Journal Pre-proof

First births after using a closed, simplified IVF culture system in a mobile IVF laboratory: a proof of concept study

Gerhardus Marthinus Boshoff , Carin Huyser , Willem Ombelet

PII: S1472-6483(25)00618-2
DOI: https://doi.org/10.1016/j.rbmo.2025.105411
Reference: RBMO 105411

To appear in: *Reproductive BioMedicine Online*

Received date: 16 June 2025
Revised date: 10 November 2025
Accepted date: 12 November 2025

Please cite this article as: Gerhardus Marthinus Boshoff , Carin Huyser , Willem Ombelet , First births after using a closed, simplified IVF culture system in a mobile IVF laboratory: a proof of concept study, *Reproductive BioMedicine Online* (2025), doi: https://doi.org/10.1016/j.rbmo.2025.105411

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo editing, typesetting, and review of the resulting proof before it is published in its final form. Please note that during this process changes will be made and errors may be discovered which could affect the content. Correspondence or other submissions concerning this article should await its publication online as a corrected proof or following inclusion in an issue of the journal.

© 2025 The Author(s). Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

Journal Pre-proof

First births after using a closed, simplified IVF culture system in a mobile IVF laboratory: a proof of concept study

Gerhardus Marthinus Boshoff [1,2*], Carin Huyser[1], Willem Ombelet [2,3]

[1] Department Obstetrics and Gynaecology, University of Pretoria, Pretoria, South Africa

[2] Faculty of Medicine and Life Sciences, University of Hasselt, Diepenbeek, Belgium

[3] The Walking Egg non-profit organisation, Genk, Belgium

*Corresponding author. E-mail address: gerhard.boshoff@up.ac.za (Gerhardus Marthinus Boshoff)

Journal Pre-proof

Abstract

Access to assisted reproductive technologies (ART) is limited and remains a significant challenge, especially in low- and middle-income countries, due to high costs and limited availability of affordable IVF centres.

Research question

Can pregnancies be achieved in woman undergoing IVF treatment, through the culture of embryos with a simplified embryo culture system in a mobile IVF laboratory?

Design

In this case series, the results of a pilot study investigating the use of a simplified, closed, low-cost embryo culture system in a prototype mobile IVF laboratory is described. A mild follicle stimulation protocol was used in all cycles for controlled ovarian hyperstimulation. Follicular aspiration was performed in a clinical setting, while oocyte fertilisation, embryo culture and embryo transfers were carried out in the mobile unit using the Walking Egg simplified embryo culture system.

Results

From 12 cycles initiated, no oocytes were obtained in one cycle, fertilisation failed in another cycle due to a low number of predominantly immature oocytes, and 10 women continued with embryo culture cycles. In 5 cycles, a single embryo transfer was performed, in the remaining 5 cycles two embryos were transferred. Five of the ten women became pregnant, one miscarried, and four had a live birth.

Conclusion

Proof of concept, even with a limited sample size, to apply mild stimulation and low-cost embryo culture in a mobile laboratory, was presented. These promising results support the use of a mobile IVF laboratory in areas where access to ART is costly and limited due to few IVF centres, mainly located in cosmopolitan centres.

KEY WORDS: accessible ART, affordable ART, Assisted Reproductive Technologies, equity, infertility care, LMICs, low-cost IVF, Low- and Middle-Income Countries, simplified IVF, mobile unit

Introduction

Access to infertility treatment and assisted reproductive technologies (ART) is unequally distributed globally, with minimal opportunities in low- and middle-income countries (LMICs) in particular (Ombelet *et al.*, 2025; World Health Organization, 2020; Polis *et al.*, 2017). This is often a neglected problem due to the paradox known as "barrenness amid plenty", where high fertility and infertility rates go hand-in-hand (Schivone and Blumenthal, 2016; Legese *et al.*, 2023). Lack of access to ART is justified in these settings due to overpopulation and limited resources (Ombelet *et al.*, 2008a).

Unfortunately, infertility and unintended childlessness in LMICs have grave consequences, such as stigmatisation, isolation, ostracization, loss of social status and intimate partner violence (Inhorn and Patrizio, 2015; Chiware *et al.*, 2020). Despite this recognized burden associated with childlessness in LMICs, many publications on the topic focus mostly on campaigns and larger picture policies, without pragmatic solutions to address the problem (Ombelet *et al.*, 2025). This lack of practical solutions to the problem of limited access to ART in LMICs is also reflected in a WHO-initiated systematic landscape analysis (Chiware *et al.*, 2020). In this publication, a review of applicable publications described that ART remain largely out of reach for most infertile couples in LMICs, primarily due to the prohibitive costs of treatment. The authors report that in LMICs, infertility and childlessness often carry severe social and psychological repercussions, which drives efforts to create more affordable in vitro fertilisation (IVF) methods. However, the implementation of low-cost assisted reproduction in LMICs is limited by the lack of high-quality outcome-based trials in these countries (Chiware *et al*., 2020).

Whittaker *et al*. (2024), drawing conclusions from their interviews with local professionals in the field of assisted reproduction, ascribe the low access to ART in LMICs mainly to high treatment cost, the distances patients must travel, too little public funding and few skilled personnel. In order to address the lack of access in LMICs, Ombelet *et al*. (2025) recently proposed the introduction of a mobile IVF laboratory, equipped to culture embryos with a simplified IVF culture system. Several papers have been published that demonstrate the efficacy and safety of such a low-cost, simplified IVF system including excellent perinatal outcomes (Van Blerkom *et al*., 2014; Ombelet *et al*., 2022a, 2022b; Ombelet *et al*., 2023a, 2023b).

The combination of a mobile IVF unit and a cost-effective IVF technique could provide a solution for many infertile couples in LIMCs. Rural and remote areas can be reached without the cost implications of setting up fully functional ART units. To explore this possibility, a prototype mobile unit was developed for use with the Walking Egg simplified culture method. In this case series, the results of a pilot study investigating the success rate after using a simplified, closed, low-cost IVF system in a prototype mobile IVF laboratory is described.

Case series

The design and commissioning of an IVF laboratory "on wheels" was performed in Pretoria, South-Africa, as part of a PhD project focusing on improving accessibility to ART, i.e. to construct a prototype laboratory, ready for use. The mobile laboratory was developed to incorporate equipment used with the Walking Egg (tWE) simplified IVF culture system (Van Blerkom *et al*., 2014), and an embryo transfer room. Equipment in the mobile laboratory included a Fertilisafe® Muti-Zone ART Workstation, Airstream® Class II Biological Safety Cabinet, 50 liter CellCulture® Incubator (without any gas connections) (ESCO Medical, Singapore, Republic of Singapore), Primostar phase contrast microscope and Stemi 508 Trino stereo microscope (Zeiss, Oberkochen, Germany) (see Figure 1 for a collage of images of the

Journal Pre-proof

mobile laboratory's interior and exterior). The planned operation of the mobile laboratory was for the unit to be used at an established medical facility equipped with a small theatre or day facility with a procedure room, where the follicle aspiration procedures could be performed. These types of medical facilities can be found in most small towns and even rural villages in South Africa, meaning that with appropriately trained staff and a mobile laboratory, IVF treatment could be offered in all but the most remote of places in South Africa. After construction of the mobile laboratory was finalised, an ART pilot study to establish proof of concept was initiated (September - November 2024).

The pilot study was performed in a rural town in the Northwest province of South Africa, simulating a real-world scenario where a mobile IVF laboratory could be used. Patients were recruited from a local IVF clinic. Couples who in recent times contacted the fertility clinic for treatment were invited to participate in the study, if they met the inclusion criteria of the project. This treatment was offered as an alternative to their regular treatment. Patients were provided full information on the project outline, and they signed informed consent to participate in the study. Participants in the study were provided medication for follicle stimulation at no cost, and laboratory fees were subsidised through the research project. The recruitment, informed consent and execution of the pilot study was according to ethical approval from the University of Pretoria's Research Ethics Committee (REC protocol 149/2021) and Hasselt University's Comité voor Medische Ethiek (CME 2023/046).

Patients were included in the pilot study based on the following criteria: female patients below 38 years old, with a blood serum Anti-Mullerian Hormone (AMH) level above 1 ng/ml and body mass index (BMI) between 18 and 36 kg/m^2, and male patients below 48 years old with a semen analysis of at least 5 million motile sperm (volume x concentration x % progressive motility). A mild ovarian hyperstimulation protocol, as described by Gianaroli, *et al.* (2022) with clomiphene citrate, low dose gonadotropins, a GnRH antagonist and an hCG trigger was provided to all patients.

From a cohort of 12 patients originally recruited to undergo follicle stimulation, one patient developed two follicles only, and no oocytes were obtained after oocyte aspiration. In a second patient, 5 oocytes were retrieved, of which only a single oocyte was mature, that did not fertilise. To evaluate the capability of embryo culture and transfer in the mobile laboratory, the remaining ten patients are being considered. The average ages of the female and male patients were 32.2 ± 4.7 and 38.9 ± 3.84 years, respectively. To note, one of the cycles was performed with an oocyte donor, and the donor's age was used to calculate the average. The original 12 patients' reproductive history is summarised in Table 1. No other medical interventions, such as laparoscopic disconnection of tubes for patients with hydrosalpinxes, were performed prior to the IVF procedure, or as part of their treatment.

Follicle aspiration procedures were performed in the local IVF clinic's procedure room, with the follicular fluid aspirated into 14 mL round bottom culture tubes (Cat no. 82341, IVFGEN, Brisbane, Australia). Aspirates were transported to the mobile laboratory in a portable incubator without any gas connection (K-Systems G95 Portable incubator, CooperSurgical Inc, Trumbull, USA). Follicular fluid was screened for oocytes, and oocytes were fertilised, with subsequent embryo culture, embryo transfer and cryopreservation, along with embryo thawing in one cycle, performed in the mobile laboratory.

Embryos were cultured using an adaptation of the tWE simplified embryo culture system (Van Blerkom *et al.*, 2014). Pre-gassed tWE tubes were provided by the tWE foundation, instead of tubes that needed to be gassed with a CO_2 generator as previously described (Klerkx *et al.*, 2014). The tWe tubes (tWE, Genk, Belgium) were supplied ready for use, pre-gassed with a tri-gas mixture and without any culture media. The use of these pre-gassed tubes was validated through testing the culture media pH using a blood-gas analyser, confirming stability of the culture media pH over a 7-day period (unpublished data). Furthermore, the tubes were used in a preliminary investigation to confirm the feasibility of culturing embryos in the mobile laboratory, where abnormally fertilised oocytes (containing ≥3 pronuclei) were cultured to the blastocyst stage (unpublished data). To prepare the tubes for embryo culture, 1ml embryo culture medium (V-ONESTEP, VitroMed, Jena, Germany) was injected into the tube using a 22G needle and 1ml syringe (V-TRANSIN 1ml SYRINGE, VitroMed, Jena, Germany). The tubes were then left in a warming oven at 37˚C for a minimum of two hours before introducing gametes to them, to allow for pH and temperature equilibration of the culture medium. Embryo transfers were performed at the mobile laboratory with ultrasound guidance (DP30 Ultrasound, Mindray, Shenzhen, China) using an embryo transfer catheter (Ryden, Kitazato Corporation, Tokyo, Japan) and 1ml syringe. All clinical procedures, such as the ovarian stimulation and follicle aspirations, as well as embryo transfers, were performed by the same Gynaecologist subspecialised in assisted reproduction, while all embryology procedures, including embryo culture, transfers and vitrification, was performed by the lead author.

Journal Pre-proof

Follicular aspirates were screened for cumulus-oocyte complexes (COCs) in the mobile laboratory, using the stereo microscope, on the heated stage (37˚C) of the Muti-Zone ART Workstation. The COCs were transferred into prepared pre-gassed culture tubes containing 1ml embryo culture medium, by injecting a single COC per tube using a 1ml syringe and 22G needle (Klerkx *et al.*, 2014, Van Blerkom *et al.*, 2014). The semen samples obtained from the male partners were processed with pre-prepared sperm washing gradients (V-GRAD, VitroMed, Jena, Germany), using standardised density gradient centrifugation (Ali *et al.*, 2022). Before insemination, the processed sperm samples were diluted to 0.5 x 10^6 motile sperm/ml using gassed embryo culture medium from a prepared tube. For a 10 000 motile sperm per ml insemination, one drop (25µl) of the diluted sperm samples was added to each of the tubes containing a COC, by injecting a needle through the tubes' stopper (see Figure 2A). The tubes were kept in aluminium blocks (Figure 2B) in the warming oven, at a temperature of 37˚C, with fertilisation evaluation performed 16-18 hours later, followed by daily embryo development evaluations up to the time of transfer or cryopreservation (see Figure 2C). The culture tubes were kept stoppered, to maintain the gassed environment throughout the culture time, and was only opened right before embryo transfer or cryopreservation, to remove the required embryo.

All the embryo transfers, except one, were performed as fresh transfers, with one transfer scheduled on day 3 of culture, due to a single developing embryo being available only, and on day 5 of culture for the other 8 patients. After transfer, the remaining embryos were cultured further to a maximum of six days after insemination and cryopreserved when reaching blastocyst quality of a sufficient grade. The one frozen embryo transfer was performed because the patient's embryos developed at a slow rate and a blastocyst was cryopreserved early on day 7 of culture only. This blastocyst was thawed and transferred to the patient on day 19 of her next menstrual cycle. Blastocysts were graded using the Gardner-Schoolcraft criteria and deemed cryopreservable for grades 3BB or higher, indicating a fully formed blastocyst, with both an inner cell mass and trophectoderm of medium to good quality (Gardner and Schoolcraft, 1999, Gardner *et al.*, 2000). On average, 6.4 cumulus-oocyte complexes were retrieved per patient, with a 67% maturation rate of the oocytes. From these mature oocytes, 79% fertilisation was achieved, and 62% of these zygotes developed sufficiently for embryo transfer or cryopreservation. Summarised outcomes per patient can be seen in Table 2.

From 10 embryo transfers, 5 patients requested two embryos to be transferred, while the other 5 had a single embryo transferred. Positive blood βHCG levels were reported for 5 of the 10

Journal Pre-proof

patients, two weeks after the embryo transfer. One pregnancy ended in a miscarriage at 7 weeks, and the other four resulted in live births. The four babies (three male, one female) were born at 38, 37, 36 and 37 weeks, respectively, weighing 2.72, 3.01, 2.32. and 2.99 kg each. Excess embryos (n=6 from 4 patients) were cryopreserved in the mobile laboratory. At the time of publication, the frozen embryos are stored in liquid nitrogen at the local IVF clinic, none has been thawed.

Discussion

In the presented pilot study, a total of 21 embryos cultured in a mobile IVF laboratory were used for either embryo transfer or cryopreservation, from a cohort of 34 fertilised oocytes, accounting for a 61.7% utilisation rate. From a group of 10 patients with embryo transfer cycles, 50% pregnancy was achieved, resulting in a 40% live birth rate.

The time has come to bring infertility out of the shadows of reproductive health research and policy, as was mentioned in editorials of the Lancet Global Health and the Lancet (Wang, 2022, The Lancet, 2024). The improvement of access to effective and safe patient-centred and evidence-based treatments is therefore imperative (The Lancet, 2024). Poor access to infertility treatment and ART is partially the result of a lack of public funding for medical services and drugs, a shortage of skilled embryologists and a lack of political awareness of the problem (Ombelet and Goossens, 2017). This problem can be addressed by reducing the high costs associated with ART in the largely private sector (Njagi *et al*., 2023) and by increasing the possibility of treatment in rural and remote areas, away from the cosmopolitan centres where private ART centres are increasingly being established (Ombelet *et al*., 2025).

The introduction and implementation of a low-cost IVF culture system without the need for a high-tech laboratory could assist non-severe male factor infertility patients (Ombelet *et al*., 2022a). In fact, although ICSI is presently the most commonly used ART procedure, several important papers have questioned the value of performing ICSI instead of IVF for unexplained, female-only and moderate male infertility (Berntsen *et al*., 2025, Wang *et al*., 2024). These publications showed that for non-severe male factor infertility, ICSI did not improve the live birth rate compared with conventional IVF, bearing in mind that ICSI is a more invasive and costly procedure with a potential increased risk to the health of the offspring. According to an analysis of the registry of the Human Fertilisation and Embryology Authority in the United Kingdom, ICSI is associated with a reduction in live births in female-only factor infertility

Journal Pre-proof

(Paffoni *et al.*, 2024). In addition, the simplified IVF system has been described to require 1000-5000 motile washed spermatozoa for insemination, with successful results, with very low fertilisation abnormalities such as dispermic penetration (Boshoff *et al.*, 2019, Ombelet *et al.*, 2022a, Van Blerkom *et al.*, 2014). This means that this system can be used in cases of mild and moderate male infertility instead of ICSI, with similar results (Ombelet *et al.*, 2022a, Van Blerkom *et al.*, 2014).

The low-cost IVF culture system in a mobile unit may be an opportunity to provide affordable diagnostic and therapeutic ART services in resource-poor countries. The simplified culture system requires less equipment than conventional IVF, resulting in substantially lower financial investment for a tWE laboratory compared to a conventional setup (Christiaens, 2018). This reduced capital requirement can enable clinics to offer more affordable rates to patients. By combining this strategy with a mobile tWE laboratory, the cost of the mobile laboratory could be distributed among multiple facilities using the laboratory in rotation. Furthermore, the simplified culture system's cost has been calculated to be less per IVF cycle than conventional IVF (Christiaens, 2018), especially when combining with mild follicle stimulation strategies, which will further reduce the final out-of-pocket expense to patients. Additionally, apart from the direct reduction in cost, the utilisation of the mobile laboratory in areas that are more accessible to patients, the patients' indirect costs, such as travel and accommodation expenses would also be less (Boshoff *et al.*, 2025).

Although the sample size in the study was limited, the results were promising. There was a noteworthy variation in the number of oocytes retrieved per patient, as well as the number of mature oocytes per patient. All patients had at least one embryo that was useable, and from the developing embryos, all blastocysts that developed were of good enough quality to be either transferred or frozen. The embryo culture results are similar, and slightly better, than previously reported with the tWE simplified culture system (Ombelet *et al.*, 2022a). Since these results are based on a very small number of patients, this cannot be seen as statistically significant, but the pilot study is successful proof of concept for embryo culture and transfer in a mobile laboratory. This may be an effective tool in areas where access to ART is limited and will be explored further in larger cohorts.

The development of the mobile laboratory is a world-first, showing that it is possible to perform IVF embryo culture in a less sophisticated laboratory environment. Improving access to ART through innovative developments such as this has been the goal of the Walking Egg NPO since

Journal Pre-proof

Journal Pre-proof

their initiation. This is one step in a long road of development and design, striving to assist people all over the world. Valuable lessons have been learned about working in such a mobile laboratory, which can and will be applied in a subsequent larger study. One of the important aspects to take into consideration is logistics and the careful scheduling of procedures. Sufficient time should be allowed between follicular aspiration procedures on the same day, to allow time to perform the aspiration procedures in the clinical complex, transport the aspirates to the mobile laboratory outside, continue with laboratory work, and return to the clinic rooms for the next procedure. Another improvement would be to have a comfortable waiting area at or near the mobile laboratory, to support the healthcare experience of patients before and after embryo transfers.

The future deployment of mobile laboratories, such as the current prototype, should take a diverse range of issues into account that could impact ART provision in LMICs, not only the logistics of performing procedures in a mobile laboratory. Some of the problems regularly found in LMICs are the lack of adequately trained personnel, as well as unreliable infrastructure that affects access to commonly available IVF-approved disposables and consumables (Chiware *et al.*, 2020, Hammarberg *et al.*, 2018, Whittaker *et al*., 2024). The latter (infrastructure) could be addressed by working with local suppliers and importers to provide the necessary items to health professionals. The former (staff) is more challenging to overcome, and any organisation, whether for profit or not, may find that they should invest in a training programme or work with local training facilities to ensure that there is sufficient trained staff to carry out the necessary procedures. Additionally, the coordination of procedures at rural medical facilities without ART background, will be a crucial element to the success of such an endeavour. Assisted reproduction is not always regulated on the same level in different LMICs. In some countries there might be restrictions in place on what medical procedures may be done in mobile facilities, and in others, specifications on the minimum qualification and registration of health care providers may be in place. The application of a mobile laboratory, such as is proposed here, will have to consider local requirements and legislation.

When investigating the use of a mobile laboratory, the mentioned stumbling blocks must be considered carefully, which could, in the short run, affect the roll-out time of such a project. However, in the long term, the use of mobile facilities might prove useful to provide a faster and easier way to deliver fertility treatment to societies with no other options available. This pilot study provides evidence that the simplified IVF technology is a safe, effective and successful low-cost method that can be implemented in settings with low or moderate resources, using mobile units."

Reference list

Ali A.H., Ajina T., Ali M.B., Mehdi M. **Efficacy of density gradient centrifugation technique (DGC) in enhancing sperm cell DNA quality for assisted reproductive technique.** Mid. East Fert. Soc. J. 2022; 27(1): 22-31. doi: 10.1186/s43043-022-00108-4.

Berntsen, S., Zedeler, A., Nøhr, B., Rønn Petersen, M., Grøndahl, M.L., Andersen, L.F., Løssl, K., Løkkegaard, E., Englund, A.L., Vestergaard, Gabrielsen, A. *et al.* **IVF versus ICSI in patients without severe male factor infertility: a randomized clinical trial.** Nat. Med. 2025; 1: 1-10. doi: 10.1038/s41591-025-03621-x.

Boshoff G.M., Ombelet W., Huyser C. **Oocyte insemination with the Walking Egg simplified IVF culture system–an investigation into reduced sperm numbers, sperm DNA fragmentation and reactive oxygen species formation.** Facts Views Vis. Obgyn 2019;10(4):191-197.

Boshoff G.M., Ombelet W., Huyser C. **Public sector access to medically assisted reproduction in South Africa: a case study.** Reprod. Fertil. 2025; 6(3): e240072. doi:10.1530/RAF-24-0072.

Chiware, T.M., Vermeulen, N., Blondeel, K., Farquharson, R., Kiarie, J., Lundin, K., Matsaseng, T.W., Ombelet, W., Toskin I. **IVF and other ART in low- and middle-income countries: a systematic landscape analysis.** Hum. Reprod. Update 2020; 27: 213-228. doi: 10.1093/humupd/dmaa047.

Christiaens, I. **Setting-up a new IVF centre: Is a "Walking Egg" centre an attractive investment?** Master thesis biomedical sciences program at the Catholic University of Leuven, Belgium. 2018.

Dhont N. **The Walking Egg non-profit organisation.** Facts Views Vis. Obgyn 2011; 3:253-255.

Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., Schoolcraft, W.B. **Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer.** Fertil. Steril. 2000; 73: 1155-1158. doi: 10.1016/s0015-0282(00)00518-5.

Gardner, D.K. and Schoolcraft, W.B. **In vitro culture of human blastocysts.** In: Towards reproductive certainty: Fertility and genetics beyond 1999, Ed: Jansen, R. and Mortimer, D. 1999; 378-388.

Gerrits, T., Ombelet, W., van Balen, F., Vanderpoel, S. (eds.) **Biomedical infertility care in poor resource countries: barriers, access and ethics.** Facts Views Vis. Obgyn 2012. Monograph: 1–90.

Gianaroli, L., Vitagliano, A., Ferraretti, A. P., Azzena, S., Terzuoli, G., Perruzza, D., Ambrosini, G., Tabanelli, C. & Magli, M. C. **IVF Lite: a smart IVF programme based on mild ovarian stimulation for good prognosis patients.** Reprod. BioMed. Online 2022; 45: 256-263. doi: 10.1016/j.rbmo.2022.04.003.

Hammarberg, K., Trouson, A., McBain, J., Matthews, P., Robertson, T., Robertson, F., Magli, C., Mhlanga, T., Makurumure, T. & Marechera, F. **Improving access to ART in low-income settings through knowledge transfer: a case study from Zimbabwe.** Hum. Reprod. Open 2018; 4: hoy017. doi: 10.1093/hropen/hoy017.

Inhorn, M.C., Patrizio, P. **Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century.** Hum. Reprod. Update 2015; 21:411-426. doi: 10.1093/humupd/dmv016.

Klerkx, E., Janssen, M., Van Der Auwera, I., Campo, R., Goossens, J., Vereecken, A. & Ombelet, W. **A simplified IVF laboratory method.** Facts Views Vis. Obgyn 2014; Monograph, 4-5.

Legese, N., Tura, A.K., Roba, K.T., Demeke, H. **The prevalence of infertility and factors associated with infertility in Ethiopia: Analysis of Ethiopian Demographic and Health Survey.** PLoS One 2023; 18:e0291912. doi: 10.1371/journal.pone.0291912.

Njagi, P., Groot, W., Arsenijevic, J., Dyer, S., Mburu, G., Kiarie, J. **Financial costs of assisted reproductive technology for patients in low- and middle-income countries: a systematic review.** Hum. Reprod. Open 2023; 2: hoad007. doi: 10.1093/hropen/hoad007.

Ombelet, W., Cooke, I., Dyer, S., Serour, G., Devroey, P. **Infertility and the provision of infertility medical services in developing countries.** Hum. Reprod. Update 2008a; 14: 605–621. doi: 10.1093/humupd/dmn042.

Journal Pre-proof

Journal Pre-proof

Ombelet, W., Devroey, P., Gianaroli, L., te Velde, E. (eds). **Developing Countries and Infertility. Arusha-expert meeting (Arusha 2007-12-15).** Spec. Issue Human. Reprod. 2008b;1–117.

Ombelet, W. and Goossens, J. **Global reproductive health - Why do we persist in neglecting the undeniable problem of childlessness in resource-poor countries?** Facts Views Vis. Obgyn 2017; 9: 1-3. PMID: 28721178.

Ombelet, W., Van Blerkom, J., Nargund, G., Van der Auwera, I., Janssen, M., Dhont, N., Bosmans, E., Boshoff, G., Vertessen, V.J., Campo, R. **Multiyear outcomes using sibling oocytes demonstrates safety and efficacy of a simplified culture system consistent with use in a low-cost IVF setting**. Reprod. Biomed. Online 2022a; 45: 481-490. doi: 10.1016/j.rbmo.2022.04.008.

Ombelet, W., Van Blerkom, J., Nargund, G., Janssen, M., Jacobs, P., Van der Auwera, I., Dhont, N., Bosmans, E., Vertessen, V.J., Campo, R. **Perinatal outcome of babies born after using a simplified culture system for IVF versus ICSI followed by conventional culturing with sibling oocytes: a multi-year prospective cohort study.** Reprod. Biomed. Online 2022b; 45:574-582. doi: 10.1016/j.rbmo.2022b.04.009.

Ombelet, W., Van Blerkom, J., Bruckers, L., Dhont, N., Nargund, G. & Campo, R. **Promising perinatal outcome after using a simplified low-cost IVF culture system specifically designed for resource-poor countries.** J Clin Med. 2023a; 12(6): 2264. doi: 10.3390/jcm12062264.

Ombelet, W., Goemaes, R., Fomenko, E., Campo, R. **Perinatal Outcome of Singletons Born after Using a Simplified Low-Cost IVF Culture System and All Singletons Born in Flanders (Belgium) between 2012 and 2020.** J. Clin. Med. 2023b; 12(11):3683. doi: 10.3390/jcm12113683.

Ombelet, W., Van Blerkom, J., Boshoff, G., Huyser, C., Lopes, F., Nargund, G., Sallam, H., Vanmechelen, K., Campo, R. **Now is the time to introduce new innovative assisted reproduction methods to implement accessible, affordable, and demonstrably successful advanced infertility services in resource-poor countries.** Hum. Reprod. Open 2025; 1: hoaf001. doi: 10.1093/hropen/hoaf001.

Paffoni, A., Vitagliano, A., Corti, L., Somigliana, E., Viganò, P. **Intracytoplasmic sperm injection versus conventional in vitro insemination in couples with non-male infertility factor in the 'real-world' setting: analysis of the HFEA registry.** J. Transl. Med. 2024; 22(1): 687. doi: 10.1186/s12967-024-05515-x.

Polis, C. B., Cox, C. M., Tuncalp, Ö., McLain, A. C. & Thoma, M. E. **Estimating infertility prevalence in low-to-middle-income countries: an application of a current duration approach to Demographic and Health Survey data.** Human Reprod. 2017; 32: 1064-1074. doi: 10.1093/humrep/dex025.

Schivone, G. B. & Blumenthal, P. D. **Contraception in the developing world: special considerations.** Semin. Reprod. Med. 2016; 34(03): 168-174. doi: 10.1055/s-0036-1571437.

The Lancet. **The fertility industry: profiting from vulnerability.** The Lancet, 2024; 404(10464): 215. doi: 0.1016/S0140-6736(24)01484-3.

Van Balen, F., Ombelet, W. (eds.) **Social Aspects of accessible infertility care in developing countries.** Facts Views Vis. Obgyn 2009. Monograph: 1–72.

Van Blerkom, J., Ombelet, W., Klerkx, E., Janssen, M., Dhont, N., Nargund, G., Campo R. **First Births with a Simplified Culture System for Clinical IVF and ET.** Reprod. Biomed. Online 2014; 28; 310-320. doi: 10.1016/j.rbmo.2013.11.012.

Wang, Y. **Infertility—why the silence.** Lancet Global Health 2022; 10(6):e773. doi: 10.1016/S2214-109X(22)00215-7.

Wang, Y., Li, R., Yang, R., Zheng, D., Zeng, L., Lian, Y., Zhu, Y., Zhao, J., Liang, X., Li, W. *et al*. Lancet 2024; 403(10430): 924-934. doi: 10.1016/S0140-6736(23)02416-9.

Whittaker. A., Gerrits. T., Hammarberg. K., Manderson. L. **Access to assisted reproductive technologies in sub-Saharan Africa: fertility professionals' views.** Sex. Reprod. Health Matters 2024; 32:2355790. doi: 10.1080/26410397.2024.2355790.

World Health Organization. **WHO fact sheet**. [September 14, 2020]. www.who.int/news-room/fact-sheets/detail/infertility.

Journal Pre-proof

Authors' roles

All authors made substantial contributions and read and approved the final version of the manuscript. The concept of a mobile laboratory originated with WO and the non-profit organisation Walking Egg. GB wrote the original draft and carried out most of the practical work reported as part of a PhD research project under the supervision of CH and WO.

Acknowledgements

The authors would like to thank Dr Pieta Geyser, Ms Chiné Lottering and the rest of the team at Le Femme Fertility Clinic in Rustenburg, South Africa. The pilot study would not have been possible without their support and active participation. We would also like to thank the companies and organisations listed in the funding section for their financial contribution to the project. Lastly, we would like to thank Mr Koen Vanmechelen for the generous donation of his time and talents to design the mobile laboratory's exterior artwork.

Funding

The study is part of a PhD project with research ethics approval from both the University of Pretoria (REC number 149/2021) and the University of Hasselt (CME2023/046), and the research is supported by the Hasselt University BOF bilateral scientific cooperation grant (reference number BOF21BL16). The prototype of the mobile laboratory was funded by the Walking Egg non-profit organisation (Belgium). The equipment used in the laboratory was loaned to the project by ESCO Technologies (PTY) Ltd. (South Africa). Disposables and consumables for the project were sponsored by Ducit (PTY) Ltd. (South Africa). The stimulation medication was partially sponsored by Delfran Pharmaceuticals (South Africa), and the Walking Egg non-profit organisation (Belgium) provided funds for the remainder of the medication and other miscellaneous items that were not sponsored.

Conflict of interest

The project design was performed with the assistance and on request of the Walking Egg non-profit organisation. WO is a director of said organisation. GB and CH have no conflict of interest to declare.

Legends to figures

Journal Pre-proof

Figure 1: Collage of (A) the mobile unit being towed, (B) exterior design, (C) laboratory area, and (D) embryo transfer room.

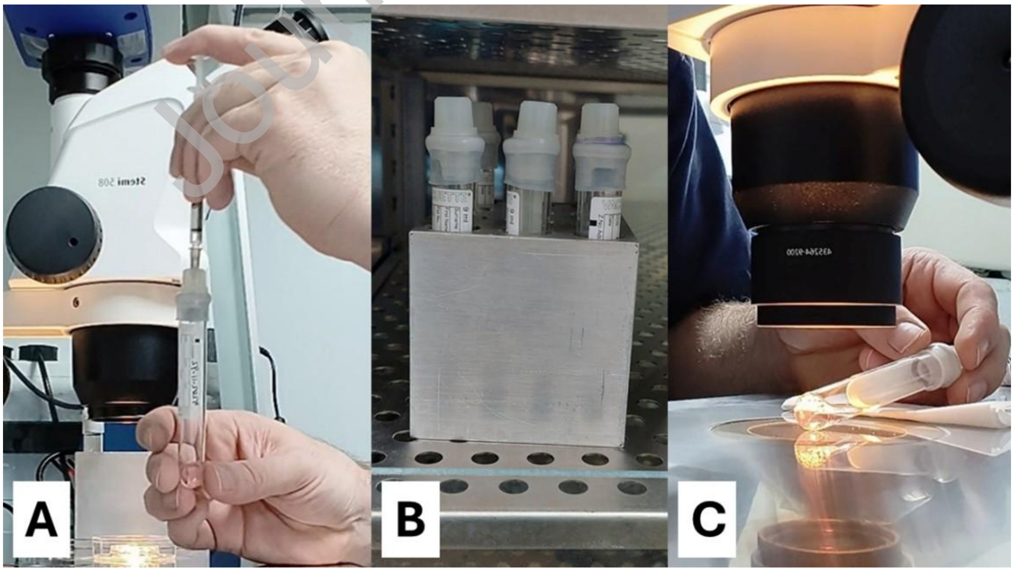

Figure 2: Closed tWE culture tubes showed (A) during oocyte insemination, (B) kept in an aluminium block to maintain temperature and (C) embryo evaluation.

Journal Pre-proof

Journal Pre-proof

Table legends

Table 1: Summary of patients' fertility history (n=12).

Patient number	Age	AMH	BMI	Infertility	Previous_ ART	Parity
1	37.6	2.6	29.0	Unexplained	None	G0P0
2	37.9	1.0	29.0	Bilateral salpingectomy	None	G2P2
3	36.5	3.7	27.4	Mild male factor	None	G0P0
4	24.3	2.3	23.4	Hydrosalpinx	None	G0P0
5	35.1	2.0	20.5	Bilateral tubal occlusion	None	G2P1 M1
6	33.4	1.4	35.8	Resistant PCOS	2xIUI	G0P0
7	33.3	4.6	27.6	Unexplained	None	G0P0
8	35.0	2.6	31.7	Bilateral tubal occlusion, PCOS	None	G0P0
9	23*/37 **	4,12*	30.5	Donor oocytes due to ovarian insufficiency	None*	G1P1 *
10	29.0	2.2	28.1	Bilateral tubal occlusion and hydrosalpinx	None	G0P0
11	29.8	2.6	28.7	Unexplained	None	G0P0
12	31.1	2.0	27.9	Mild male factor	None	G0P0
Average (SD)	32,2 (4,7)	2,6 (1,0)	28,3 (3,7)			

* Donor; **Recipient

Table 2: Embryology and transfer outcomes of embryos cultured in the mobile laboratory.

Patient number	Oocytes	Mature oocytes	Fertilised	Blastocysts	Transferred	Cryopreserved	Outcome
1	7	3	2	1	0	1	Negative*
2	3	3	2	2	2	0	Live birth
3	1	1	1	1**	1	0	Live birth
4	5	3	1	1	1	0	Negative
5	6	4	4	2	2	0	Negative
6	7	5	4	3	2	1	Live birth
7	19	11	9	4	2	2	Negative
8	9	8	7	3	1	2	Live birth
9	1	1	1	1	1	0	Miscariage at 8 weeks
10	6	4	3	3	2	1	Negative

* Cryopreserved embryo transferred in a subsequent cyle, embryo thaw and transfer performed in mobile laboratory

** Day 3 embryo transfer, blastulation assumed based on positive pregnancy

Mr. Gerhard Boshoff is an embryologist in the Private Sector of South Africa and joint PhD candidate at the Universities of Pretoria and Hasselt. Mr Boshoff is involved in embryology training and his research interests lies in access of ART in resource poor communities and the practical application of ART.

Journal Pre-proof

Key message

The proof of concept of a mobile laboratory, using the Walking Egg simplified embryo culture system, to provide ART services. The first births through the use of such a mobile laboratory showcase the possibility to offer ART procedures in an alternative manner that can make MAR more accessible.