, a reasonable number of embryos could be cryopreserved, and the number of twin pregnancies was low (TABLE 2).

It is reassuring that the fertilization rate was significantly better in the SCS group compared with the ICSI group when all oocytes are taken into account, as previously reported by *Li et al. (2018)* for non-male factor infertility. The maturational state is unknown in the SCS group when allocated as intact COC, while in the ICSI group only metaphase II oocytes were inseminated, accounting for 77.9% of the oocytes randomly allocated to ICSI. It is important to reiterate in a study such as this, with the unbiased, random allocation of COC at retrieval, the proportion of immature and metaphase II oocytes should be largely the same in both groups, but fertilization rates with ICSI are based only on metaphase II oocytes, which is not the case for the SCS. Consequently, the fertilization rates/retrieved oocyte with low sperm numbers seen in the SCS would likely be higher if immatures were excluded.

The primary reason that the number of cryopreserved embryos was significantly higher in the ICSI group occurred because as noted above: (i) more embryos were allocated to ICSI as a maximum of eight oocytes were apportioned to the SCS per treatment cycle and (ii) in case of an odd number of oocytes, the extra egg was inseminated by ICSI. Therefore, it is not surprising that significantly more ICSI embryos were available for cryopreservation.

With respect to the economic and practical issues associated with use of the SCS method, the dissimilarity in costs when setting up and running a conventional high-tech IVF laboratory or a SCS laboratory have previously been examined. The discounted cash flow (DCF) method was chosen to evaluate the investment. The SCS laboratory clearly showed the highest net present value (NPV) and was identified as the most attractive investment in this study (*Christiaens, 2018*). NPV is the difference between the present value of cash inflows and the present value of cash outflows over a period of time.

Collectively, the present findings with a larger patient population than earlier (*Van Blerkom et al., 2014*) are reassuring with respect to the effectiveness of this system as a culture platform that can be readily reproduced in other (multicentre) studies. Further validation of outcome offers the real potential to open up a new era in the application of IVF to a much larger proportion of the world's infertile population. In this regard, the SCS could become an important breakthrough in

terms of human rights, equity and social justice for infertile couples in LMIC (*Fathalla et al., 2006*; *Johnson et al., 2014*; *Ombelet, 2011*; *Pennings et al., 2009*; *Vayena et al., 2009*).

No difference was found in ongoing pregnancy rate, implantation rate and miscarriage rate between the SCS and ICSI in a selected group of normo-responders with no severe male factor involved. Considering the ongoing pregnancy rates per transferred embryo, plus the economic advantage of the SCS system, it could offer a real solution to a larger number of patients with low access to ART due to financial restraints and in areas where IVF laboratory costs are the limiting factor to providing reproductive healthcare services.

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