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Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

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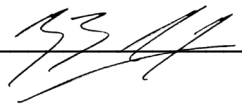
Declaration of authorship

“I declare that the thesis, which I hereby submit for the degree Doctor of Philosophy in Reproductive Biology at the Faculty of Health Sciences, University of Pretoria and Doctor of Philosophy in Biomedical Sciences at the Faculty of Medicine and Life Sciences, Hasselt University, is my own work and has not previously been submitted by me for a degree at another university.”

The PhD researcher and the UHasselt supervisor hereby formally declare that the research conducted for the purpose of this PhD thesis was executed in accordance with the principles of good scientific conduct, as stipulated in the UHasselt Integrity charter, the UHasselt charter supervisor – PhD Researcher, the UHasselt Integrity Policy and the UHasselt guidelines for the use of (generative) AI in research.

The author asserts that this PhD thesis is made Open Access immediately upon submission. The full text is publicly available without restrictions.

Signature  Date: 20 February 2026

Dedication

I would like to dedicate this thesis and the outcomes from this project to my family, who is my inspiration and my reason for perseverance. To my wife Ilana, and my two children, Liané and Erich, without your understanding and support I would not have been able to get this far.

Declaration: Publications

Journal articles published as first author:

- Boshoff GM, Ombelet W, and Huyser C. 2025. Public Sector access to Medical Assisted Reproduction in South-Africa: A case study. *Reproduction and Fertility*, 6 e240072, DOI: 10.1530/RAF-24-0072.
- Boshoff GM, Huyser C, and Ombelet W. 2025. First births after using a closed, simplified IVF culture system in a mobile IVF laboratory: a proof of concept study. *Reproductive BioMedicine Online*; In press. DOI: 10.1016/j.rbmo.2025.105411.

Journal articles submitted as first author (under review):

- Boshoff GM, Ombelet W, and Huyser C. 2025. Point-of-care Assisted Reproduction: assessing a mobile IVF laboratory. *South African Medical Journal.*

Authors contributions:

GB conceptualised and wrote the draft manuscripts under the supervision of CH and WO, who also critically reviewed and provided input to revise the manuscripts.

Journal articles published as co-author (Annexure A):

- Ombelet W, Van Blerkom J, Nargund G, Van der Auwera I, Janssen M, Dhont N, Bosmans E, Boshoff GM, Vertessen VJ and Campo R. 2022. Multiyear outcomes using sibling oocytes demonstrates safety and efficacy of a simplified culture system consistent with use in a low-cost IVF setting. *Reproductive Biomedicine Online*, 45(3), pp.481-490, DOI: 10.1016/j.rbmo.2022.04.008.
- Ombelet W, Van Blerkom J, Boshoff GM, Huyser C, Lopes F, Nargund G, Sallam H, Vanmechelen K, and Campo R. 2025. 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. *Human Reproduction Open,* 2025(1), p.hoaf001, DOI: https://doi.org/10.1093/hropen/hoaf001.

Declaration: Additional research outputs

Outcomes from the research have been presented at several international conferences and meetings, as described below:

Oral presentations

Presentation title	Conference	Organizing institutions	Location	Presentation date
The mobile IVF laboratory	Access to Infertility Care in South Africa	• University of Pretoria • Hasselt University • The Walking Egg non-profit organization	Faculty of Health Sciences, University of Pretoria, South Africa	28/10/2023
Get the IVF lab moving – design, construction and furbishing of a mobile IVF laboratory	Anthropological Contributions to SRHR Future(s): From Theory to Practice and Back	• University of Amsterdam • Amsterdam Institute for Social Science Research (AISSR)	Anthropology Department, University of Amsterdam, The Netherlands	05/07/2024
ART on the move: the realization of a mobile IVF Lab in South Africa	Re-worlding Reproduction: Navigating Emerging Knowledge, Politics, and Justice	• University of Pretoria • University of Amsterdam • Monash University • University of the Witwatersrand	Faculty of Health Sciences, University of Pretoria, South Africa	18/09/2024
A demographical analysis of patients in the public sector of South Africa over a six-year period: Who returns for ART procedures and who doesn't?	SASREG Congress 2024: Infertility care a multidisciplinary approach	• Southern African Society of Reproductive Medicine and Gynaecological Endoscopy (SASREG)	Coastlands Umhlanga Hotel, Durban, South Africa	09/03/2024
The mobile IVF unit at a glance	The Walking Egg on Wheels symposium	• The Walking Egg non-profit organization • Hasselt University	La Biomista, Genk, Belgium	02/07/2025

Poster presentations

Poster title	Conference	Organizing institutions	Location	Presentation date
P-717 First pregnancies after using a simplified low-cost IVF system in a newly designed mobile IVF laboratory: a pilot-study.	ESHRE2025: 41st Annual meeting	European Society of Human Reproduction and Embryology (ESHRE)	Paris Expo Centre, Paris, France	29/06/2025 – 02/07/2025
P03.057 Pilot study: First pregnancies after using a simplified IVF system in a mobile IVF laboratory.	XXV FIGO World Congress of Gynecology and Obstetrics	International Federation of Gynecology and Obstetrics (FIGO)	Cape Town International Convention Centre, Cape Town, South Africa	05/10/2025 – 09/10/2025

Abstracts of the poster presentations:

Boshoff G, Huyser C, Ombelet W. P-717 First pregnancies after using a simplified low-cost IVF system in a newly designed mobile IVF laboratory: a pilot-study. *Human Reproduction*. 2025. 40(Supplement_1): deaf097-1023, DOI: 10.1093/humrep/deaf097.1023.

Boshoff G, Ombelet W, Van Blerkom J, Campo R, Geyser P, Huyser C. 2025. 1 P03.057 Pilot study: First pregnancies after using a simplified IVF system in a mobile IVF laboratory. In: Poster Presentations. *Internation Journal of Gynaecology & Obstetrics*, 171:121-53, DOI: 10.1002/ijgo.70503.

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Executive Summary

The availability of Assisted Reproductive Technologies (ART) is sorely lacking in Low-and-Middle income countries. Barriers to ART include overly high costs, and few ART facilities within an acceptable travelling distance. The research project focused on access to ART, and is presented in three parts, namely (i) an investigation of patients undergoing ART procedures in the public sector of South Africa, (ii) the design and construction of a mobile IVF laboratory, and (iii) a proof-of-concept study showcasing the use of the mobile IVF laboratory.

In the first study, patients who underwent diagnostic evaluations, but did not return for therapeutic procedures, were investigated and compared with patients who followed through with therapeutic procedures. Their income status, access to medical aid and distances required to travel to the ART facility was considered. Additionally, the time between the first diagnostic and the first therapeutic interventions was investigated in the subpopulation of patients who returned for ART procedures. The comparison of data showed that patients from lower income groups continued less often with therapeutic ART than patients with higher incomes. The low-income patients who did return for therapeutic procedures took longer to progress from the diagnostic to the therapeutic phase of their treatment.

A mobile IVF laboratory was presented as a tool to be used to improve access to ART. This was a world-first endeavour, to equip a trailer for use as an IVF laboratory. The design and selection of equipment is described, as well as the process to construct the laboratory. After the mobile laboratory was completed and equipped, all equipment was verified to be functional through a series of quality control tests. The study concluded with a pilot study, confirming the IVF culture system's functionality through the culturing of abnormally fertilised oocytes to the blastocyst stage. In October 2023 the mobile laboratory was showcased in Pretoria, South Africa during the symposium "Access to Infertility Care: South Africa", where participants were able to visit the newly constructed mobile IVF laboratory.

After completion of the mobile IVF laboratory, a proof-of-concept study was performed to show the real-world application of the mobile IVF laboratory. From a cohort of 10

patients who had embryos cultured in the mobile IVF laboratory, 5 patients tested positive for βHCG and eventually four healthy babies were born. The findings were reported in a research article as well as two poster presentations at international conferences, i.e. the European Society of Human Reproduction and Embryology's 41st Annual meeting in Paris, France, as well as the FIGO XXV World Congress of Gynecology and Obstetrics in Cape Town, South Africa. During the latter congress, the mobile lab was moved to Cape Town and available at the congress venue for viewing. The prototype mobile IVF laboratory was proven to be successful in terms of IVF embryo cultures with the simplified Walking Egg IVF culture system. This type of laboratory can be further investigated to be used as a vehicle to provide ART services to rural areas.

The thesis concludes with a discussion on the manner in which mobile IVF laboratories can be used to improve access to ART. Two examples are provided, to describe how these mobile IVF laboratories can be used in the private as well as the public sector. Further recommendations are made, to engage with the South African National Department of Health to improve access to ART in South Africa, as well as training of medical professionals working in the field of medical assisted reproduction.

Layman's summary

The project was performed in three parts. First, the return for treatment in the public sector, by people trying to become pregnant, was looked at. The project showed that patients who do not earn a lot of money have a higher chance of not coming back for the necessary treatment than people who have more money. Also, the people with more money will return to the hospital faster, than those without a lot of funds.

The second and third parts of the project focused on the building and testing of an IVF laboratory in a trailer. This trailer laboratory was designed specifically for the project, and this is the first laboratory of this kind in the world. The way that the laboratory was designed is explained, and details are given about the problems that was encountered during this process.

After the laboratory was built, patients were given the chance to use it for assisted reproduction. From a group of 12 patients who began with treatment, 10 had successful egg retrievals and there were embryos that was grown in the trailer laboratory. The embryos' growth and progress went well and after the project was done, 4 of the 10 ladies had healthy babies born from the project. This showed that the idea of an IVF laboratory in a trailer is useful and works well in practice.

The thesis ends with a chapter that explains how laboratories in trailers can be used to make it easier for people to get treated when struggling to become pregnant. Examples are given to explain in what way private doctors can use the trailer laboratory, and also how public hospitals can use it. Advise is given on how the South African Government should use the information from the project to improve the way people can get help when wanting to become parents.

Flemish laymen’s summary

Het project werd in drie delen uitgevoerd. Eerst werd de terugkeer van onvruchtbare koppels voor behandeling in de publieke sector onderzocht nadat de diagnose werd gemaakt. Het project toonde aan dat patiënten die niet veel verdienen een grotere kans hebben om niet te terug komen voor de nodige behandeling dan mensen die meer verdienen. Ook keren onvruchtbare koppels met meer geld sneller terug naar het ziekenhuis dan minder begoede koppels.

Het tweede en derde deel van het project focusten op de bouw en het testen van een IVF-laboratorium in een container. Dit trailerlaboratorium werd specifiek voor het project ontworpen, en het is het eerste laboratorium in zijn soort ter wereld. De manier waarop het laboratorium werd ontworpen, wordt uitgelegd, en er worden details gegeven over de problemen die tijdens dit proces werden ondervonden.

Nadat het laboratorium was gebouwd, kregen patiënten de kans het te gebruiken voor geassisteerde voortplanting. Bij 10 patiënten konden we embryo’s bekomen die dan in het trailerlaboratorium werden gekweekt. De ontwikkeling van de embryo's verliep goed en na afloop van het project waren 4 van de 10 patiënten bevallen van gezonde baby's. Dit bewees dat het idee van een IVF-laboratorium in een aanhangwagen in de toekomstperspectieven opent.

De thesis eindigt met een hoofdstuk dat uitlegt hoe laboratoria in trailers kunnen worden gebruikt om IVF meer bereikbaar te maken op plaatsen waar het op dit ogenblik niet kan. Er worden voorbeelden gegeven om uit te leggen hoe privé-artsen het trailerlaboratorium kunnen gebruiken, en ook hoe openbare ziekenhuizen het kunnen inzetten. Tot slot geven we een advies hoe de Zuid-Afrikaanse regering de informatie van dit project zou kunnen gebruiken om IVF meer bereikbaar te maken voor meer onvruchtbare koppels.

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List of Abbreviations

ART	Assisted Reproductive Technology
COCs	Cumulus-oocyte complexes
IVF	*in vitro* fertilization
LMICs	low- and middle-income countries
MAR	Medical Assisted Reproduction
NHI	National Health Insurance
NDoH	National Department of Health
NPO	non-profit organisation
QC	quality control
PPP	Private-Public-Partnership
RBL	Reproductive Biology Laboratory
RSA	Republic of South Africa
SASREG	Southern African Society of Reproductive Medicine and Gynaecological Endoscopy
SBAH	Steve Biko Academic Hospital
TVOA	trans-vaginal oocyte aspiration
TTP	time to procedure
tWE	the Walking Egg
uHasselt	Hasselt University
UP	University of Pretoria

Chapter 1: Introduction

1.1. Thesis background

“Children are the hope that gives birth to a people!” according to Pope Francis, during his opening of the third General states of birth in 2021.[1] He also said that “most young people want to have children”.[1] Unfortunately, this is not something that is possible for all. Having children is an important milestone and not having the autonomy to decide when and how many children to have, can be devastating for some.[2-4]

Being able to have children is recognised as a basic human right.[5] Not being able to do so due to infertility, which is a recognised disease, is affecting millions of couples worldwide.[6-8] The exact prevalence of infertility is difficult to determine, with many investigators using different models to attempt to find an answer to the question.[7,9,10] Globally, the average prevalence of infertility is calculated to be at least 10%, but in some Sub-Saharan African regions, infertility is reported to affect 20 to 30% of the population.[9,11,12] With an averaged prevalence of infertility of 10% worldwide, and taking various factors in consideration, the number of Assisted Reproductive Technology (ART) treatments required per million population, per year, can be calculated.[13] These factors include, but are not limited to, the proportion of the population that is of child-bearing age, an uptake of 50% of people with infertility seeking help, and the percentage of people with infertility who would benefit from ART.[13] Based on the calculation, a minimum of 1500 ART cycles is required per million individuals in any population, per year.[14-16]

Apart from the ongoing prevalence of infertility that is inherently part of any population, the postponement of childbearing has been increasing the mean age at first birth of many countries, with the number of women over the age of 40 seeking fertility treatment to assist them to become mothers increasing.[17] Because of family planning policies focused on contraception to avoid unintended pregnancies, and the mitigation of population growth, fertility rates have been steadily declining globally. An increasing number of countries’ fertility rate is dropping below the replacement level.[18] The global necessity for ART seems to be increasing and the calculation indicating 1500 ART cycles per million population needs to be updated, to keep up to date with global changes and the development of technologies.[15,18]

Unfortunately, infertility treatment and Medical Assisted Reproduction (MAR)[a] is not as universal as the disease itself, with people in low- and middle-income countries (LMICs), especially, not having sufficient opportunities to access the treatment they need.[6,8,9,19-21] The International Committee for monitoring ART report a global utilisation of ART in 2018 of 639 cycles per million population.[22] The authors reported that there was a large regional variation, with the highest utilisation reported in the Middle East at 5732 cycles/million population, and the lowest in Africa at 73 cycles/million population.[22]

This thesis will focus on access to ART in South Africa, with emphasis on the public sector and specifically the Reproductive Biology Laboratory (RBL) facility at Steve Biko Academic Hospital (SBAH) in the Gauteng province. The description of a potential solution to improve access to ART services, in the form of a mobile *in vitro* fertilization (IVF)[13] laboratory, will follow. The design and construction of a mobile IVF laboratory using a simplified IVF culture system will be described. The thesis will conclude with the results from a proof-of-concept study showing the feasibility of culturing embryos in the prototype mobile IVF laboratory that flowed from the previous section.

1.2. Problem statement

The availability of sufficient ART facilities has a major negative impact on the access to MAR in South Africa.[23] The limited provision of ART is exacerbated in the public sector of South Africa.[24,25] The effect of ART expenses on patients' realised access to MAR is widely commented on and assumed.[21,24-27] However, the follow-through of patients from the diagnostic to therapeutic phase of ART is not well defined and scarcely reported on.[28]

The number of ART facilities in South Africa is well known to be insufficient to provide enough ART cycles for the country's population.[23,25] The way in which this should be addressed is unclear, other than increasing the number of trained health care

[a] The terminology of MAR and ART is often used interchangeably, with a distinct difference between the two phrases.[17] The first, MAR, is the broader term, covering all medical interventions that is used to assist persons on their journey to parenthood, such as ovulating inducing drugs and artificial insemination, while the latter, ART, is a more focussed definition of the procedures where *in vitro* manipulation of gametes and embryos is involved after trans-vaginal oocyte aspiration (TVOA), which would include embryo culture, transfer, biopsy and vitrification, but exclude sperm intra-uterine insemination (IUI).[13]

practitioners in the field of MAR.[26] An investigation of the provision of ART services in South Africa, and specifically the public sector, is needed to determine plausible areas of improvement. This should be focussed to determine shortcomings that can be remedied to increase the service delivery of ART facilities in the public sector. A focused investigation on the demographic profiles of patient failing to return for ART services after diagnostic evaluations, compared to those who do return, would shed light on the patients that are missing out on the opportunity to have ART procedures performed that is available to them. Data from such a report can be of benefit to policy makers, to make the necessary changes to encourage patients to return for ART procedures after their diagnostic evaluations has been completed.

Furthermore, the severe lack of ART facilities in South Africa needs to be addressed. The proven track record of mobile laboratories in other fields have paved the way to determine if the same can be used in ART. By designing and testing a prototype mobile IVF laboratory, the first step towards readily available ART laboratories can be taken. The future use of such mobile laboratories can be investigated further, once the culture of embryos in such an environment has been proven to be possible and practical.

1.3. Aims and Objectives

1.3.1. Aims

The aims of this research project were to (i) investigate the demographic profiles of patients accessing ART at RBL, SBAH, (ii) design, contract assembly and validate the use of a mobile IVF laboratory, and (iii) perform a proof-of-concept evaluation, to successfully culture human embryos in the prototype mobile laboratory.

1.3.2. Objectives

The objectives of the research were to:

- Determine and compare the demographical profiles of patients undergoing diagnostic ART investigations only vs. those who return for therapeutic ART procedures, at Steve Biko Academic Hospital, Gauteng, South Africa.
- Design and supervise the construction and furnishing of a mobile IVF laboratory.
- Validate the functionality of the mobile IVF laboratory through a pilot study, culturing patient embryos in the mobile IVF laboratory.

1.4. Study area and methodology

The project was subdivided into three studies, each addressing one of the study objectives. The first study evaluated ART usage in the public sector of South Africa, while the second and third studies explored a possible tool to improve ART service delivery through the use of a mobile IVF laboratory. Each study's methodology is described in a summarised manner, with detailed descriptions of the study's materials and methods in the relevant manuscripts in Chapters 3-5.

1.4.1. Study 1: Public sector ART in South Africa

The provision and usage of ART services in the public sector of South Africa was explored in the first study. The study was performed retrospectively at the RBL, SBAH. All data was manually captured by the researcher and statistically analysed with the assistance of a biostatistician. Patients who visited the RBL to investigate their reproductive health status, as identified by the male partner providing a semen sample for analysis, was incorporated in this study. Sampling was from 01 January 2015 to 31 December 2020. Patients' return for therapeutic ART procedures (TVOA or IUI) was recorded to 31 December 2021 to allow at least 12 months' time for patients to return after their diagnostic investigation.

The couples' combined monthly income, medical aid status and town of residence was captured. Based on the demographic data obtained, the patients were categorised on (i) income (Low-, Medium-, or High-income), (ii) whether they were eligible for subsidised ART, (iii) if they have medical aid cover, and (iv) their distance to travel to SBAH (<50km, 51-100km, 101-250km, >250km). Couples who initiated ART investigations at SBAH without continuing to therapeutic ART procedures (Group 1) vs. those that followed through with therapeutic ART procedures (Group 2) were investigated. Within Group 2, the time from initial diagnostic evaluation to first therapeutic procedure, termed time to procedure (TTP) was calculated. All parameters listed were compared to identify differences in the accessibility and usage of ART procedures to patients from different demographic groupings and locations. The collection and processing of data, as well as the outcomes of the study, is presented in Chapter 3 of the thesis.

1.4.2. Study 2: Conceptualisation of a mobile IVF laboratory

The second study focussed on the design and construction of a prototype mobile IVF laboratory. The study concluded with a pilot study as a quality control of the Walking Egg (tWE) simplified IVF culture system used in the mobile laboratory. A sequential process (Figure 1.1) was followed, to determine what is required in the mobile IVF laboratory. Identified aspects of the mobile laboratory was then used to guide the design and construction.

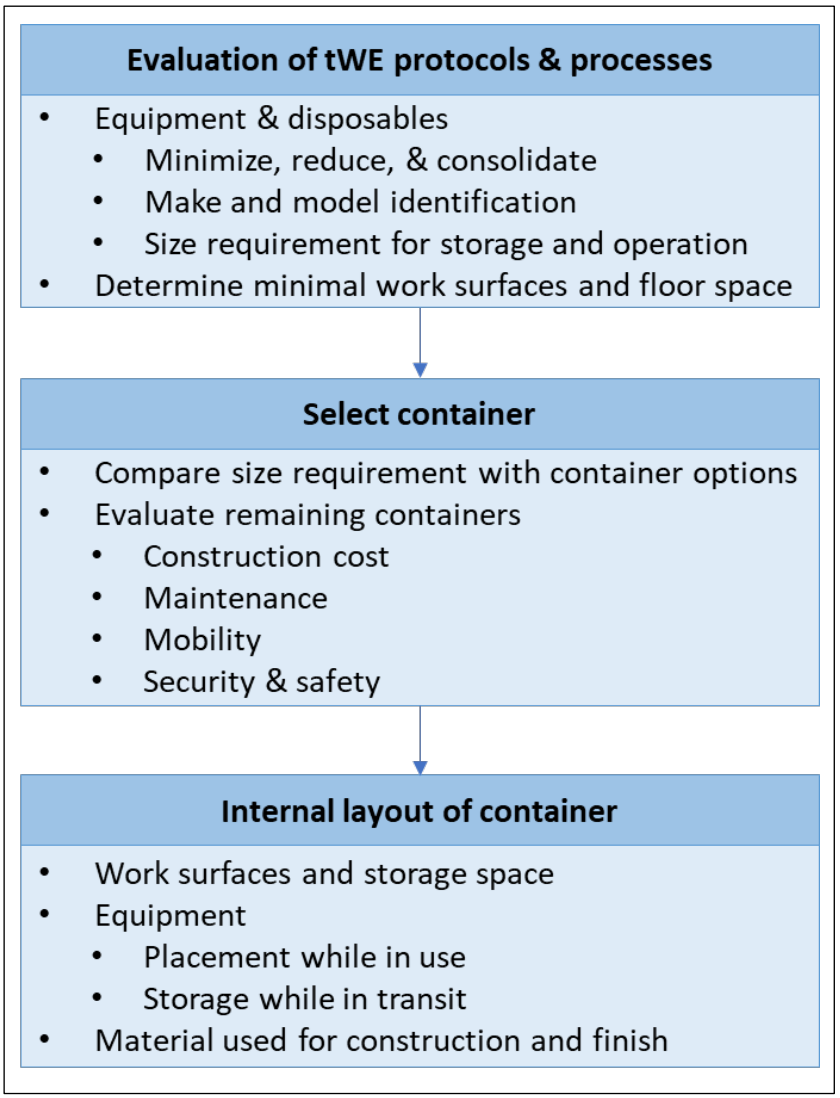

Figure 1.1: Sequence of events for the design of a prototype mobile IVF laboratory.

After the laboratory design has been finalised, a supplier was identified to build the prototype, followed by installation of all equipment. The prototype mobile laboratory was outfitted and tested at SBAH. The tWE simplified IVF culture system's individual components, such as the temperature and pH regulation of the culture tubes, and

insemination with low numbers of sperm, was validated during the researcher's MSc project.[29] The equipment in the mobile laboratory was evaluated through a series of quality control (QC) checkpoints that included:

i) Operational testing of individual equipment,
ii) sterility of embryo culture tubes after assembly, and
iii) pH of equilibrated embryo culture tubes.

After the equipment validations were finalized, a pilot study was launched, using non-viable (containing 3 pro-nuclei) fertilized oocytes (n=22 from 14 patients), to demonstrate embryo development in the tWE culture system and operations in the mobile laboratory. After informed consent was obtained from the patients (described in section 1.4.4 and see Annexure B for a copy of the patient information and informed consent document), the 3PN zygotes were vitrified and warmed in the mobile laboratory when the pilot study commenced. The warmed zygotes were placed into pre-equilibrated tWE embryo culture tubes and subjected to standard tWE simplified embryo culture and evaluation. Embryo evaluations were performed to confirm ongoing embryo development, including cellular division past the 8-cell stage to compaction and blastulation. There was an expectancy of at least 50% of these abnormal zygotes to initiate cleavage events.[30] Compaction and blastulation of the derived genetically abnormal embryos (test group) were compared to that of sibling embryos cultured in parallel in the RBL's conventional IVF laboratory (control group). Blastulation of the test group had to surpass 50% of the control group's blastulation.[29] The mobile IVF laboratory's design, commissioning, and testing is described in detail in Chapter 4 of the thesis.

1.4.3. Study 3: Patient IVF cycles in the mobile laboratory

A prospective proof-of-concept study (study 3) was performed to confirm the ability culture human embryos in the prototype mobile IVF laboratory constructed during study 2. To simulate a real-world scenario, the proof-of-concept test was performed in Rustenburg, a rural town 112km from SBAH. To safeguard patients' ART cycles, the mobile laboratory was used at La Femme fertility clinic, one of the only two ART facilities in Rustenburg. A precautionary arrangement was made with the fertility clinic to move embryos to their conventional IVF laboratory if any problems arose at the

mobile IVF laboratory, that could have a negative impact on embryos being cultured in the mobile laboratory. By performing the study at this location, the impact of having the mobile laboratory in a rural environment could be tested, with the safety net of the fertility clinic's conventional IVF laboratory available nearby. No unforeseen circumstances arose, which necessitated moving embryos to the conventional IVF laboratory, and all embryo culture cycles were performed from start to finish in the mobile IVF laboratory.

Patients from the fertility clinic, who has not yet undergone ART procedures, were considered for the study. Those who met the inclusion criteria for both the male and female partner of the study was approached, and the study was explained to them. Upon their acceptance to participate in the study, they were given an informed consent document (see Annexure B) to sign. Twelve patients were recruited for the study. The female patients were provided with the necessary medication for mild ovarian stimulation, with regular monitoring of the ovarian response by the fertility subspecialist at La Femme fertility clinic, and his clinical staff. At such time that sufficient follicular development was identified, a TVOA procedure was scheduled for the female patient.

The TVOA was performed in the fertility clinic's procedure room, and the follicular fluids were collected in 14ml round-bottom tubes, which were closed and transported to the mobile laboratory in a portable incubator at 37˚C. In the mobile laboratory, the follicular fluid was screened for the presence of cumulus-oocyte complexes (COCs), which were inseminated in prepared tWE embryo culture tubes. Embryo culture, with subsequent embryo transfers and cryopreservation of excess embryos were performed in the mobile laboratory. Embryo culture outcomes, including oocyte fertilization, embryo development and usage rates, were considered to show proof-of-concept of the mobile laboratory. A detailed procedural description is provided in Chapter 5 of the thesis.

1.4.4. Ethical considerations

The study protocol was submitted for review and approved by the University of Pretoria (UP) Faculty of Health Sciences and Hasselt University (uHasselt) PhD committees, as well as the UP Ethics committee (protocol number 149/2021) and the uHasselt

Comité voor Medische Ethiek (protocol CME2023/046). The committee approvals can be found in Annexure C (PhD Committee) and D (Ethics committee).

Participation by patients in either the pilot study (Study 2) or the proof-of-concept study (Study 3) was confirmed by the completion of written informed consent documents (see Annexure B), after reading study information pamphlets and discussion with the researcher. All data gathered was anonymised and kept confidential with access only by the researcher and his supervisors.

As part of Study 2, there was no benefit to the patients, apart from altruistic reasons to assist in research. This study also had no risk to the patients, as they would only give permission to delay the discarding of abnormally fertilised oocytes to a timepoint after the zygotes were cultured for 5 days in the mobile laboratory. In Study 3, the patients benefited from the study due to cost savings to their ART treatment. Medication for ovarian hyperstimulation was provided to the patients at no cost, and all laboratory fees were waived. Patients only had to pay a facility fee to use the aspiration room at the local fertility clinic.

1.4.5. Sponsorship and resources

The various sections of the research project were funded individually, as described below. The travel and living expenses for the researcher to visit Belgium, to comply with requirements of a joint PhD agreement, were awarded to the researcher through the “Bijzonder Onderzoek Fonds” Bilateral Cooperation programme, at uHasselt (BOF-BILA agreement BOF21BL16).

Given that study 1 was retrospective, no direct costs were incurred. Information technology equipment (computer, printer, internet accessibility) was provided via UP.

During Study 2, the construction of the mobile laboratory was financed by tWE non-profit organisation (NPO), and equipment in the mobile laboratory was provided on loan to the project by ESCO Technologies Pty (Ltd), as described in the manuscript in Chapter 4. The pilot study performed as part of Study 2 was funded jointly through the RBL research fund and tWE NPO. The proof-of-concept performed in Study 3 was

funded by Delfran Pharmaceuticals (medication), Ducit Pty (Ltd) (disposables), and tWE (day-to-day operational cost and sundries).

1.5. Layout of thesis

The thesis is constructed as a preamble, with six chapters and annexures. The preamble contains the study title, declarations by the student, acknowledgements, an executive summary of the work, a table of contents, and lists of figures, tables and abbreviations.

Chapter 1 introduces the thesis, containing a background of the field of assisted reproduction with a focus on access to care. The thesis background is followed by a problem statement, research aims and objectives. The chapter concludes with a short description of the study area and methodology followed during the research, and an explanation of the layout of the thesis.

A review of relevant topics that is addressed in the thesis is provided in Chapter 2, discussing the rationale behind the research study. Chapters 3-5 presents three research articles with specific details of each section's materials and methods, as well as the outcomes and discussion of each section. Each manuscript addresses one of the study objectives.

Chapter 6 contains a combined synthesis of the entire project, consolidating the previous sections and reflecting on limitations of the research, as well as future applications.

Chapters have their own reference sections, with the presented manuscripts' references captured as part of the manuscript. The thesis concludes with some relevant additional information in the Annexures.

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Chapter 2: Literature review

The treatment of fertility and infertility has been documented since ancient times and the concept of medical intervention to make reproduction possible has therefore been around for ages, albeit in very primitive ways in the beginning [1,2]. The practice of MAR has developed in modern times to a multidisciplinary field, where medical doctors, scientists, psychologists, and many others work together to treat different fertility problems.[3-5] A greater part of this field is direct intervention through the *in vitro* manipulation of gametes, which is called ART.[4] Since all ART procedures are performed *in vitro*, the ART laboratory forms an essential part of MAR facility.[6,7] The distinction between MAR and ART is important when describing MAR or ART facilities. The first, MAR facilities, is a medical practice where a team of health professionals offers an array of services for the medical treatment of fertility issues, and can include ART, or not. The ART facility is the place where gamete manipulation takes place, i.e. the ART laboratory.[6] When considering the number of facilities available for patients, one has to be clear about which of the two is being referred to.

Performance of ART cycles are often used as a gauge of access to MAR in a population.[8,9] An analysis of the number of ART cycles per million people in a population has historically been used to provide a crude measure of the ART and MAR utilization rate in said population.[10-12] According to this calculation, and taking into consideration factors such as only 50% of infertile couples seeking medical assistance, 5% of infertile couples have tubal obstructions, and 5% have sever male infertility, etc., an average number of 1500 ART cycles is needed for every 1 000 000 people.[10]

2.1. Access to ART in South Africa

The provision of ART in South Africa began, along with many other countries, in the early 1980s.[13-15] The first ART facilities in this country was found in the public sector.[14,15] However, even then the financial support of the, at the time, new field of assisted reproduction was not a priority to the government.[14] This meant that many of the medical professionals who performed the first ART cycles in South Africa soon established private ART facilities to continue their work.[14] This trend has continued since then, with most of the fertility treatments in South Africa happening in the private sector.[16] At present, there are approximately 36 ART facilities in South Africa, mostly found in large cities (figure 2.1).[17-19]

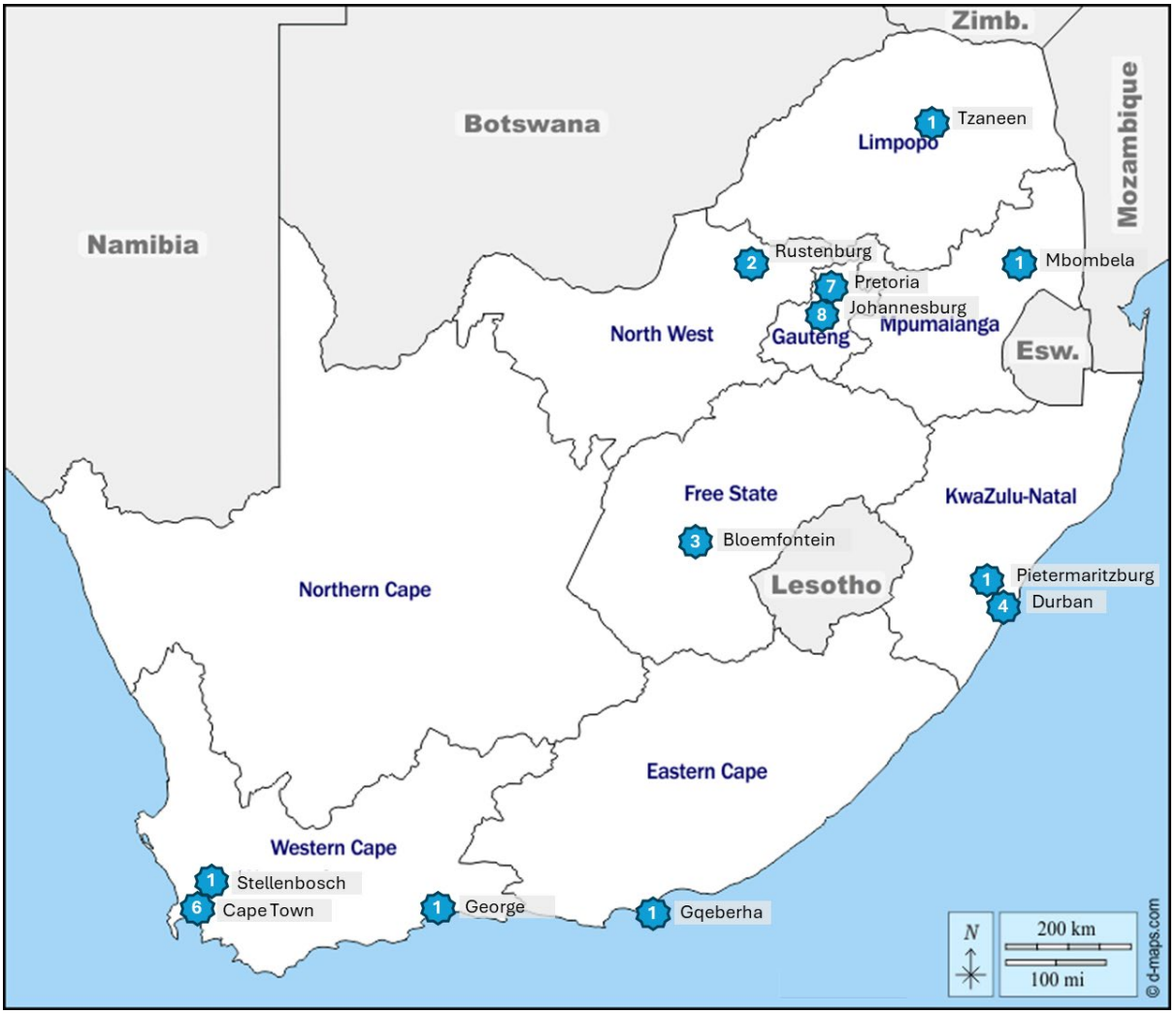

Figure 2.1: Map of South Africa depicting the number and location of Medical Assisted Reproduction facilities.

There is a significant difference in cost to patients between the two sectors, with the public sector ART facilities offering services at a lower price.[15,16] Based on personal experience, ART facilities in the public sector do not have to charge patients for overhead costs such as staff salaries, professional insurance, or building rental, which reduces tariffs as compared to the private sector. Additionally, in the private sector the companies are owned by stockholders, who wishes to see a return on investment. This causes the ART facility to be operated as a business that needs to be lucrative, and in turn relates to patients being charged more. All South African citizens are allowed to access procedures at public sector ART facilities, and patients with lower-bracket incomes are offered services at lowered cost due to government subsidisation.[20] When a patient enters the public health system, proof of their current income is requested. Based on this, patients are categorised according to their income level and

procedural payments to the hospital is staggered per income group.[20] Patients with the highest income and access to medical aid has to pay the most, with no government subsidisation, whereas the lower income groups are subsidised.[20] This subsidy system is described in full in the manuscript presented in Chapter 3 of this thesis.[21] However, even though ART in the public sector is more affordable, some patients more readily prefer to frequent private sector facilities. This can be due to the low number of public sector ART facilities available and patients not knowing that these facilities exist,[17] or a general preconceived notion that service in the private sector is of better quality than that in the public sector.[22]

The incidence of infertility in South Africa is not well documented and based on fairly outdated accounts, is estimated that approximately 15-20% of couples of reproductive age are infertile.[23,24] The prevalence of tubal factor infertility is mentioned as the highest contributor of infertility (approximately 60%), and about 40% of cases reporting on male factor infertility to various degrees.[23,24] According to the latest data available, in 2021 the South African ART utilization was 9439 ART cycles in a population of 59.4 million people.[17] This calculates to a utilization of 159 ART cycles/million population, which can be reduced to 138 cycles/million population for South African citizens when taking into account that 1216 of the ART cycles were reported to be for cross-border care.[17] This type of reproductive tourism is fairly common in South Africa.[25] Whereby, South Africa is one of the few countries in Sub-Saharan Africa with a well-established MAR presence. Combinedly, South Africa, Nigeria, and Ghana account for more than 63% of ART facilities in Sub-Saharan Africa reporting to the regional registry in 2022.[26] The calculated ART utilization in South Africa equates to less than 10% of the national requirement of 1500 ART cycles/million population.

Any health system should promote four elements to support the use of a specific health service, namely the availability, accessibility, acceptability and quality of the service being offered.[8] In the calculation that determines the minimum requirement of 1500 ART cycles/million population, the acceptability of ART procedures has already been accounted for, through assuming an uptake of only 50% of patients in need if ART

seeking the necessary assistance.[10] In South Africa, accreditation[b] of ART facilities is not mandatory, but strongly advised, and all 19 facilities who submitted data to the national registry is listed on the national society's webpage as "Centres of excellence", for which accreditation is a requirement.[17,18] Therefore, the quality of ART services being offered in South Africa is regulated to achieve a minimum standard. With acceptability and quality accounted for, the last two elements needed to support access to MAR in South Africa is availability and accessibility.

The availability of ART services effectively speaks towards physical accessibility and the number of ART centres available to a given population, which are often found in metropolitan areas or high population cities.[27,28] In established regions of Europe, the USA and Latin America, the average number of ART cycles performed per centre per year calculate to 398, 500 and 429 respectively.[29] In order to provide the necessary 1500 ART cycles/million population, this equates to 3.7, 3.0, and 3.5 ART facilities required per million population in these respective areas. In comparison, in South Africa in 2021, there were known to be 23 ART facilities providing ART procedures to a population of 59.4 million people, i.e. 0.4 ART facilities per million population, each performing an averaged 498 ART procedures per year.[17] Unfortunately, this still leaves the South African population with a shortage of 155 ART facilities to make up the required number to provide sufficient ART procedures for the population.

Additionally, the physical accessibility of a health service entails that the service has to be available within reasonable reach of the patients.[28,30] In the previous report of the national registry, the number of ART cycles per facility was reported with an indication of four out of the sixteen facilities providing 500 ART cycles or more per year.[31] Three of these sixteen participants are public sector health providers, i.e. the centres at Groote Schuur (Cape Town), Steve Biko (Pretoria) and Tygerberg (Cape Town) hospitals.[31] Unfortunately, the public sector units are known to be among those centres performing less than 500 ART cycles per year.[32,33] The private sector dominates MAR in South Africa, with three active public service ART facilities, one in Gauteng and two in the Western Cape Province.[16] Patients from any of the other six

[b] Voluntary accreditation of ART centres by the Southern African Society for Reproductive Medicine and Gynaecological Endoscopy (SASREG), which include mandatory reporting of ART cycle data to the South African Registry for Assisted Reproductive Technology.[18]

provinces in South Africa must travel over at least one provincial border to access ART services in the public sector.[34]

Apart from the lack of sufficient ART facilities having a negative impact, accessibility is another high-ranking factor that explains the dissimilarity between the demand for ART cycles and the number of cycles being performed.[35-37] This accessibility primarily relates to whether ART services are available to a person, but economic factors also play a role.[28,26] In Sub-Saharan Africa, the cost for assisted reproduction is often named as one of the major obstacles for patients to overcome.[33,36,38]

Similarly to the rest of Sub-Saharan Africa, in South Africa the cost of ART is also a barrier to access for patients.[33,39,40] An ART cycle in South Africa, with oocyte aspiration and IVF, can range from at least R30 000 (≈1500€) to more than R110 000 (≈5500€) in the private sector (conversion from ZAR to Euro was calculated as R20:1€, based on the rounded average exchange rate in 2025).[40-43] Marked differences exists between ART cost in the private and public sectors, and few medical aid schemes providing benefits for ART.[15,16,32,40]

According to a study by Dyer and colleagues (2013), the cost of ART at one of the public sector ART facilities in South Africa represents a calculated catastrophic out-of-pocket payment (an expense that threatens the household's financial survival) for 1 out of 5 patients in general, and fifty percent of patients when considering the poorest third of the population.[44] Importantly, these estimates relate primarily to the costs of ART procedures themselves and do not encompass the diagnostic evaluation required before treatment can be considered. Diagnostic procedures represent an additional financial commitment that may influence patients' ability or willingness to proceed beyond initial evaluation. Although public and private sectors costs for investigative and therapeutic procedures may differ considerably, proceeding from diagnostic to ART procedure in either sector may be beyond the financial means of many households. A small sample of middle- to lower-income patients at a public-sector ART centre in South Africa showed that patients presenting for diagnostic services had an average income 38% lower than those who continued to therapeutic ART procedures.[15]

Apart from the low number of ART cycles being offered, South Africa is ranked as one of the three most economically unequal regions in the world, where the 55% or more of the national income is earned by 10% of the population.[45] Through the combination of services that is already not readily available, and the disproportionate distribution of service in an unequal society, there is no surprise that access to ART services in South Africa is sorely lacking.[32,39,40,46] The cost of ART in the public sector appears to be a significant barrier to the access of therapeutic services and therefore merits critical examination. To explore the progression of patients from diagnostic to therapeutic procedures, Chapter 3 of this thesis presents the data from a retrospective study, spanning six years, that compared the demographic profiles, including income class, region or province of residency and access to medical aid, of patients who entered the public health sector seeking assistance to become pregnant.

2.2. The Walking Egg simplified embryo culture system

To curtail laboratory fees, an affordable laboratory setup was developed through the Walking Egg (tWE) NPO.[47,48] The NPO, founded in 2010, “aim to strengthen fertility care through innovation and research, advocacy and networking, training and capacity building, and service delivery”.[49] Since their inception, the NPO has performed many projects and publications to achieve this goal, and a timeline of events showing some of their milestones achieved is depicted in Figure 2.2.

The affordable laboratory setup is focused on the use of the simplified tWE IVF culture system. Through the use of embryo culture in closed, gassed tubes, expensive laboratory equipment such as microprocessor-controlled tissue culture incubators and large area air filtration systems are not required.[47] This design significantly reduces the laboratory setup cost, which in turn amounts to a more affordable ART procedure being offered to patients. The simplified IVF system was investigated and successfully applied by the Belgian group lead by Prof Willem Ombelet.[47,50] In an analysis performed at the Universities of Leuven and Hasselt, the cost to establish a conventional ART laboratory was approximately double that of a tWE laboratory.[51] Additionally, the cost of disposables and media used for tWE embryo culture calculated to less than a third the cost of conventional ART with IVF or ICSI.[51]

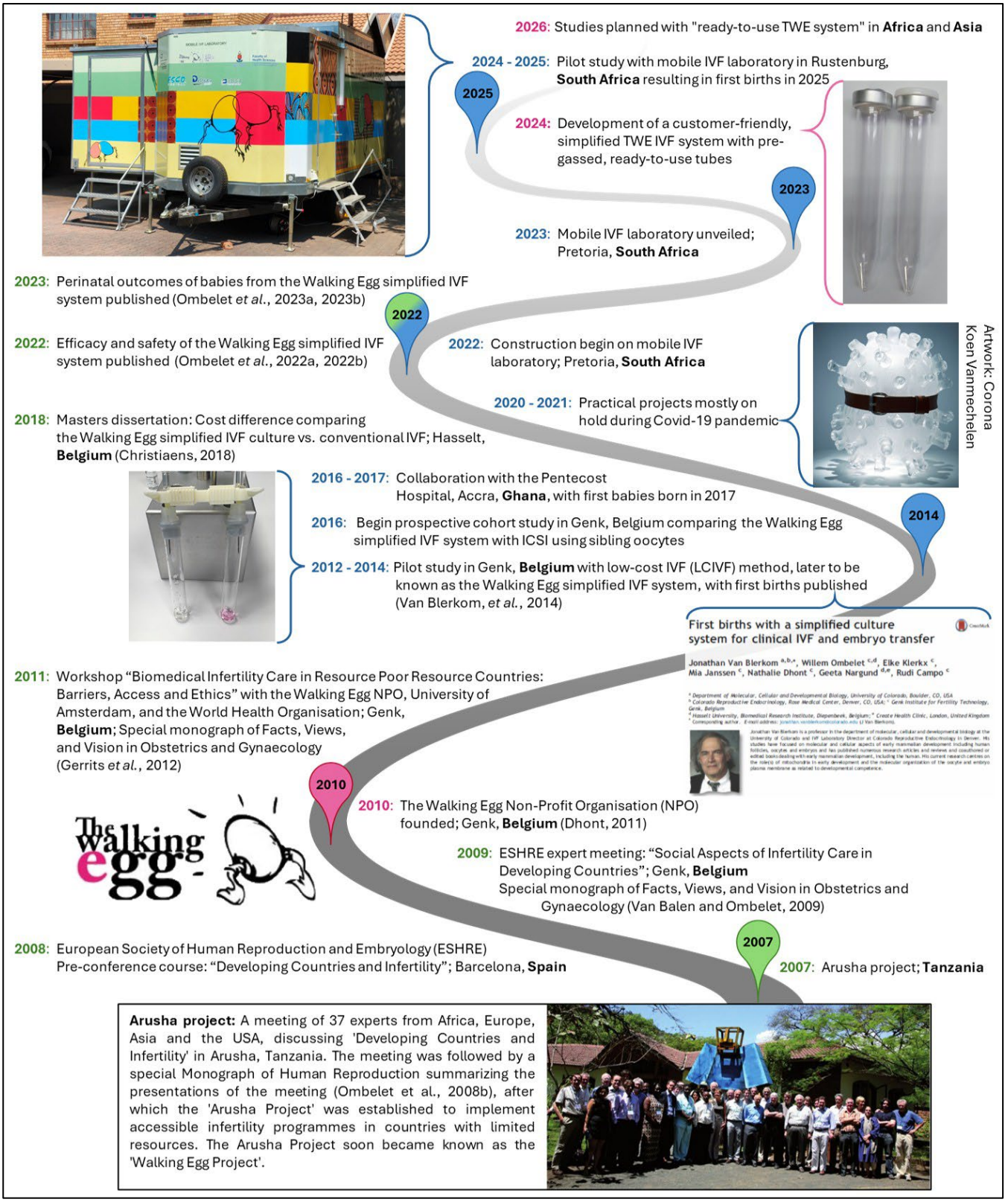

Figure 2.2: Milestones in the road to improve access to Medical Assisted Reproduction in Low-and Middle-Income Countries, with focus on the Walking Egg NPO. Developmental and academic milestones indicated in blue, research projects in orange, and Walking Egg organisational specific items in green.

Quality control aspects of the simplified tWE IVF culture system has been investigated at the RBL by the researcher, during his master's degree research project.[52,53] The setup of a simplified tWE laboratory at SBAH has been considered and equipment at the RBL was used to investigate crucial aspects of the tWE simplified IVF culture system, such as temperature control, pH stability and the sperm insemination protocols proposed by tWE. [52,53]

More recently, the efficacy and effectiveness of this embryo culture system have been reported on by the group led by Ombelet. In two separate manuscripts published in 2022, they reported on the safety and efficacy, as well as the perinatal outcome of babies born using the tWE simplified IVF culture system vs babies conceived through ICSI and conventional embryo culture.[48,54] The researcher was a co-author of one of these articles, which was published during the PhD term and a copy thereof can be found in Annexure A of this thesis.[54] Two additional publications by Ombelet and colleagues in 2023 described the promising perinatal outcomes of babies born after using the tWE simplified IVF culture system as compared to all ART babies born in Belgium (2013-2018) and all singletons born in Flanders (Belgium) (2012-2020).[55,56]

The tWE simplified IVF culture system has been proven to be a feasible option for embryo culture, that can be implemented as a less expensive option in low-cost laboratories.[56] Implementation of this culture system can be done in resource poor countries across the globe.[57] The culture system's closed tubes design reduces the exposure to external elements and formed an integral part of the second section of the PhD, the realisation of a mobile IVF laboratory.

2.3. Mobile health units

The necessity for more ART facilities in South Africa have been described, and this requirement is echoed in Sub-Saharan Africa.[36,58,60] The setting up of an ART facility, especially the IVF laboratory required for ART procedures required, is very expensive and an array of factors needs to be considered.[15,35] A single laboratory that can be used by multiple ART facilities will ease the financial burden of commissioning an ART laboratory for each centre.[60]

Gametes and embryos are transported between facilities regularly, but the distance and time needed for transport impacts on the practicalities and feasibility of this model.[60-63] Therefore, implementing a mobile laboratory could provide support to multiple MAR facilities while avoiding the risk of transporting gametes or embryos over long distances.[64] This model was also described in an article that the researcher co-authored, which was published during the PhD term and a copy thereof can be seen in Annexure A of this thesis.[57]

Mobile medical units are modified vehicles that are stocked with specialized medical equipment to provide medical services to out-of-reach areas and are employed in various medical disciplines.[65,66] This mode of providing health services to rural areas is implemented worldwide, in both high-and low-resource settings.[65-70] In India, the utilization of mobile medical units are even considered to improve access to reproductive and child health services, albeit more focused on family planning and contraceptive services.[65,71]

In South Africa, mobile medical facilities are regularly used to provide basic health care to rural communities.[72] These mobile health clinics are often provided by non-governmental organisations, or as a collaboration between governmental and non-governmental organisations.[73] Some examples of these include mobile health units for general health checks (www.starforlife.org/south-africa/), or specialised diagnostic mobile wellness clinics developed to test patients' HIV and TB status (www.nacosa.org.za). Mobile facilities are also used for therapeutic interventions, such as basic dental procedure and issuing spectacles after eye tests (www.mobilehealthclinics.org.za). Mobile medical services are offered in various forms in South Africa and can range from as small as Iyeza Express' bicycle courier delivering chronic medication,[74] to as large as Roche's Phelophepa[c] Healthcare Train,[75] and anything in between, such as a temporary setup in a tent,[76] mobile clinics in vehicles and trailers,[77,78] setting up a temporary facility in available open space such as school halls,[76,79] or even fully equipped medical facilities in large trucks.[30,76,78] Some examples of such medical facilities can be seen in Figure 2.3. Unfortunately, no such mobile facilities are available for ART, in either South Africa, or

[c] Phelopheba, meaning "Good clean health" in Sesotho

elsewhere in the world.

Figure 2.3: Examples of different modes of mobile medical services in South Africa, including (A) a backpack used by Iyeza Express bicycle couriers,[73] health clinics in a (B) tent,[72] (C) small truck,[80] (D) trailer,[81] and (E) bus,[78] as well as (F) the Phelophepa Healthcare Train.[75]

The application of a mobile medical unit needs to be carefully planned to accommodate a niche in the market previously unmet, while remaining financially viable.[66] Applying the principle of mobile medical units on assisted reproduction, a mobile IVF laboratory can service multiple medical centres. The mobile laboratory can visit these centres in a scheduled manner, allowing local clinicians to recruit and batch patients who qualify for ART intervention, until such time that the mobile laboratory frequents the facility.

2.4. Innovation in assisted reproduction

Introducing novel concepts in ART requires careful consideration of ethical, regulatory, and quality control requirements. Primarily, patient safety must be ensured, while maintaining laboratory standards, monitoring relevant outcomes, and avoiding risk, particularly when implemented in resource-constrained settings.[82,83] Unfortunately, this process is not always followed, since there is substantial pressure from intended parents to try new procedures and methods to have successful ART outcomes.[82]

The roll-out of new ART procedures tends to be focussed on advances in technology, such as artificial intelligence-based embryo selection, and pre-implantation genetic testing.[83,84] Often, the additional cost of these new technologies are not taken into consideration when provided as add-ons.[83,85] Additionally, the research outcomes usually is more concerned about short-term success rates, than long-term outcomes of the babies born.[82,83] With the safety and efficacy of the treatment proven, experimental treatment is recategorized as being innovative treatment options, but not yet established.[84] Over time, with enough use, these adjuncts can easily be misconstrued as being established standard of care procedures.[84,85]

To ensure appropriate pre-clinical and clinical validation of the simplified tWE IVF culture system in a mobile laboratory, the mobile laboratory presented in this thesis has undergone a wide variety of quality control evaluations. The design, contracting of assembly, and outfitting of a mobile IVF laboratory using the tWE simplified IVF culture system was performed and reported on in Chapter 4 of this thesis. Furthermore, the testing and proof-of-concept results of the use of the mobile IVF laboratory is described in Chapter 5 of the thesis.

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Chapter 3: Public sector access to medically assisted reproduction in South Africa

The first objective of the research was to investigate access to ART in the public sector of South Africa. This was performed through an investigation on the demographic profiles of patients undergoing ART. The objective was achieved through a retrospective study that specifically considered the patients at the RBL, SBAH, Gauteng, South Africa. Even though the data was obtained from a single ART facility, the location of the facility in the northern half of the country was deemed relevant to represent a large portion of the population. With the only other two public sector units in South Africa situated in Cape Town, on the southern-most tip of the country, the facility at SBAH is the only public sector ART facility available to people in the northern half of the country.

Patient records from the RBL were used to identify differences between patients following through with ART procedures, or not. The patients' income, access to medical aid, and distance to travel to the RBL was considered. Additionally, within the cohort of patients who returned for ART procedures, the time that elapsed between their first diagnostic evaluation and their first therapeutic procedure was determined. Comparisons were made between demographic profiles and the time to procedure. The outcome of this investigation is presented in Chapter 3 in a manuscript format.

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REVIEW

FERTILITY CARE IN LOW- AND MIDDLE-INCOME COUNTRIES

Public sector access to medically assisted reproduction in South Africa: a case study

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This paper forms part of a special series on Fertility care in low- and middle- income countries. The guest editors for this series are Associate Professor Willem Ombelet (University of Hasselt, Belgium) and Dr Federica Lopes (University of Dundee, UK).

Abstract

In South Africa, approximately 10% of the calculated need for medically assisted reproduction is being met due to limited access and unequal availability of these services. To facilitate understanding of challenges associated with access to assisted reproduction, a retrospective case study spanning 6 years was performed at one public sector hospital in South Africa offering these services. Demographic profiles, including income, region of residency and access to medical insurance, of patients seeking assistance to become pregnant were investigated. Patients were categorised as those who underwent diagnostic investigations only vs those who returned for therapeutic procedures, and the difference in demographic profiles between the two groups was determined. This investigation showed that patients from the lower-income classification group, without medical insurance, tend to return for therapeutic procedures less often than those with a higher income and medical insurance, even though these low-income patients qualify for a therapeutic procedure subsidy. An inverse relationship existed where patient numbers decreased as their travel distance increased, but patients who were required to travel further for assisted reproductive therapy tended to return for these procedures more often than patients who resided closer to the medical facility. In conclusion, access to medically assisted reproduction facilities is critically undersupplied and limited in the region. In order to ease the travel distance of patients, alternative primary diagnostic routes with accessible clinics are needed. In addition, costs of therapeutic procedures in the public sector should be re-evaluated to be offered at affordable rates for marginalised patients.

Lay summary

In South Africa, about 10% of patients who need assistance to become pregnant are being helped. To better understand this phenomenon, researchers considered information about patients from a public sector hospital in South Africa. This includes how much money the patients earned, how far they travelled to the hospital and whether they had medical insurance. The patients were grouped into those who requested initial investigations but never returned for treatments, and those who returned for medical treatment. The differences between these groups were then evaluated. The research showed that people with less money tend to abandon further treatment more often, or take

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longer to return, than those with more money. The conclusion drawn is that assisted reproductive therapy is too expensive and that more IVF clinics are needed, using cheaper and simpler procedures of the same quality.

Keywords: access; assisted reproduction; developing countries; LMIC; MAR; patient demographics; treatment progression

Introduction

Medically assisted reproduction (MAR) has a centre-stage role in addressing infertility and assisting individuals and couples to achieve their reproductive goals (Afferri *et al.* 2022, Seiz *et al.* 2023). The optimal coverage of assisted reproductive technology (ART) is estimated at 1,500 ART cycles per year per million individuals in any given population (ESHRE Capri Workshop Group 2001, European Society of Human Reproduction and Embryology 2023). However, in South Africa, the South African Registry for Assisted Reproductive Technologies (SARA) reported that only approximately 10 and 13% of this calculated need was being met in 2015 and 2019, respectively (SARA 2019, 2024). (These percentages were calculated according to 7,950 total ART cycles reported for a population of 55 million people, and 9,439 total ART cycles for a population of 59.4 million people, respectively for 2015 and 2019, reporting on the period that the current study is focused on (SARA 2019, 2024)). In addition, the 2015 report did not indicate how many of these ART cycles were utilised by foreign patients. The 2019 report stated that more than 1200 ART cycles were initiated for cross-border care, resulting in lower ART utilisation by South African citizens, as South Africa is known to be a global hub for reproductive tourism in Sub-Saharan Africa (SARA 2019, 2024, Pande 2020, Moll *et al.* 2022, Whittaker *et al.* 2024).

South Africa faces significant challenges in providing adequate MAR, resulting in limited access and unequal availability of these services (Dyer *et al.* 2020, Connolly *et al.* 2021). In South Africa, accessibility to MAR (i.e. ART, surgical procedures, hormonal treatment and other interventions) is among the highest-ranking factors explaining the dissimilarity between the demand for ART cycles (intra-uterine insemination or ovarian aspiration with subsequent embryo culture) and the number of cycles being performed (Ombelet & Goossens 2017, Adamson *et al.* 2018, Ombelet & Onofre 2019, Dyer *et al.* 2020). Accessibility primarily relates to whether ART services of acceptable quality are available to a person or couple, and their ability to access these services from an economic and sociocultural viewpoint (Adamson *et al.* 2018, Dyer *et al.* 2020).

The geographic availability of ART services effectively speaks towards the number and placement of ART centres available to a given population. Although participation in SARA is not mandatory, the data provided is believed to be representative of most of the country's MAR provided, especially the 2019 report. The registry reports indicate an increase in reporting from 16 centres in 2015 to 19 centres in 2019, of a total of 23 known ART units in South Africa (SARA 2019, 2024). In the 2015 report, the SARA group indicated that only four of the 16 centres reported performing 500 ART cycles or more per year, but there is no indication whether any of these centres were in the public sector (SARA 2019). However, it is known that ART in the private sector is dominant in South Africa and that the public sector units are most likely not among those performing more than 500 ART cycles per year (Connolly *et al.* 2021, Whittaker *et al.* 2024). Only three participants in the national ART registry publications are from public health sector academic hospitals, situated in two of the country's nine provinces, with one in the Steve Biko Academic Hospital (SBAH) in the Gauteng province, and the other two in the Groote Schuur and Tygerberg Hospitals, respectively, both in the Western Cape province (SARA 2019, Government of South-Africa 2024). The SBAH unit is located north of the central region of the country, while the other two ART public service providers are situated close to the southernmost point of the country, approximately 1,450 km from SBAH, as calculated using Google Maps (Huyser & Boyd 2013). Patients from any of the other seven provinces in South Africa are required to travel through at least one provincial border to access ART services in the public sector (Government of South-Africa 2024). In a country of 1,219,813 km^2, domestic travel implicates substantial distances to cover (Government of South-Africa 2024). Travelling from any of the other provinces ranges from 265 to 1,743 km to reach a public sector ART unit (Government of South-Africa 2024).

Apart from the well-documented lack of ART centres in the public sector of South Africa, the affordability of reproductive health screening and subsequent ART procedures should also be considered. South African medical professionals working in the field of MAR agree that access to ART should be increased (Whittaker *et al.* 2024). In Sub-Saharan Africa, the cost of MAR is often named as one of the major obstacles to overcome (Adamson *et al.* 2018, Botha *et al.* 2018, Dyer *et al.* 2020, Connolly *et al.* 2021, Njagi *et al.* 2023, Whittaker *et al.* 2024). According to studies by Dyer and colleagues (2013 & 2017), the cost of *in vitro* fertilisation (IVF) at one of the public sector ART centres in South Africa represents a calculated catastrophic cost (an expense that is so much more than a household can afford that it threatens the household's financial survival) towards out-of-pocket payment for IVF in 20% of patients in general, and this

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increases to 50% of patients when considering the poorest third of the population only (Dyer *et al.* 2013, 2017). This does not even take into account the cost of explorative procedures to determine the couples' aetiologies.

Expenses for investigative and therapeutic procedures may differ considerably between the public and private sectors, with occasionally subsidised MAR treatments in the public sector for low-income patients who are South African citizens without medical insurance (Matsaseng 2016, Connolly *et al.* 2021). The measure and type of subsidisation for ART procedures in the public sector differ across provinces and are based on provincial budget allocations. In addition, national and provincial human resource management, i.e. moratorium on posts, salary adjustments, retention of skills and infrastructure, minimises patient intake artificially. ART subsidisation therefore fluctuates annually and is also impacted by the different levels of ART procedures offered by the four different public sector facilities. Patients are indirectly subsidised through the coverage of infrastructure costs and salaries by public sector facilities, which can account for as much as 29% of the procedural cost according to one publication (Matsaseng 2016). Patients are exempted from having to cover these hospital expenses, or there is a stratified hospital fee that takes the patients' income into account (Matsaseng 2016, Gauteng Department of Health 2023, Karaga *et al.* 2023). Direct subsidisation to patients usually includes a co-payment by patients, but the amounts and manner of subsidy changes from facility to facility and between provinces (Matsaseng 2016, Dyer *et al.* 2017, Gauteng Department of Health 2023). In general, even though the proportions differ, the subsidisation of MAR in the South African public sector allows for some patients to pay less for the facility fee at the hospital, while having to cover most, if not all, of the medication cost and laboratory fees from their own out-of-pocket funds (Matsaseng 2016, Dyer *et al.* 2017, Gauteng Department of Health 2023). The medication and laboratory fees can contribute to a significant proportion of the total ART cost, with laboratory fees reported to contribute as much as 35–48% of the direct expenses related to ART, while medication accounts for another 28% (Huyser & Boyd 2013).

At the time of the case study, the policy in the Gauteng province was that subsidisation of hospital fees, including ART cost, was calculated according to the patient's income. Patients are classified into one of four categories, namely full payment (PP/PH/PF/PM), subsidised (H1/H2/H3), receiving free services (H0) or exempted to pay any fees (HG), according to the Gauteng Department of Health 'Patient Classification Policy Manual' (Gauteng Department of Health 2023). A patient is required to supply documentation during the initial registration to declare their level of income, i.e. a payslip/bank statement or complete a declaration of income form, or, if unemployed, provide an affidavit to this effect. This information is used to assign them to the relevant subsidisation categories (and subcategories). Full-paying patients are those whose annual income is above a set threshold for the current year, or are externally funded, not South African (with some exceptions) or have private medical insurance. Subsidised patients are grouped from H1 to H3, with patients in these categories receiving an 80%, 80% & 70% (H1, H2 & H3, respectively) subsidisation of costs associated with primary health care services. Although H1 and H2 categorised patients are eligible for the same percentage of subsidisation, H1 patients are subsidised for a more comprehensive list of medical procedures than H2 patients, listed as tariff categories described in the Gauteng Department of Health's 'Uniform Patient Fee Schedule'. In addition, patients classed as H0 and HG are exempted from paying for primary health care services and include, but are not exclusive to, patients without medical insurance who receive pension or social grants, are pregnant, less than 6 years of age or fall within the other criteria described in the policy manual. Patients are required to provide updated information yearly to review their classification status (Gauteng Department of Health 2023). These subsidies can relate to a substantial, almost 80%, reduction in the clinical and laboratory procedural fees for transvaginal ovarian aspiration, embryo culture and transfer. However, the subsidies exclude the payment of medication for controlled ovarian hyperstimulation, a significant portion of the total cost of ART (Njagi *et al.* 2023). Therefore, even when subsidised, the out-of-pocket costs for patients to bridge the financial gap from diagnostic to ART procedural costs may still be beyond the means of many households (Connolly *et al.* 2021, Njagi *et al.* 2023). Sampling of a small number from a middle-to-lower income group of patients at a public sector ART centre in South Africa indicated that the average income of patients attending the centre for diagnostic purposes only is 38% less than those continuing to therapeutic ART procedures (Huyser & Boyd 2013).

Through the understanding of these challenges, policymakers and healthcare professionals can work towards developing strategies to improve access to MAR in South Africa (Whittaker *et al.* 2024). The impact of the cost of ART provision in the public sector, which evidently impedes access to therapeutic ART services, requires scrutiny. This is underlined by the opinions expressed by medical professionals in the field of MAR in South Africa, who all agree that better access to ART is needed (Whittaker *et al.* 2024). To achieve the impact of ART expenses on access to MAR, we performed a retrospective study spanning 6 years at one of the three public sector hospitals in South Africa offering MAR. These data represent a case study focused on a single hospital in South Africa. However, this facility is the only public sector hospital providing MAR services in the northern half of the country and is known to perform the most ART cycles of all the public sector hospitals in South Africa. This facility's data can therefore account for more than half of the country's reported public sector ART

activities (Karaga *et al.* 2023) and the authors consider the outcomes to be representative of a substantial proportion of MAR in the public sector of South Africa.

The demographic profiles of patients who entered the public health sector seeking assistance to become pregnant were compared, including income class, region or province of residency and access to medical insurance. The patients were categorised as those who underwent diagnostic investigations only vs those who also returned for therapeutic procedures, to determine the difference in demographic profiles between the two groups.

Materials and methods

Recent provision and usage of ART services in the public sector of South Africa were explored retrospectively, with all information extracted from patient files at the Reproductive Biology Laboratory, SBAH, Pretoria, Gauteng, South Africa. The University of Pretoria's Faculty of Health Sciences Research Ethics Committee approved the project as part of a comprehensive investigation into ART provision to marginalised patients (REC 149/2021).

The registration history of couples who initiated an investigation into their reproductive health status at SBAH was investigated (n = 1,679; first diagnostic visit 01 January 2015–31 December 2020). Demographic data (Fig. 1) were captured from clinical files, including i) income according to combined monthly income, then grouped per income class (low: €350, middle: €350–1,495, high: >€1,495), ii) qualification for subsidised care at SBAH, iii) access to medical insurance by either one of the partners and iv) the distance travelled to SBAH (<50 km, 51–100 km, 101–250 km, >250 km). Income groupings were calculated based on the study population's quartile ranges according to the couple's combined income as disclosed to the ART unit during their first visit. Private medical insurance classification was included as informative data supportive of the patients' income classification. Payment for ART was not covered by any medical aid scheme in South Africa over the time period of this research study, as the first medical aid to provide this benefit launched it in January 2021 only (Discovery Health 2021). The selection of distance groupings was to include patients from within the same metropolitan area in the first category, patients from outside this area but from the same province in the second category, patients from adjacent provinces in the third and all others in the last category. In addition, the couples' nationalities (South African or non-South African) were recorded for commentary purposes. However, since income and distance to travel were calculated from a quoted South African income and residing address, nationality was not considered when analysing demographic comparisons.

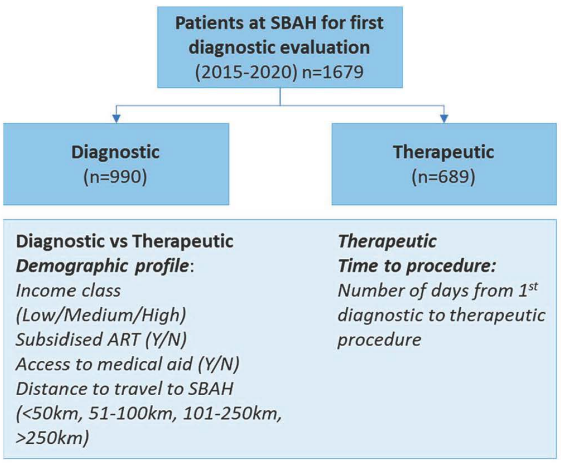

Figure 1

Allocation of patients to the diagnostic and therapeutic groups, based on the return for therapeutic procedures, with an indication of all demographic criteria evaluated.

The couples' initial diagnostic visit date and first therapeutic procedure date (if applicable) were captured, and the study population was grouped according to those with diagnostic evaluations only (not proceeding to therapeutic ART procedures; diagnostic group, n = 990) vs those that did return for therapeutic ART services (therapeutic group, n = 689). During data capturing, a 12-month cut-off time was considered sufficient to allow opportunity for patients to return for follow-up procedures, whereby patient recording stopped at the end of December 2020, and their return for therapeutic procedures was captured up to the end of December 2021. Within the therapeutic group, the time in number of days from initial diagnostic evaluation to first therapeutic procedure, termed time to procedure (TTP), was calculated. Using this information, demographic comparisons were made and discussed between various subpopulations.

The study population was split into two groups (diagnostic and therapeutic), for which one continuous variable (patient income), one ordinal variable (distance to travel) and two categorical variables (access to subsidy and medical insurance) were considered. Furthermore, the patients' income groupings, which were calculated according to the study's quartile ranges, were used for descriptive purposes and considered as an ordinal variable. Variables were compared using the Student's 2-sample t-test and mean values with standard deviations reported, while the ordinal and categorical variables were evaluated using the Wilcoxon rank-sum and Fisher's exact tests respectively, and data reported as frequencies and percentages. Nationality was noted as additional information. Nonetheless, most non-South African patients supplied a South African residential

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address, and the entire study population was considered as a group. Statistical analyses were performed by a biostatistician from the University of Pretoria (School of Medicine, Faculty of Health Sciences) using STATA 17 statistical software.

Results

A summation of the parameters investigated for the entire study population can be seen in Table 1, with the overall average income reported, as well as the average income per classification group, in Euro. The study population's average monthly income of €1,337.58 was observed to be similar to the 2018 South African average monthly income of €1,344.91 (Natalie 2023). All values reported in Euros are derived from South African Rand converted by ZAR16.016:€1 as per the average exchange rate from 2015 to 2020 (Nedbank Group Economic Unit 2024). Since the patient income groupings were calculated based on the study population's quartile ranges, the average income of the three groups differed significantly ($P < 0.001$), as would be expected. A substantial difference was calculated between the low- and high-income groups' average earnings, of which the former, at €209.26 per month, was a mere 7.2% of the latter's €2,905.76 per month. The other parameters are reported as the total number of patients per subcategory, as well as the percentage breakdown of each category. Patients were predominantly South African, with 80% of couples indicating both partners as South African nationals.

Table 1 Summary of patient groups according to demographic categories. Data are presented as mean ± SD or as *n* (%).

Categories	Values
Income in euros	
All	1,337.58 ± 1,306.75
Low	209.26 ± 145.29
Middle	1,019.31 ± 455.06
High	2,905.76 ± 1,555.54
Income class	
Low	358 (21%)
Middle	884 (53%)
High	437 (26%)
Subsidised IVF	
Yes	797 (47%)
No	882 (53%)
Access to medical insurance	
Yes	706 (42%)
No	973 (58%)
Distance to travel	
<50 km	728 (43%)
51–100 km	564 (34%)
101–250 km	212 (13%)
>250 km	175 (10%)
Nationality	
Both RSA	1,348 (80%)
Male RSA, female foreign	43 (3%)
Male foreign, female RSA	57 (3%)
Both foreign	231 (14%)
Returned for therapeutic procedure	
Yes	689 (41%)
No	990 (59%)

Higher patient income is known to provide access to medical insurance opportunities, where financial resources enable a person to afford higher insurance brackets (Discovery Health 2021). In addition, according to the subsidisation policies, this parameter is directly linked to the patients' income level. The relationships were confirmed in the study population with Pearson's correlation testing, indicating that there are significant medium relationships between income and access to medical insurance (positive relationship, $r = 0.37$, $P < 0.001$) and subsidisation (negative relationship, $r = -0.47$, $P < 0.001$). The same relationships were also observed when considering income according to the subgroupings, with 71 and 6% of patients in the high- and low-income groups having medical insurance, respectively. In Fig. 2, a radar chart depicts the distribution of patients with and without medical insurance as plotted over the three income categories. The two-dimensional chart displays the multivariate data on axes starting from the same mid-point. In the middle-income group, access to medical insurance was evenly distributed, with 57% of patients in this category presenting with medical insurance. A similar trend was observed in the subsidised IVF group classification (see Fig. 3), where 94.48% (753 of the 797) of eligible patients were from the low- and middle-income groups, while 93.88% (828 of the 882) of those who did not qualify for a subsidy were from the middle- and high-income groups. A significant difference ($P < 0.001$) was noted between the income groups when considering those who do not receive subsidised ART but have access to

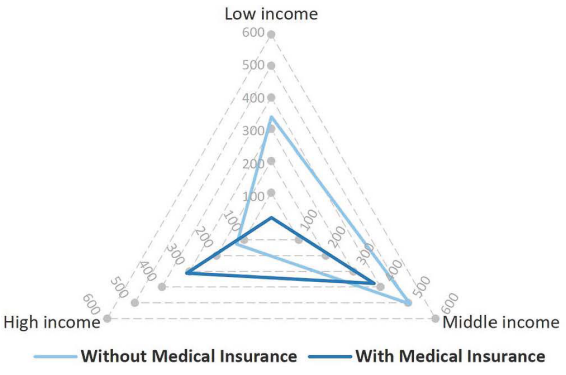

Figure 2

Radar chart (range 0–600) of patient numbers according to the triage of patients' income classification (high, middle or low) vs their access to medical insurance.

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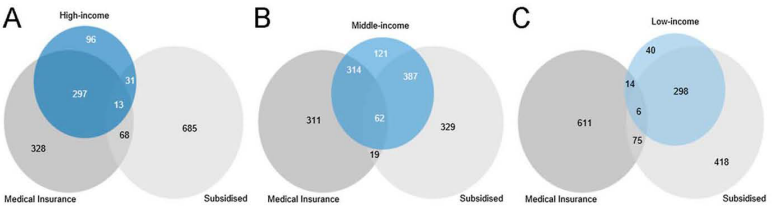

Figure 3

Venn diagrams of patient numbers showing the overlaps between access to medical insurance and subsidised ART procedures as well as (A) high-, (B) middle- and (C) low-income patients.

medical insurance (68%:36%:4% for high-, middle-, and low-income, respectively; Fig. 3). Due to the relationship between income and the two variables access to medical insurance and subsidisation being driven by income, further analyses used income as a single variate, with the assumption that the same relationships with income would be in place with access to medical insurance and subsidisation.

An inverse association was observed in the study population between the number of patients and travelling distance categories (n = 728 patients: <50 km; n = 564: 101–250 km; n = 212: 101–250 km; n = 175: >250 km). This was especially more noticeable in the diagnostic vs the therapeutic group, as shown in Fig. 4.

Comparing the diagnostic and therapeutic subpopulations, 689 couples (41% of the overall study population) returned for therapeutic procedures, as seen in Table 1, with a summary of the patients' demographic distributions for the two groups seen in Table 2. A significant difference (P < 0.001) was seen in the return rate of patients who were or were not eligible for subsidised ART procedures (35 and 46%, respectively; Table 2). Similarly, patients from the low-income class who do not have medical insurance were less likely to return for therapeutic ART procedures than their high-income counterparts with medical insurance (30 vs 52%, P < 0.001; Table 2). The total number of patients in the therapeutic and diagnostic groups

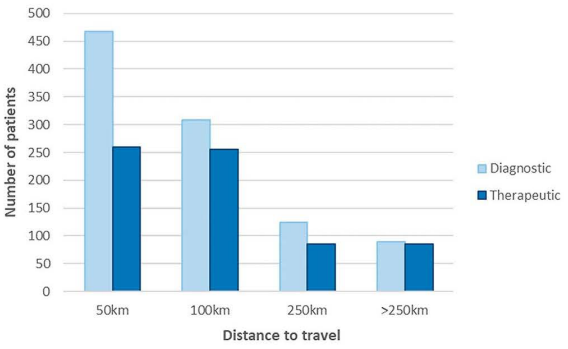

Figure 4

Graphical representation indicating a decrease in patient numbers as distance to travel increases, per subcategory diagnostic and therapeutic.

within specific income classifications, grouped according to the distance to travel, is indicated in Table 3. An income histogram (€200 brackets) for the diagnostic and therapeutic groups is displayed in Fig. 5, emphasising that patients within the diagnostic group are mostly earning below the study population's average monthly income of €1,337.58. Patients who earn less than the study population's average monthly income did not return for therapeutic procedures in 75% of cases, compared to 49% of cases when patients earn above the average (P < 0.001).

The relationships between the patients' likelihood to return for therapeutic procedures, their income and the distances that must be travelled were also considered. A small, significant positive relationship (r = 0.11, P < 0.001) was observed between income and the patients' return for therapeutic procedures. In addition, a very small, significant positive relationship (r = 0.06, P = 0.013) was identified between the distance to travel and return for procedures. No significant relationship was observed between the patients' income and the distance they travelled.

Within the therapeutic group, the average time to procedure (TTP) was 240 days from the patients' first diagnostic evaluation to the first therapeutic procedure.

Table 2 Distribution of patients according to diagnostic and therapeutic groupings with reference to demographic categories income class, subsidisation, access to medical insurance and distance to travel.

	Diagnostic	Therapeutic
Income class		
Low	252	106
Middle	521	363
High	217	220
Subsidised IVF		
Yes	518	279
No	472	410
Access to medical insurance		
Yes	371	335
No	619	354
Distance to travel		
<50 km	467	261
51–100 km	309	255
101–250 km	125	87
>250 km	89	86

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Table 3 Number of patients in the diagnostic and therapeutic groups, subdivided (i) per income group, (ii) distance to travel and (iii) an indication of the percentage diagnostic vs therapeutic patients per category.

	0–50 km		51–100 km		101–250 km		>250 km	
	Diagnostic	**Therapeutic**	**Diagnostic**	**Therapeutic**	**Diagnostic**	**Therapeutic**	**Diagnostic**	**Therapeutic**
Low income	138 (78%)	38 (22%)	79 (65%)	43 (35%)	23 (61%)	15 (39%)	12 (55%)	10 (45%)
Middle income	233 (65%)	127 (35%)	161 (53%)	140 (47%)	76 (62%)	47 (38%)	51 (51%)	49 (49%)
High income	96 (50%)	96 (50%)	69 (49%)	72 (51%)	26 (51%)	25 (49%)	26 (49%)	27 (51%)

With a median of 171 days, the distribution of patients' TTP in four quartiles (<103, 104–170, 171–303, >303 days) is displayed per income group in Fig. 6 and per distance to travel category in Table 4. Patients from the low-income group returned for therapeutic procedures after more than the study population median of 171 days in 67% of cases, while only 42% from the high-income group took this long (P < 0.001). The relationships between TTP, income and distance to travel showed a small but significant negative relationship (r = 0.12, P = 0.002) between TTP and income, while there were no significant relationships between TTP and distance to travel or income and distance to travel.

Discussion

Data from the current case study confirmed that a large disparity exists in earnings of patients frequenting a public sector ART unit. The inequality in the income of the low-income group, earning only 7% of the wages of the high-income group, showcased the severity of the situation. The patients' access to medical insurance and eligibility for subsidised ART procedures is linked to their monthly income, with the high- and low-income groups being representative of the patients with and without medical insurance, as well as not being subsidised and being subsidised respectively. The data indicated that patients in the low- income group, who are eligible for subsidisation and do not have access to medical insurance, have an approximately 70% drop-out rate to proceed from diagnostic to therapeutic procedures. In comparison, patients from the high-income group, who do have medical aid and are not eligible for subsidisation, return for therapeutic procedures in more than 50% of cases. Considering this difference in the number of patients returning for therapeutic procedures, a compelling argument can be made that the lack of funds and the cost of procedures have a direct impact and influence on patients' decisions. The failure to return for therapeutic procedures is not necessarily an abandonment of the patients' fertility journey. Some of the non-returns might be due to patients transferring to another fertility unit, either in the public or private sector, or deciding to adopt a child (Wiersema *et al.* 2006, Maxwell *et al.* 2018, Weis 2021). With patients who earn less than the study population's average monthly income not returning for therapeutic procedures more often than patients who earn above average, the cost of procedures might be a factor when patients decide to return for MAR therapy. This is especially concerning when considered that 61% of patients earn below the study population's average of €1,337.58.

Apart from being less likely to return for therapeutic procedures, low-income patients who do return for therapeutic procedures tend to progress slower to MAR therapy than their high-income counterparts. This can be witnessed via the incidence of a significantly higher

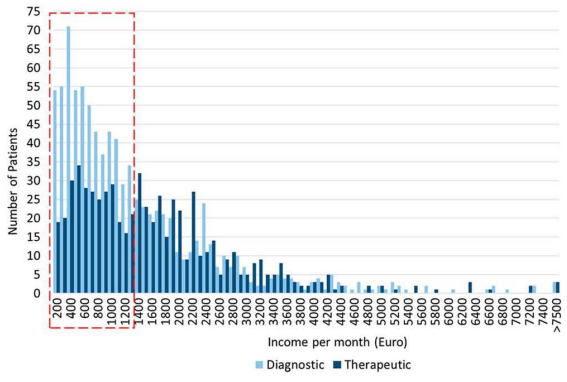

Figure 5

Number of patients in the diagnostic and therapeutic groups with income in €200 brackets. Patients below the population average indicated in a red block.

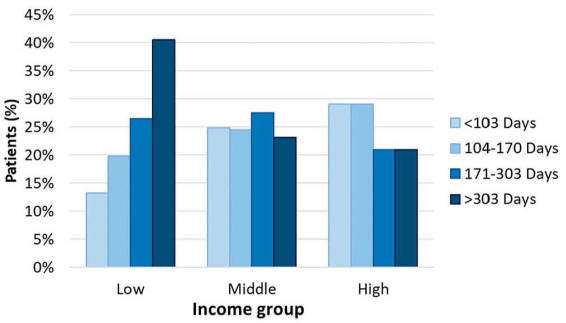

Figure 6

Therapeutic patients' time to procedure according to four quartiles, per income group.

Table 4 Number (%) of patients returning for therapeutic ART services per time to procedure quartile (days) plotted over the distance travelled, with an indication of the percentile distribution per distance category.

Time to procedure	0–50 km	51–100 km	10–250 km	>250 km
<103 days	53 (20%)	70 (27%)	22 (25%)	29 (34%)
104–170 days	71 (27%)	64 (25%)	22 (25%)	11 (13%)
171–303 days	65 (25%)	60 (24%)	22 (25%)	28 (33%)
>303 days	72 (28%)	61 (24%)	21 (24%)	18 (21%)

percentage of patients from the low-income group returning for therapeutic procedures after more than the study population median time than from the high-income group. One might consider that patients from the low-income group require more time to budget and obtain funds to afford the necessary treatment, which is oftentimes an expense they cannot recover from (Dyer *et al.* 2017).

When considering the sensitive nature of MAR, a female's optimal period to conceive is directly and inversely linked to her age (Liao *et al.* 2019, Pantos *et al.* 2020, Yang *et al.* 2023). According to Pantos *et al.* (2020), a single year's difference in age relates to a statistically significant decrease in positive hCG and clinical pregnancy rates in patients 35 or 36 years old. With the study population's average time between therapeutic procedures already being almost 6 months long, any delay causing a patient to wait longer than this to return for a therapeutic procedure, as is seen in more than half the cases in the low-income group, could have a negative impact on the patient's probability to achieve a pregnancy. In addition, according to Speksnijder *et al.* (2024), the cumulative ongoing pregnancy rate after IVF of patients from lower socioeconomic environments was lower than their higher socioeconomic counterparts. This finding was based on the cumulative ongoing pregnancies of their study group in the Netherlands using a neighbourhood socioeconomic status calculation. Applying these two detrimental factors, increasing age and reduced cumulative ongoing pregnancy rate, a low-income patient who takes more than a year or two to return for the needed therapeutic intervention would have a cumulative diminished probability of a successful outcome. The high cost of MAR has an impeding effect and causes low-income patients to take longer to fulfil their wishes to become parents, and in some cases, denying them from achieving this goal at all.

An interesting phenomenon is seen when considering the combination of the patient's income class and distance that they must travel for MAR. Patients from the lower income groups and with the shortest distance to travel (<50 km) do not return for therapy more often than those with the greatest travel distance (>250 km), showing a prominent decreased therapeutic return rate in the group with the least distance to travel. The necessity of multiple visits to an ART centre during a single ART cycle, combined with the lack of geographic accessibility of ART units, implies additional cost for travel and accommodation. The build-up of expenditures may be a significant and possibly unsurpassable extra expense for these patients (Huyser & Boyd 2013, Karaga *et al.* 2023). The shift in patient numbers might be an indication that patients who are required to travel further are less likely to initiate any form of MAR investigations, while those who initiate a MAR journey are committed and budgeted for the necessary expenses required for therapeutic procedures. The significant distance decay in patient numbers again reiterates the fact that more ART centres are needed in close proximity to be reasonably accessible.

The data reflects upon and is a reality check for a public sector hospital in South Africa, but the loss or fallouts emphasise inequalities relevant for all areas where MAR is not readily available or subsidised. This includes most middle- to low-income countries, especially those in Africa (DeWeerdt 2020, Afferri *et al.* 2022, 2024, Archary *et al.* 2023, Njagi *et al.* 2023, Seiz *et al.* 2023). Therefore, this is a call for action for MAR to be made more accessible to patients from lower income groups. The need for access to MAR to be increased is uniformly agreed upon by South African medical professionals in the field of MAR, but the way to do so is not so easy to agree upon (Whittaker *et al.* 2024). Strategically, this could be achieved through i) increasing support by means of subsidised treatment cycles, ii) reducing the cost of MAR and iii) increasing the number of IVF centres and medical professionals trained in MAR to reduce travel distances and associated expenses (Ombelet & Onofre 2019, Chiware *et al.* 2020, Afferri *et al.* 2024, Whittaker *et al.* 2024).

Some of these barriers might be overcome by the use of telemedicine, which has improved dramatically due to the Covid-pandemic, while keeping to high practice standard of care (Townsend *et al.* 2020, Prinsen 2023, Tran *et al.* 2024), or through basic investigative diagnostic procedures at remote clinics. Access to ART can be promoted through simplified diagnostic and ART procedures (Ombelet *et al.* 2025). For one such method, embryos cultured in the Walking Egg simplified IVF culture system have been reported to perform the same, and better, in the lab than sibling embryos resulting from ICSI and conventional culture when comparting fertilisation rate, with similar ongoing pregnancy, implantation and miscarriage rates after embryo transfer (Ombelet *et al.* 2022*b*). This embryo culture system is described to be effective for most patients in need of ART, only excluding those with moderate to severe male factor infertility or known fertilisation failure after IVF (Van Blerkom *et al.* 2014, Ombelet *et al.* 2025). The perinatal outcomes of babies born from this system were also compared to babies resulting from conventional culture in the same laboratory in Belgium (Ombelet *et al.* 2022*a*), singleton

babies born from IVF/ICSI cycles in Belgium (Ombelet *et al.* 2023*b*) and all singleton babies born in Flanders, Belgium (Ombelet *et al.* 2023*a*). On all accounts the preterm birth and low birth weight rates were found to be favouring the simplified culture system, with similar (Ombelet *et al.* 2023*a*), if not better, results reported (Ombelet *et al.* 2023*a*,*b*). In addition, mild ovarian hyperstimulation has been shown to be an effective treatment option for good prognosis patients (Gianaroli *et al.* 2022, Nargund *et al.* 2022). Combining a low-cost simplified IVF culture system with mild ovarian stimulation could dramatically reduce the cost to patients for MAR, making MAR even more accessible to a large proportion of patients (Nargund *et al.* 2022, Ombelet *et al.* 2025).

Combining these strategies with the implementation of mobile ART laboratories can provide patients services at multiple medical facilities without the financial cost of setting up a laboratory at each facility (Büsing *et al.* 2021, Kroes *et al.* 2019, Kushnir *et al.* 2022,Ombelet *et al.* 2025). The reduced setup cost for a simplified IVF culture laboratory, further decreased through the shared costs by multiple facilities, would favourably lower patient fees. The use of a mobile ART laboratory is an academic exercise at this point (Ombelet *et al.* 2025). For the use of this type of laboratory to be implemented, a feasibility study would have to be performed, to establish the practicality of this plan. This would have to consider the necessary infrastructure requirements, based on the type of laboratory, availability of disposable items and consumables, and cost effectiveness to set up and operate this vehicle with sufficiently trained staff. The deployment and type of mobile laboratory should be carefully considered, to take into account the availability and willingness of trained medical professionals to work independently but within a team in such a mobile laboratory environment. When sufficient qualified health professionals are not available, which might well be the case in LIMC, training facilities should be established or current training programmes expanded to include the use of mobile laboratories in their repertoire. Experienced independent and self-employed consultants could also upskill to manage a mobile ART laboratory and be available to assist in the batching of patients on a regular basis. Furthermore, regulatory differences between countries and areas necessitate knowledge of the local legal requirements before the placement and operation of mobile laboratories.

In South Africa, a National Health Insurance (NHI) Act was accepted in 2023 and the Bill signed into law by the President of the Republic on the 15th of May 2024 (RSA Parliament 2023, National Department of Health 2024, RSA Government 2024). After signing the NHI Bill into law, the inevitable health system reform that should follow such a major policy change provides the ideal environment to address inequities and disparities to infertility treatment (Whyle & Olivier 2024). The aim of the NHI Act is to provide South Africa with universal access to health care services, as described in the NHI White Paper and the Constitution of South Africa (RSA Parliament 2023, RSA Government 2024). The NHI Fund will be the main purchaser and payer of healthcare services, with a defined package of services covered. For treatments and medicine not covered by the NHI, private medical insurance companies may still provide complementary cover while patients without medical insurance will have to pay for these services out-of-pocket (RSA Government 2024).

The NHI Act states that 'in terms of section 27(1)(a) of the Constitution everyone has the right to have access to health care services, including reproductive health care' (RSA Government 2024). While this has the potential to improve access to reproductive health services, infertility treatments are not explicitly listed as a covered service and the extent to which infertility treatments will be covered remains unclear (RSA Government 2024). Historically in South Africa, the focus on sexual and reproductive health has often been more on family planning to avoid unwanted pregnancies and on abortion than to assist patients who require MAR (Cooper *et al.* 2016, Lince-Deroche *et al.* 2016, Lince-Deroche *et al.* 2019). The gathering of commentaries and current discussions after the signing of the NHI Bill is the ideal time for stakeholders to act and ensure that access to MAR, and the lack thereof, is noticed by policy makers while there is a chance to address this inequity. This should be expanded further, to address the international requirement to address the problem of MAR being inaccessible to all and overly expensive (Kroes *et al.* 2019, Kushnir *et al.* 2022, Wang 2022, The Lancet 2024). Subsidisation of MAR needs to be addressed at the level of policy makers for regions with limited resources and large income disparities (Botha *et al.* 2018, Chiware *et al.* 2020, Connolly *et al.* 2021, Njagi *et al.* 2023). Furthermore, to reduce the cost of MAR, both diagnostic and therapeutic procedures should be simplified and affordable. This is achievable through low-cost IVF processes with mild ovarian stimulation (Gianaroli *et al.* 2022, Nargund *et al.* 2022) in tandem with subsidised medication (Njagi *et al.* 2023), combined with simplified IVF systems (Christiaens 2018), telemedicine and the use of mobile IVF laboratories (Kroes *et al.* 2019, Kushnir *et al.* 2022).

Declaration of interest

The following interests are relevant: the data presented are part of a larger study performed during the principal author's PhD research. All the authors are associated with the Walking Egg non-profit organisation. W Ombelet is an Associate Editor of *Reproduction & Fertility* and was not involved in the review or editorial process for this paper, on which he is listed as an author.

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Author contribution statement

The principal author contributed to all aspects of the research, including data gathering, processing and write-up. All the authors were involved in development of the research plan, conceptualising of information and write-up of the article.

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Chapter 4: Point-of-care Assisted Reproduction: development of a mobile IVF laboratory

In the research background and review, the lack of sufficient ART facilities in South Africa, as well as the impact of ART cost drivers on access to MAR, has been discussed. These factors create a barrier for patients to access needed medical attention when they struggle with infertility. In Chapter 3, this was further investigated for patients in the public sector of South Africa. The demographic profiles of patients attending the RBL, SBAH was considered and the results demonstrated that patients with lower incomes are more likely to not return for the ART treatment required. Furthermore, when they do return, the patients from the low-income brackets take longer to proceed from diagnostic to therapeutic ART. There appears to be a direct inverse association between a patient's income and their progression to ART in a timely fashion. This indicates that the cost of ART, even in the public sector, is still an obstacle to patients' access to the required ART procedures.

The challenges of too few ART facilities and expensive costs may be addressed through the use of mobile IVF laboratories. Although mobile medical facilities can be seen in many different fields, the provision of ART treatment is not yet available, and this proposed mobile IVF laboratory would be a world first. Chapter 4 presents the design and manufacture of such a mobile IVF laboratory prototype, as was the second objective of the project. The process of selecting an appropriate vehicle for the mobile laboratory, the necessary equipment, as well as the eventual construction, installation and testing of the mobile laboratory, is presented in manuscript format.

The manuscript titled "Point-of-care Assisted Reproduction: development of a mobile IVF laboratory" has been submitted to the South African Medical Journal (proof of submission in Annexure E). This journal is a peer reviewed, general medical journal publishing leading research impacting clinical care in southern Africa. The selection of this journal was to promote the visibility of this world-first design, of a mobile IVF laboratory, in a local journal.

Title: Point-of-care Assisted Reproduction: development of a mobile IVF laboratory

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Abstract:

Access to assisted reproduction does not cover the national requirement in South Africa and, where available, is very costly. To offer an alternative approach to this problem, a mobile *in vitro* fertilisation laboratory was designed, constructed, and tested. The laboratory was intended to utilise the Walking Egg simplified embryo culture system, a robust, closed *in vitro* fertilisation system. The laboratory design included all necessary equipment to perform semen processing, embryo culture, transfer and embryo. The design and construction of the laboratory is discussed in detail, highlighting the major steps in the process and setbacks encountered.

After construction and installation of equipment was completed, the laboratory underwent various quality control procedures. Lastly, a pilot study with abnormally fertilised oocytes was performed to assess the embryo culture environment in the laboratory. The cleavage and blastulation rate of embryos derived from abnormally fertilised oocytes were compared with those of sibling oocytes in a conventional *in vitro* fertilisation laboratory. The embryo development of the test group surpassed the minimum criteria of 50% that of the control group, confirming that embryo culture in the mobile laboratory is possible. In the final discussion and conclusion, the potential use of the mobile laboratory is considered, with reference to perform assisted reproduction cycles at multiple facilities.

Introduction:

What can be done to improve access to Assisted Reproduction Treatment (ART) in South Africa? Less than 10% of the required ART cycles necessary are being offered in South Africa,[1] and the country is ranked as one of the three most economically unequal regions in the world.[2] Therefore, combining a service that is not readily available with the disproportionate distribution of such services in an unequal society, cause restrictive access to ART services in South Africa and a hefty price-tag.[3, 4]

Could a mobile IVF laboratory, used in conjunction with a low-cost IVF system, be the answer to provide access and cut costs of treatment? According to Whittaker *et al.* (2024), some medical professionals are of the opinion that mobile medical units for assisted reproduction could improve accessibility.[5] The use of mobile medical facilities have increased considerably in the past decades.[6-9] This enable medical interventions in a variety of settings, whereby the modified vehicles are stocked with specialized equipment to provide medical services to out-of-reach areas and are employed in various medical disciplines.[6, 7, 9] In India, mobile medical units are used to provide family planning and contraceptive services, whereby access to reproductive and child health services were improved.[6, 10] In South Africa, mobile medical units are used to offer some reproductive health services in rural communities, such as screening for sexually transmitted infections, dispensing contraceptives, and providing information, advice and support.[11, 12] However, no information is available on any mobile units offering assisted reproductive services.

Weighing up costs in South Africa, an ART treatment in 2025 can range from R30 000 to R110 000,[13, 14] with significant differences between the public and private sectors.[15, 16] These amounts increase significantly with the additional procedural costs for more advanced procedures, such as intra-cytoplasmic sperm injection (ICSI) and pre-natal genetic testing.[17] The laboratory fee portion of this cost can amount to up to 48% of the procedural cost, calculated from the sum of culture media and other disposables, as well as the laboratory set-up cost and staff remunerations.[16]

Reducing the cost of ART through laboratory requirements and the implementation of a milder, less costly, ovarian stimulation strategy was addressed as early as 2008 by Ombelet and co-workers.[18] The authors stated that 'Before ART can be made more accessible, many challenges will need to be negotiated [in Sub-Saharan Africa]. These relate to funding,

geographical barriers and to the infrastructure required for ART'.[18] To curtail laboratory fees, an affordable laboratory setup was developed through the Walking Egg (tWE) non-profit organisation (npo) (Genk, Belgium; www.thewalking egg.com).[19, 20] Revisiting the origin of ART where embryos were cultured in tubes (hence the colloquial term test-tube babies), closed-off embryo culture tubes containing the necessary gas and culture media was researched and the tWE simplified embryo culture system was coined.[19] With the use of the simplified embryo culture system, expensive laboratory equipment such as microprocessor-controlled tissue culture incubators and large area air filtration systems are not required.[19] This design significantly reduces the laboratory setup cost, whereby a more affordable ART procedure can be offered to patients in a less expensive laboratory setting.[21]

A cost-benefit analysis of a laboratory using the simplified embryo culture system and a high-technological conventional ART laboratory proved the simplified laboratory to require 50% less seed-funding.[22] Additionally, the cost of disposables and media used for the simplified embryo culture system, were less than a third of the cost of conventional ART with or without ICSI.[22] The design, costs and construction of a mobile laboratory to house the simplified IVF culture system, as well as quality control of the functionality of the system after commissioning, is described in context to ART applications. The project was performed as part of a PhD project focusing on improving accessibility to ART. Ethical clearance for all sections of the PhD study was obtained with approval from both the University of Pretoria's Research Ethics Committee (protocol number 149/2021) and the Hasselt University's Comité voor Medische Ethiek (ref number CME2023/046).

Laboratory design

A structured approach was employed to plan and design the mobile IVF laboratory. Initially, the largest items, including equipment required in the laboratory was identified, followed by a consideration of types of vehicles and appropriateness of designs. After the concept vehicle was identified by all parties involved, the design was finalized, including the selection and placement of equipment. This was followed by the construction and testing of the mobile laboratory.

Minimum space requirement

To determine the main equipment items required for the mobile laboratory, consideration was given to the primary services that would be provided. The focus was to be able to perform IVF

embryo culture in the laboratory, using the tWE simplified culture system, while also considering what other ART procedures cold be accommodated using the same equipment. With the use of the tWE culture system, embryos are cultured in closed, pre-gassed culture tubes containing equilibrated embryo culture medium. Gametes are introduced into these tubes with a needle and syringe and cultured in a warming oven without the need to supply medical grade gas to an incubator (see Figure 1). Based on these principles, an IVF workstation with a stereo microscope, warming oven, and fridge was identified as essential for the mobile laboratory to enable IVF embryo culture.

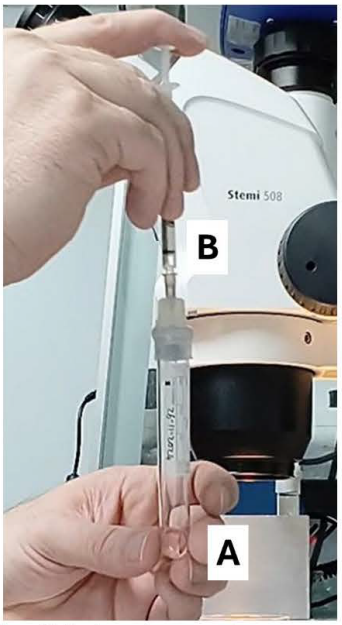

Figure 1: The Walking Egg simplified embryo culture system in use, showing (A) a pre-gassed culture tube containing embryo culture media, with gametes being inserted through the stopper using (B) a needle and syringe.

Additionally, for semen processing before IVF, an upright phase-contrast microscope, Class II Biological Safety Cabinet, and centrifuge would have to be included in the laboratory design. The sperm processing equipment would also allow for standard semen analyses and semen processing for intra-uterine insemination. Additionally, minimum requirements include the cryopreservation of excess embryos, using a liquid nitrogen dewar for storage of the cryopreserved material. Herewith, the thawing of frozen embryos for transfer was added to the possible services that would be provided by the mobile laboratory.

Since the trans-vaginal oocyte aspiration is an invasive procedure with a risk of medical complications, which is usually performed under anaesthesia,[23] the decision was made to exclude this procedure in the design of the mobile laboratory. Therefore, a theatre or procedure room should be available at the host medical facility where the mobile IVF laboratory will be stationed. This will accommodate the oocyte aspiration procedure. For this procedure room, an ultrasound of sufficient quality to visualise the aspiration process would be needed, along with an aspiration pump and gynaecological surgery bed. The ovarian follicular fluid collection would be performed in this area and the tubes in which the fluid is collected should then be transported to the mobile laboratory in a portable warming oven at 37˚C. In the mobile laboratory, the fluid will be screened for oocytes, followed by insemination and embryo culture.

An IVF workstation equipped with a stereo microscope is required for the screening of the follicular fluid, embryo evaluation, the removal of the embryos from the culture tubes, loading into the embryo transfer catheters prior to embryo transfer, cryopreservation, and thawing. The embryo transfer procedure was therefore earmarked to be performed at the mobile laboratory. A gynaecological examination bed and ultrasound was therefore included in the list of minimum equipment for the mobile laboratory. Lastly, to provide clean working conditions, and maintenance of sterility during embryo culture and transfer, the necessity of a wash-up and dressing area in the mobile laboratory was identified.

Based on the equipment and procedures identified (see table 1), the minimum space requirement of the mobile laboratory was (i) a laboratory area large enough to house the equipment listed, (ii) an embryo transfer area, (iii) a dressing room with wash-up facilities, and (iv) sufficient storage space to transport the needed disposable items.

149 Table 1: Equipment identified for a mobile IVF laboratory

Laboratory	
Equipment	**Procedures**
IVF Laminar flow cabinet	Oocyte and embryo evaluations Embryo transfer catheter loading Embryo cryopreservation and thawing
Warming oven	tWE Embryo culture
Medical fridge	Cold-chain disposables for all procedures
Class II Biological Safety Cabinet	Semen analysis Semen cryopreservation
Centrifuge	Semen processing
Upright microscope	Semen analysis
Stereo microscope	Embryo evaluations
Storage cabinet	Disposable storage
Work surface	Administrative work
Transfer room	
Item	**Procedure/Use**
Gynaecological procedure bed	Embryo transfers
Ultrasound	Embryo transfers
Cabinet	Storage of disposables for embryo transfers
Light	Embryo transfers
Dressing room	
Item	**Procedure/Use**
Washing basin	Wash hands
Battery and converter	Solar power back-up
Storage cabinet	General storage
Liquid nitrogen dewars	Cryopreservation and storage

150

151

152 *Vehicle options considered*

153 Mobile medical facilities are used in various forms and levels of mobility. This include fully
154 mobile units that are motorised, which can move autonomously, or towed by another vehicle.
155 Another option was modular units that are prefabricated sections which can be assembled
156 where and when needed. These options were considered and shortlisted to (i) a self-propelled
157 truck with the laboratory built into the cargo box, (ii) a semi-trailer that can be hauled by a
158 truck horse, (iii) a laboratory built inside a shipping container, (iv) a minibus similar to an
159 ambulance, or (v) a trailer that can be towed by a standard sized car. Each of these options was
160 evaluated based on the cost to purchase or manufacture, the amount of refurbishing needed to
161 convert to a mobile medical laboratory, the mobility of the unit, and the space available. A
162 summary of these findings can be seen in Table 2. In the table, the first section indicates the
163 parameters considered colour coded to indicate the most (green) and least (red) ideal options,
164 while the second section shows a visual representation of the type of build for ease of reference.

The costs indicated are shown as determined in 2020 when the laboratory design was performed and provides an indication of the difference in costs between different options, rather than a current price estimate. The construction of the mobile laboratory was funded by the tWE npo. To preserve the concept of affordable IVF, the project budget was indicated to be as low as possible, while maintaining the ability to culture embryos in a safe and reliable environment.

Table 2: Summary of options considered for mobile IVF laboratory (cost as identified in 2020), with photo images of possible design options.

	Cost	Furbished	Mobility	Space
i: Truck	R 1 750 000	Basic	Very high	Limited
ii: Semi-trailer	R 500 000	None	Medium	Large
iii: Shipping container	R 30 000	None	Low	Large
iv: Minibus	R 600 000	Basic	High	Very limited
v: Trailer	R 300 000	Good	Medium	Medium

i: Truck

ii: Semi-trailer

iii: Shipping container

iv: Minibus

v: Trailer

The self-propelled truck (option i) was ruled out due to high purchase cost, while the truck-trailer (option ii) was excluded because of the combination of cost and low mobility, with a truck having to be sourced every time the laboratory will be moved. The shipping container (option iii) was favourably considered as an alternative semi-permanent placement of a laboratory but did not fulfil the requirement as a fully mobile medical laboratory. The minibus (option iv) and trailer (option v) were deemed to be the most likely possibilities. However, the cost of the minibus, combined with the smaller proportions, which would restrict movement and placement of equipment, was identified as possible complications. Lastly, the trailer (option v) was considered to be the most promising solution, providing more space at an

affordable cost. One drawback identified to use a trailer was that a towing vehicle would be needed to move the laboratory, with the size, and weight of the trailer determining the minimum requirements for such a towing vehicle.

According to the space requirements described (laboratory, embryo transfer, and dressing areas), an initial design was identified using a standard trailer, where the necessary equipment could be fitted (see Figure 2a). However, the space available would be severely limited with no dressing area, and no exit from the laboratory area while a patient is in the transfer room. An alternative solution was considered, using a trailer and towing vehicle as a combined unit, where the towing vehicle could be furnished similarly to an ambulance, to act as an embryo transfer room, while the laboratory was custom designed to be accommodated in the trailer (Figure 2b). For the indicated size of trailer needed, a large body type towing vehicle, such as a pick-up truck or sports utility vehicle, with towing capacity of at least 2 tons is required. These types of vehicles can be rented at a daily rate, but to use the towing vehicle as an embryo transfer room, one would have to be bought and refurbished, which would add an additional R1 000 000 to the total price. Lastly, a solution to the limited space problem was identified through the design of a modular trailer. This trailer would create additional space when parked, using collapsable panels that can unfold to form an additional room (Figure 2c), and fold inwards during transport or when not used. The final design also made provision for (i) solar panels to be installed on the roof of the trailer, with sufficient lithium batteries with the capacity to run the mobile laboratory for a full day, in case of a power failure, (ii) a secured cage with air ventilation to place liquid nitrogen canisters, (iii) a water tank on top of the trailer to supply a hand washing basin, and (iv) an alarm system that can send messages to designated phones and emails if there is a power failure, there is movement in the laboratory, a door is opened, or equipment is alarming.

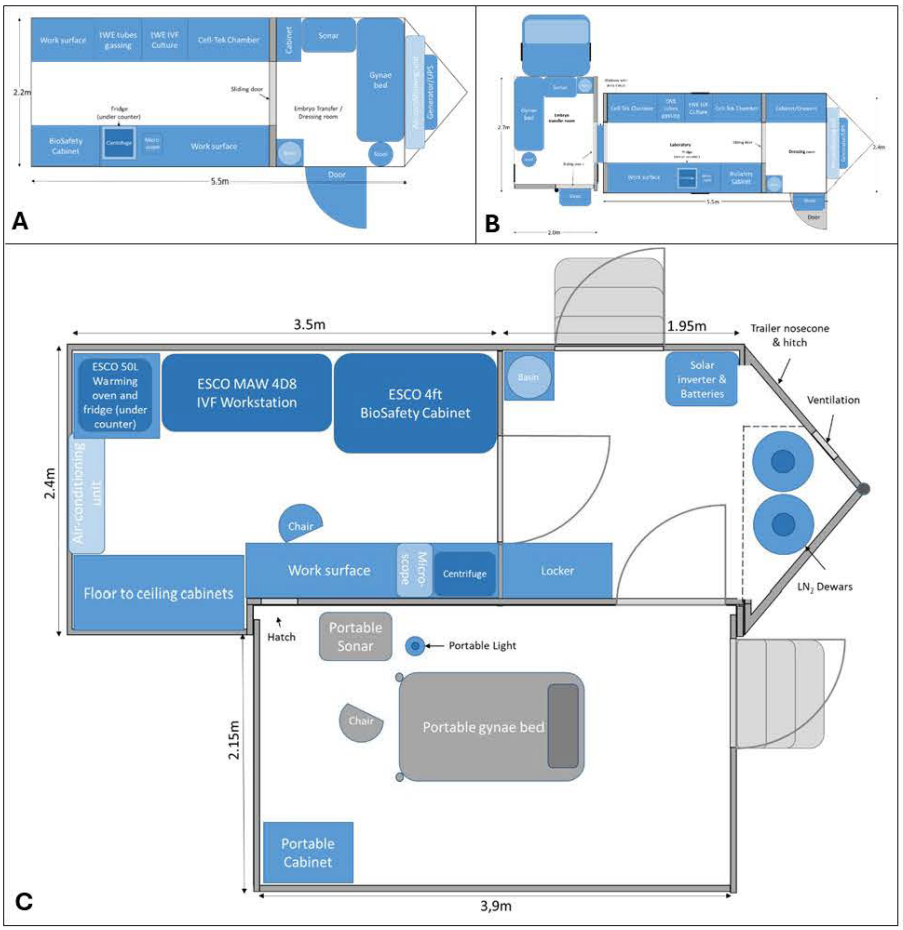

Figure 2: Designs considered for a mobile IVF laboratory using (A) a trailer and towing vehicle together, (B) a standard trailer, or (C) a trailer that can expand on one side.

Construction of the mobile laboratory

Building a trailer with room extension

After the laboratory design was completed, a local supplier was sought to construct the trailer according to specifications provided. This included approaching the engineering department of a local university, dedicated laboratory design private companies, and various trailer manufacturers. The engineering department considered assisting with the project as a post-graduate project for one of their students, however this project would have focussed on the suspension and testing of the trailer, which would have placed considerable strain on the project timeline and budget. The professional laboratory design companies were willing to participate but estimated costs for the entire laboratory with equipment exceeded R4 000 000. Finally, standard trailer or caravan manufacturers were identified as the ideal option for a cost-effective solution to build a prototype mobile medical laboratory. Several trailer manufacturers were

227 approached, and most were discouraged by the design that incorporated a section that can
228 unfold to form an additional room. However, a local manufacturer, MSF Trailers (Pretoria,
229 South Africa; www.msftrailers.co.za), was found that was willing to take on the project and
230 offered a quotation that was deemed acceptable. The final construction of the laboratory was
231 commissioned in April 2023, with a promised turn-around time of 4-6 weeks.
232
233 Construction was initiated, building the trailer up from the chassis (see Figure 3), which was
234 constructed with steel beams (Figure 3A1), incorporating attachment of a double axel and
235 strong hitch, with stainless-steel floor placed over the chassis (Figure 3A2). The body of the
236 trailer was built out of panels made from a double layer chromadec, 50mm apart, pressure
237 bonded to a thick core expanded polystyrene insulation. The interior cabinets and work surfaces
238 were custom built using stainless steel sheet metal (Figure 3B), for ease of cleaning. During
239 construction of the trailer, the initial build of the chassis and base went smoothly, however, the
240 fold-out room's assembly (Figure 3C) proofed more difficult than anticipated due to the weight
241 of the panels. The first issue was related to the floor section, which had to be constructed out
242 of a double layer of chromadec, to safely support the movement of people using the room. This
243 panel weighed more than 150kg and would pose a safety concern if dropped unsupported
244 during assembly of the second room. Therefore, a winch capable of carrying a load of more
245 than 1 ton was installed on the roof of the trailer, with a cable to support the floor while lifting
246 and dropping. The second complication was that the gas lifters supporting the roof section was
247 not able to lift the entire panel, even though these gas lifters were at maximum capacity. The
248 roof was shortened by approximately 300mm, to reduce the weight off the panel. In addition,
249 to provide more room for gas lifter movement and leverage, the door to the embryo transfer
250 room (Figure 3C3) was positioned approximately 200mm from the trailer body. Construction
251 of the mobile trailer took much longer than anticipated, and the final prototype was released
252 from the manufacturer six months after being commissioned.
253

Figure 3: Construction of the trailer that would be converted into a mobile laboratory, showing the trailer's (A) chassis and floor, (B) stainless steel interior cabinets, (C) ET room extension with roof and side panels in the open and closed positions, and (D) the fully constructed trailer.

Post-construction, some structural concerns emerged, which included water leaks in the dressing room and at the doors after heavy rainstorms, the weight of the trailer exceeding the towbar maximum limit, and sub-standard welds on the chassis. The trailer was returned to the manufacturer in November 2023, to repair the identified snags. The necessary improvements were completed and the trailer released again in June 2024.

The design and construction of the IVF laboratory were performed in conjunction with specialists from ESCO Technologies (PTY) Ltd. (Midrand, South Africa;

www.escolifesciences.com), who generously loaned equipment for the mobile medical laboratory. A design for the outside branding of the mobile laboratory was specially design for the trailer by the well-known Belgian artist Koen Vanmechelen (www.koenvanmechelen.be), who is also a director of the tWE npo.

After construction was completed at MSF Trailers, the mobile laboratory was towed to the ESCO Technologies (PTY) Ltd workshop (Figure 3D) where branding of the trailer (Figure 4A) and installation of equipment (Figure 4B) was completed. The equipment provided by ESCO Technologies (PTY) Ltd. included a Fertilisafe® Muti-Zone ART Workstation, Airstream® Class II Biological Safety Cabinet, 50 liter CelCulture® Incubator (without any gas connections), HR1-140S-8 Medical Refrigerator, Airsteam PURE upright air filter (ESCO Medical, Singapore, Republic of Singapore), as well as a Primostar phase contrast and Stemi 508 Trino stereo microscope (Zeiss, Oberkochen, Germany). Additionally, an Olarm Dual Wi-Fi and Sim alarm system (AA Alarms (PTY) Ltd., Pretoria, South Africa) was installed (Figure 4C) and connected to the laboratory equipment, electricity supply, movement sensors, and door sensors. All large equipment was secured with bolts passing through the floor to the chassis of the laboratory, to prevent movement during transportation. When moving the trailer, smaller equipment, such as microscopes, were secured in the original padded transport boxes it was delivered in and placed in the laboratory's cupboards in such a way that the boxes could not move around in the cupboards.

Figure 4: Branding (A) of the outside of the mobile laboratory, while installation of equipment (B) and an alarm system (C) is performed on the inside of the trailer.

The mobile laboratory was completed in time for the symposium "*Access to Infertility care in South Africa*", hosted at the University of Pretoria (Pretoria, South Africa) on the 28th of October 2023, where a multi-discipline group of attendees from all over South Africa participated actively during the days' sessions. During lunchtime and after the day's program was concluded, attendees were encouraged to visit and walk through the mobile IVF laboratory that was showcased in the parking area outside the lecture hall (see Figures 5&6).

Figure 5: The mobile IVF laboratory showcased at the symposium in October 2023.

Figure 6: The inside of the mobile IVF laboratory, showing (A) the laboratory area and (B) the embryo transfer room.

Quality control testing of constructed laboratory

After the symposium and launching of the mobile IVF laboratory, a series of quality control tests was initiated to prepare the unit for clinical use. First, the laboratory was cleaned per standard laboratory protocols, using appropriate approved cleaning products. To remove micro-particles in the laboratory environment, the IVF workstation, biological safety cabinet and air filter was kept running after the laboratory was manually cleaned. Air sampling and culture of agar plates for bacterial and fungal contaminants were conducted of the laboratory and embryo transfer room air, as well as the various work areas in the laboratory. Air samples from the IVF workstation, biological safety cabinet and warming oven were free of any growth after a 7-day incubation period (at 37˚C), while the air of the laboratory and embryo transfer room presented with 2 and 5 colony forming units respectively, and no fungal cultures.

Equipment installed by technical staff from ESCO Technologies (PTY) Ltd were verified for temperature regulation (warming oven, fridge and IVF workstation surface), as well as airflow and filtering (IVF workstation and biological safety cabinet). The warming oven's temperature was monitored with the warming oven's built-in thermometer, as well as an external digital thermometer (GMH G3230, Greisinger, Regenstauf, Germany) and a liquid thermometer (Red Spirit Thermometer, -10/110°C, Lasec®, Cape Town, South Africa) placed inside the warming oven. A detailed quality control system designed to be used with Google Forms was implemented, where all these temperatures, as well as the operation of all other laboratory equipment were monitored daily. Temperature fluctuations of equipment that would house biological material were logged over a 10-day period. During the validation period, the warming oven and the surface of the IVF workstation both maintained temperatures steadily at 37˚C, with fluctuations of less than 0.1˚C observed. The quality control monitoring continued during active embryo culture cycles in the mobile laboratory.

Validation of embryo culture in the mobile laboratory

To confirm the viability of human embryos in the mobile laboratory, using the Walking Egg simplified IVF culture system, a pilot study was conducted using abnormal supernumerary fertilized oocyte destined to be discarded. Fertilized oocytes with three or more pronuclei that is genetically abnormal and would not be used for embryo transfer, still has the potential to develop into blastocysts and can be used to validate the embryo culture conditions of a culture system.[24] After obtaining written informed consent from patients, oocytes from conventional IVF culture that was identified to have three or more pronuclei was cryopreserved and used in

a pilot study to culture embryos in the mobile IVF laboratory.

Oocytes containing ≥3 pronuclei (n=22 oocytes from 14 patients) were utilized for the pilot study. These zygotes were obtained from conventional IVF culture in a public sector IVF laboratory and vitrified with the Kitazato vitrification method (Kitazato Corporation, Tokyo, Japan) as soon as possible after fertilization evaluation, before pronuclei faded. A control group of sibling oocytes from the 14 embryo culture cycles, that showed normal fertilization (n=106 oocytes) were cultured in microdroplets of embryo culture media (V-ONESTEP, VitroMed, Jena, Germany) in a timelapse embryo culture incubator (EmbryoScope, Vitrolife AB, Gothenburg, Sweden). The embryo culture cycles continued for up to six days after insemination, to the point where embryos were transferred to the female patient or cryopreserved. The data from these normally fertilised oocytes was recorded for comparison with the embryo culture outcomes of the abnormally fertilised oocytes. After the mobile laboratory passed all quality control measures, a flask of liquid nitrogen containing the frozen zygotes were moved to the mobile laboratory. The zygotes were thawed with the Kitazato warming method (Kitazato Corporation, Tokyo, Japan). The thawed zygotes were placed into pre-equilibrated tWE embryo culture tubes containing 1ml embryo culture media (V-ONESTEP, VitroMed, Jena, Germany) and cultured for five days. Embryo evaluations were performed to confirm ongoing embryo development, including cellular division past the 8-cell stage to compaction and blastulation. There was an expectancy of at least 50% of these abnormal zygotes to initiate cleavage events, with continued development to compact and eventually blastulate. According to Uzun *et al.*, embryos developing from abnormally fertilized oocytes should develop into blastocyst at a rate of at least 50% that of sibling embryos derived from normally fertilised oocytes.[24]

From the test and control groups, respectively, 21 & 105 of the zygotes initiated embryonic development. From these cleavage stage embryos, 13 & 84 embryos compacted, with 10 & 78 of the compacted embryos developing further to the blastocyst stage. Comparing the embryo culture data between the two groups, all the parameters evaluated confirmed that the embryos derived from abnormally fertilized oocytes developed sufficiently to surpass the target of 50% the development of embryos derived from normally fertilized oocytes. This data verified that the embryo culture system in the mobile IVF laboratory can facilitate cellular development from the oocyte to blastocyst stage (see Figure 7).

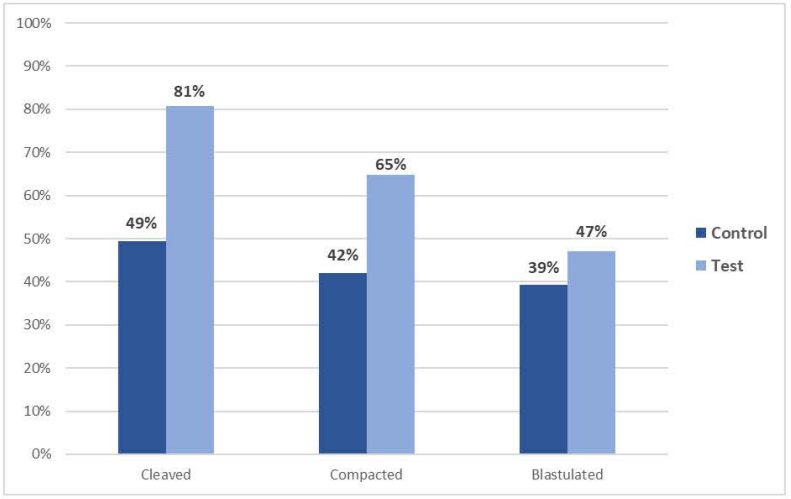

Figure 7: Comparison of embryo development indicators cleavage, compaction and blastulation rates, between the Control (50% development rate of embryos derived from normal fertilized (2PN) oocytes[24]) and Test (embryos derived from abnormal fertilized (≥3PN) oocytes) groups.

Discussion & Conclusion

To accommodate the increasing demand for ART services, not only in South Africa but also in Sub-Saharan Africa and other Low- and Middle-Income Countries, more ART providers are required.[25-27] Due to the high cost of setting up an ART unit, especially the laboratory section, careful financial planning is required, taking a myriad of factors in consideration.[16, 28] A possible model to increase the number of ART centres, without the financial burden of setting up an ART laboratory for each centre, is a single laboratory which can provide a service to multiple facilities.[29] The transport of gametes and embryos in culture is performed in many instances.[30, 31] Distances will, however, impact on practicalities and feasibility of this model.[29, 32, 33] Therefore, the old saying of "*If the mountain will not come to Muhammad, then Muhammad must go to the mountain*"[34] applies, whereby a single laboratory can provide support to multiple infertility care units through the establishment of a mobile ART laboratory.

The application of a mobile medical unit has to be carefully planned to accommodate a niche in the market previously unmet, while remaining financially viable.[35] A mobile laboratory can visit multiple centres in a scheduled manner, allowing local clinicians to recruit and batch patients who qualify for ART intervention, until such time that the mobile laboratory frequents the facility. A mobile IVF laboratory is probably best suited to incorporate a simplified IVF culture system, such as the tWE simplified embryo culture system.[21] This simplified culture system reduces the cost per cycle,[22] and because it is a closed system can function in a less

stringently controlled environment than conventional embryo culture.[20] However, the tWE simplified IVF culture system can only provide ART services to patients who fall within the suited criteria, i.e. suitable for IVF insemination.[20, 21] This exclude patients with severe male factor infertility, where ICSI is necessary, when insemination is performed with testicular aspirated sperm, or when prenatal genetic testing is required.[21] With these exclusions in mind, the tWE simplified embryo culture system can still be used to provide MAR to most patients, with the remainder of patients being referred to tertiary level institutions with conventional IVF laboratories.[36] This model can be used to alleviate the burden on the few IVF facilities in South Africa,[37] which are tasked to provide Medical Assisted Reproduction (MAR) services to the more than 60 million people in the country.[38]

The manner in which a mobile IVF laboratory can be used optimally is open for interpretation, to be adapted according to circumstances and local requirements. One possibility is through the sharing of the mobile laboratory by multiple ART centres. Patients would be seen continuously for diagnostic purposes and clinical interventions, with IVF cycles batched until such a time as the mobile laboratory is available. Oocyte retrieval can be performed at the local facility's theatres or similarly prepared procedure rooms. The gametes should be transported under controlled conditions to the mobile laboratory, for oocyte fertilization, embryo culture and transfer. Once all recruited patients have been treated at a location, the mobile laboratory would proceed to the next medical centre, where a batch of patients were prepared by a team of local health care professionals. Herewith, four to six ART centres can utilize the laboratory per year, in a twelve-week rotation cycles (see Figure 8). The cost for such a laboratory would then be shared between the centres, alleviating the financial burden.

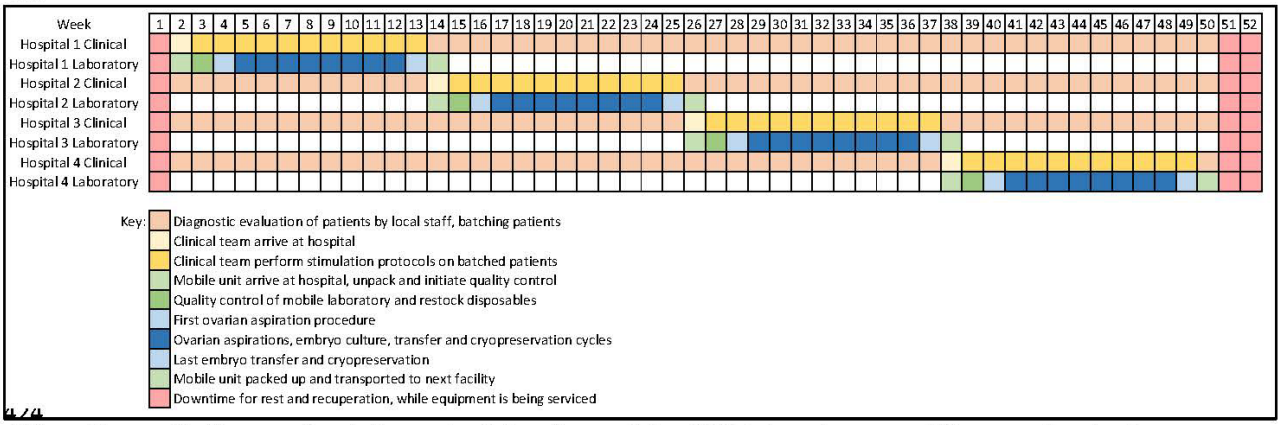

Figure 8: Proposed rotation schedule of a mobile IVF laboratory providing service to four independent ART centres.

The prototype mobile IVF laboratory described in this paper is a world-first in design. The unit was built locally and furthermore tested to function as a substitute IVF laboratory, albeit a simplified mobile version. The concept of an IVF laboratory on wheels, is currently further validated through the initiation of full IVF cycles using the tWE simplified embryo culture system in a rural setting. The proof-of-concept confirms the realization to perform embryo cultures and transfers in a mobile IVF laboratory. This can be a valuable tool in a South African context and globally, where access to MAR is limited due to few IVF centres that are mainly found in large cosmopolitan centres.

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Chapter 5: First births from a mobile IVF laboratory: proof-of-concept results

In the manuscript presented in Chapter 4, the design and construction of the prototype mobile IVF laboratory was described. The operation of all equipment has been validated, and a pilot study described that was used to confirm that the IVF culture system in the mobile laboratory could successfully culture embryos up to the blastocyst stage. At this point, there was a need to confirm in a more definitive manner that the prototype mobile IVF laboratory would be able to perform in a real-world environment. To achieve the third objective of the research, a proof-of-concept study was performed.

The mobile laboratory was used in a rural town in South Africa, to perform IVF and embryo culture cycles for a small cohort of patients. The town is 110km away Pretoria, with an estimated population of just over 600 000 people. (https://worldpopulationreview.com/cities/south-africa/rustenburg) There are two ART facilities in the town, and the ART procedures in the mobile laboratory were performed in conjunction with one of these local IVF clinics. This arrangement was made to have this facility available as a safeguard, should embryos have to be moved to a regular ART laboratory. However, the laboratory operations were performed completely in isolation from the local IVF laboratory, and at no point was there a need for any gametes or embryos to be moved to the facility's laboratory. All procedures were performed with the mobile IVF laboratory as the sole provider of ART services. This included the processing of gametes, as well as the culture, transfer and cryopreservation of embryos. The outcome from this proof-of-concept study is presented in manuscript format in Chapter 5.

The manuscript titled "First births after using a closed, simplified IVF culture system in a mobile IVF laboratory - proof of concept results" has been submitted to Reproductive Biomedicine Online (proof of submission in Annexure E), a peer reviewed open access journal with a 3.5 impact factor. The manuscript has passed the peer review process, has been accepted for publication by the journal, and will be in print as soon as final type setting is completed.

Journal Pre-proof

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

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

Running title: Proof of concept results: First births after using a closed, simplified IVF culture system in a mobile IVF laboratory

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Abstract (208)

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 success rate after using 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 supports the use of a mobile IVF laboratory in areas where access to ART is too costly and limited due to few IVF centres, mainly located in large 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 stigmatization, 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 couple 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 fertilization (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 CelCulture® 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 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 can 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).

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

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 was 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 the 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 fertilize. 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.

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	G2P1M1
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

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 verified 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 viability to culture 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.

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 fertilization 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.

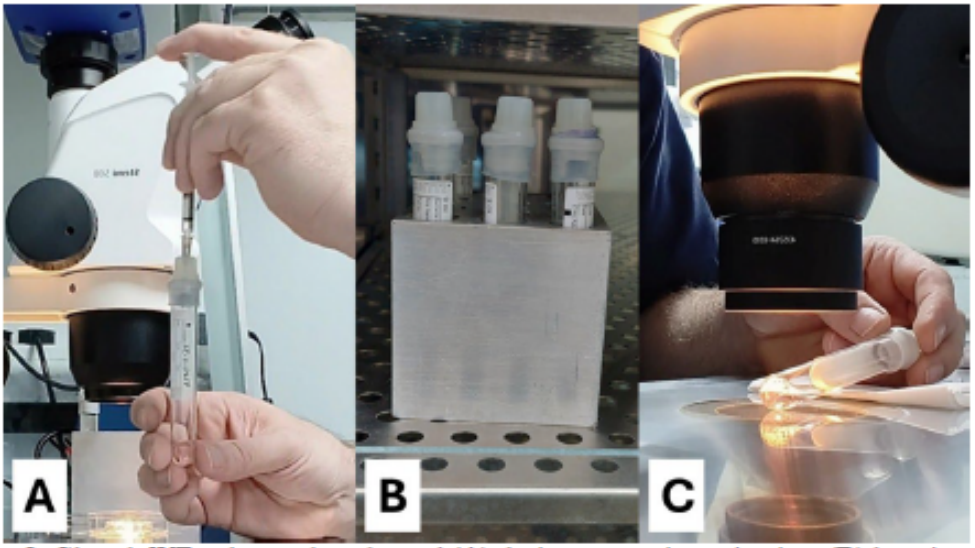

Figure 2: Closed tWE culture tubes showed (A) during oocyte insemination, (B) kept in an aluminium block to maintain temperature and (C) embryo evaluation.

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 B13BB or higher, indicating at least fully a 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% fertilization was achieved, and 62% of these zygotes developed sufficiently for embryo transfer or cryopreservation. Summarised outcomes per patient can be seen in Table 2.

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

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 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% utilization 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 the most commonly used assisted reproductive technology today, 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 (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 or create a financial cushion to repay loans or reimburse investors. 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 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, whether in mobile units or low-cost IVF laboratories.

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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.

Chapter 6: Synthesis of the findings

6.1. Background

People in general resist change. This can be due to perception, how they see the world, or to change the way they do certain things or believe how certain things can and should be done.[1] This resistance to change has dogged the field of MAR from the very beginning. Long before the birth of Louise Brown, the first IVF baby, Robert Edwards was busy with research that lead towards his role in ART, and published an article in the Lancet in 1965.[2,3] He described the maturation of human oocytes, something that very little was known about at the time, and the response from the Lancet was that they "could not see the point of the work".[4] When Edwards, Steptoe, and Purdy was struggling for 10 years to achieve the first IVF birth, and even after the first birth, widespread opposition was found in both the medical field and society in general.[4-8]

In contrary to the opposition encountered at the beginning of IVF, once the world accepted ART, this medical field boomed and is currently a multi-billion-dollar industry.[7,9,10] The treatment options have been standardised and the safety and efficacy of standard ART has been well documented.[7] However, with the growing quality and complexity of ART, patients' bills are also rising, and the cost of ART is listed as one of the major barriers for patients.[11] The world has mostly accepted ART as a viable option to treat infertility, and in the race to improve and gain an edge above competitors, the use of additional treatments and procedures, known as add-ons are increasing rapidly.[12-14]

Unfortunately, with the fast growth of the MAR industry and the costs involved, there are geographical regions that are left behind, with minimal access to reproductive health treatments. Also, when such services are available, the procedures are too expensive for a great number of people.[15,16] Cost effective methods have been proposed to address some of the barriers, but since the industry is commercially driven by profit, these solutions are finding a hard time to procure a foothold in the industry.[17,18] The industry might have forgotten the humble beginnings in Oldham,[4] and became used to technologically advanced protocol with a focus on commercial, profit-margin chasing incentives.[19] The thought of changing to alternative cost-

effective methods is unthinkable. When there are support for simplified or cost-effective strategies, the support is often found to be theoretical only, with no actionable assistance. Pioneers are often wished well, but no actual support is provided to them.

Unfortunately, even though evidence-based results were provided through the current project to show the successes achieved, the road to achieve these successes has not always been a smooth one. This became apparent in the way that the mobile IVF laboratory's validation testing was received. The research protocol was approved by the relevant research and ethics committees, however, during implementation of the proposal several roadblocks were encountered. The first version of the research plan was to perform a comparative study, with patients in the public sector having the choice of conventional IVF or IVF in the mobile laboratory. In this protocol, patients who opted to have their embryo culture performed in the mobile IVF laboratory would have received all laboratory procedures free of charge. However, the public sector hospital where this would have happened did not agree for the project to proceed to recruit patients. An amendment to the research protocol was requested, where all patients had to be offered a fully subsidised ART cycle, including ovarian stimulation and all treatment costs, paid for through the research project. The financial demands of this change placed a substantial burden on the research budget.

The research protocol was amended, committee approvals of the relevant committees was obtained, and funds and sponsors were found. However, at this point, an additional requirement was requested. The public sector hospital stipulated a proof of medical insurance to be provided, indemnifying the hospital from any claims or liability from any of the patients, or babies born from the project, that would extend past the project end date for an additional 21 years. Additionally, no support was allowed to be provided during standard working hours by employees in the public sector. Embryologists, fertility specialists or nurses from the hospital, all health professionals who wishes to assist, would have to do so after hours or use their annual leave to take time off to observe or work on the project.

The researcher, with the assistance of the University of Pretoria's Insurance Management division, spent months trying to find an insurer willing to provide such cover, to no avail, and eventually yielded that the request was impossible to comply

with. The research protocol was amended a second time, after consulting with private sector ART providers who were willing to assist. After a period of 22 months of rewriting the protocol, finding additional funding, and assistance from the University of Pretoria's Deputy Dean of Research and Postgraduate studies, the Research Office, Finance Department, Research Ethics Committee, and various UP employed individuals, the protocol was approved by the research and ethics committees, and eventually the mobile IVF laboratory validation was performed in the private sector as described.

The implementation of any new system is bound to encounter some pushback, but the level of resistance encountered in the public sector was an eye-opening experience. The reasoning behind the requests was always described to appear logical, but the reality is that fear of the unknown, and resistance to accept an alternative model of doing a procedure, was a major hindrance to promote a project directly in line with the public sector agenda to provide equitable access to health services to all people and communities without exposing them to financial hardships.[20] In the discussion and conclusion of this thesis not only the results obtained will be discussed, but also the implementation of a mobile IVF laboratory, and foreseen difficulties to overcome will be commented on.

6.2. Summary of key findings

6.2.1. Study 1: Public sector ART in South Africa

The first study showcased the population profiles of patients who followed through or did not return for ART after an initial consultation. Couples were grouped according to Low-, Middle-, or High- income brackets. Patients from the Low-, Middle-, and High-income groups had access to medical aid in 6%, 43%, and 71% of cases, respectively. Only forty-seven percent of all the patients were eligible for subsidised ART treatment. The study population's income was identified as a representative factor of the combined profile of patients, i.e. their income, access to medical aid, and eligibility for subsidised ART cycles. Patients who earned less than or more than the study population's average income returned for therapeutic procedures in 25% and 51% of cases, respectively.

The average income of the population, as well as the Low-, Middle-, and High-income groups, and the percentage of patients from each of the groups who returned for ART is shown in Table 6.1. The reported Euro values for this study are derived from ZAR and converted R 16.016:1 €, which was the average exchange rate in the study period 2015 to 2020.[21] Both Euro and ZAR values are given due to this being a joint PhD between the Universities of Pretoria and Hasselt.

Table 6.1: Averaged monthly income in South African Rand (R) and Euro (€) of the study population and income groups, with each group's return rate for ART.

Income	ZAR (€)	Return for ART
Average	R 21 422.75 (1 337.58 €)	41%
Low	R 3 351.58 (145.29 €)	30%
Middle	R 16 325.29 (455.06 €)	41%
High	R 46 538.59 (1555.54 €)	50%

The time for patients to return for therapeutic ART, i.e. from the first diagnostic to the first therapeutic procedure, described as TTP, was calculated to be 240 days on average (median TTP of 171 days, and the third quartile starting at 303 days). The percentage of patients (per income group) where the TTP exceeded the median and third quartile times is depicted in Table 6.2.

Table 6.2: Percentage of patients from different income-groups returning for ART above the population median and 3rd quartile time.

Income	TTP >median (171 days)	TTP >3rd quartile (303 days)
Low	67%	41%
Middle	51%	23%
High	42%	21%

6.2.2. Study 2: Conceptualisation of a mobile IVF laboratory

From the design and construction of the mobile laboratory, no statistical data was obtained. Valuable information was gathered during the design and construction process, which can be applied in the future when additional mobile IVF laboratories are needed. The culturing of embryos in the prototype mobile IVF laboratory was confirmed using the simplified tWE IVF culture system and non-viable (3PN) oocytes (n=22 oocytes from 14 patients). From the 22 zygotes, 21 cleaved of which 13 started compaction and 10 proceeded to form blastocysts.

6.2.3. Study 3: Patient IVF cycles in the mobile laboratory

Twelve couples were recruited to participate in the proof-of-concept study to showcase the use of a mobile IVF laboratory in a real-world scenario. All the necessary ART procedures, from the screening of follicular fluid for oocytes, to the sperm preparation for insemination, oocyte insemination, and embryo culture, transfer and cryopreservation, were performed in the mobile laboratory. During these procedures, operational and logistical complexities were identified.

Of the initial 12 patients recruited, one patient did not have any COCs after the TVOA procedure, and another attempt resulted in a single mature oocyte, which did not fertilize. Embryos from the remaining 10 patients were cultured in the mobile IVF laboratory. The average number of oocytes per patients was 6.4, of which 4.3 were metaphase II oocytes, with a 79% fertilization rate and 62% of the resulting embryos were used for transfer or cryopreservation. From 10 embryo transfers, 5 patients presented with a positive βHCG test result, and 4 out of these 5 pregnancies resulted in healthy live births.

The use of the mobile IVF laboratory provided insight in day-to-day operations of a mobile laboratory. This experience can be applied when planning future ART cycles using a mobile IVF laboratory.

6.3. Discussion

This project was established to examine barriers to equitable access to MAR in South Africa and to explore the improvement of ART services using a mobile IVF laboratory. This was achieved by a sequential design, considering (i) the demographic and socioeconomic factors influencing patient progression from diagnostic to therapeutic ART procedures, (ii) the feasibility of designing and constructing a low-cost mobile IVF laboratory using tWE simplified IVF culture system, and (iii) proof-of-concept evidence demonstrating the ability of such a mobile laboratory to function effectively in a real-world clinical setting. The findings from these studies can be applied to the problem of improving access to MAR not only in South Africa, but internationally in LMICs.

The first study considered the demographic and socioeconomic factors that affects the progression from diagnostic to therapeutic ART services in the public sector of South Africa. While previous literature consistently acknowledges cost as a barrier to ART in LMICs, there is little to no information to explain at which point patients stop to explore their treatment options.[16,22,23] This study provides quantitative evidence from a South African public sector ART unit about the transition from diagnostic to therapeutic ART treatment, and how this is influenced by socioeconomic factors.

Large inequalities in patient income were observed, affecting access to ART services in the public sector ART unit investigated. A marked difference in income was observed between patient groups, with the Low-income group earning only 7% of the income of the High-income group. This inequality aligns with broader national patterns, as South Africa remains one of the most unequal societies globally.[24,25] The study showed that patients' return for therapeutic treatment can be correlated with their income level. While more than half of High-income patients returned for therapeutic procedures, approximately 70% of patients in the Low-income group did not return for treatment after initiating diagnostic investigations, even though 61% of patients earn less than the study population's average income. This raises the question, which part of the population is being assisted with their reproductive journey in the public sector?

Not all of the patients who failed to follow-through with ART procedures in the public sector can be ascribed to inaccessible treatment options, some may have opted to find treatment in the private sector.[26,27] However, the results from the study strongly suggest that economic factors are at least partially the cause for patients to drop out from ART treatment in the public sector. This correlates with literature on the topic of barriers to ART. The cost of ART is often cited as either the largest or one of the largest contributors to lack of access to ART.[15,16,28,29] In South Africa, public sector ART units provide ART services with an out-of-pocket co-payment by patients.[30,31] Irrespective of which subsidisation category these patients belong to, there are some expenses that has to be covered by the patients. This include culture media and solutions used in the ART laboratory, with a short a shelf-life, which cannot be processed timeously though the public sector's procurement administration. Unfortunately, the treatment expenses are often so high that even affluent patients struggle to cover the necessary payments, and low-income patients are effectively excluded.[30,32,33] To determine the

real financial accessibility of ART, the patient's perspective on affordable fees needs to be considered. Are the procedural costs, along with the additional associated costs, affordable based on their socio-economic status, disposable income, and access to subsidy or reimbursement?[11,34]

The data from the study also provides evidence that there are major constraints in the public sector, causing long waiting times to return for therapeutic interventions. Increased waiting times, especially for patients from lower income brackets, could possibly reduce patients' chances of successful outcomes, as the patients' age impacts on their fertility.[35-37] The findings emphasize the need for policy-level intervention, especially during this time of health reform in South Africa, through the induction of the National Health Insurance.[38] With the cost of ART recognised as a barrier to infertility care worldwide, these findings can be applied to all areas where ART is not readily available or subsidised. Most LMICs struggle with this issue of inaccessible ART.[39-41] Unfortunately, highlighting such an acknowledged impediment does not bring anyone closer to solving said problem. According to medical professionals in South Africa, working in the field of MAR, improving access to ART in the Republic of South Africa (RSA) can only be solved when issues related to (i) human resources, i.e. the number of trained medical personnel with experience in MAR, (ii) laboratory services, and (iii) medication, is addressed.[42]

While the outcome of the first study underscored the need to improve access to ART, the second and third studies of the project focussed on a practical way to accomplish this. Study 2 documented the world-first design, construction, and initial testing of a mobile IVF laboratory using the tWE simplified IVF culture system. The IVF culture system, which replaces costly incubators and other laboratory equipment through the use of sealed, gassed culture tubes, has already been shown to support fertilisation, implantation, pregnancy, and perinatal outcomes comparable to conventional IVF culture systems.[43,44] The simplified tWE IVF culture is ideal for implementation in a compact environment, such as a mobile IVF laboratory.

During construction, several logistical challenges were identified, such as the trailer's size and weight. Additionally, to balance a low-cost option for construction with the required medical-grade quality workmanship proofed to be challenging. Specific

complications that were encountered was that the manufacturing company did not have the expertise on hand to model the design first and calculate the structural requirements. This resulted in a trial-and-error based model, that added several months to the manufacturing process. The manufacturer struggled for example to find heavy-duty gas-lifter strong enough to lift the embryo transfer room's roof during unfolding of the side room, which resulted in the redesign of the fold-out section with smaller panels and a shorter roof section. Additionally, water leaks in the IVF laboratory were only realised after a heavy rainstorm after the mobile IVF laboratory was handed over from the manufacturer. The trailer then had to be returned to the workshop for repairs, with a simulation of rain to test the repairs.

Other known risks that were identified and addressed were (i) unreliable electricity supply, (ii) temperature regulation inside the mobile laboratory, (iii) theft, and (iv) safe storage of liquid nitrogen.

(i) The unreliable electricity supply is due to regular unscheduled and scheduled power outages which can last for hours. To make provision for this, solar panels were installed on the roof of the mobile laboratory, which in turn was connected to an inverter and battery. This battery, when fully charged, can provide the mobile laboratory with electricity for at least 12 hours after a power shutdown, allowing for sufficient time to restore power to the laboratory. In case of failure to restore power within the 12-hour window period, a small electricity generator was available.

(ii) Since the laboratory had to be parked uncovered in full sunlight for the best effect of the solar panels, temperature regulation, and specifically overheating, was considered a risk. To prevent this, the air-conditioning unit built into the laboratory was running constantly during the day, even without a person present in the laboratory. The air-conditioning was set to keep the laboratory temperature at 23˚C.

(iii) Due to negative crime statistics in South Africa, the theft of equipment, or forced entry into the mobile laboratory was also considered a possible threat. The laboratory was installed with an alarm system with an audible emergency alarm, as well as send a message to the researcher, if any of the doors were opened, or if there was movement inside the laboratory when

the alarm was armed. Additionally, the laboratory was always parked in close proximity to a 24-hour manned night watch station, with arrangements made with the night watch to contact the researcher in case of the alarm sounding, or any other concerns.

(iv) Lastly, for the safe storage of liquid nitrogen, a small area of the laboratory's front, away from the main laboratory section, was cordoned off with a steel mesh cage. A ventilation hatch was provided at this area, where nitrogen could dissipate out of the laboratory, should there be a problem with the liquid nitrogen dewar. After the repairs to the mobile laboratory to prevent water leaking, and all other concerns were addressed, the prototype mobile IVF laboratory was eventually deemed ready for *in vitro* testing.

Alternative suppliers should be sourced for future developments and deployment of multiple mobile units. The various optional designs for a mobile IVF lab should also be further investigated and researched using the current prototype as basis or starting point, considering the intended service application, location and patient population. The prototype was constructed and proven to be functional, through the quality control testing of the equipment and culturing of abnormally fertilised oocytes. Furthermore, the proof-of-concept testing of the mobile laboratory was confirmed through the culture and transfer of embryos in the mobile laboratory, which finally cumulated in the birth of 4 healthy babies. Combinedly, Study 2 and 3 provides the first practical demonstration that high-quality IVF laboratory procedures can be decentralised and transported.[45] The practical operation of Study 3 in a rural town in South Africa provided very insightful information on the logistics and operations of a mobile IVF laboratory.

With a prototype mobile IVF laboratory successfully constructed and tested, the question of implementation to improve access to ART remains. How can such a mobile IVF laboratory with a simplified IVF culture system compete in a world of high-technology ART where patients demand the best, and is willing to pay for all kinds of add-ons? The answer is that there is no need to compete in this manner. The mobile IVF laboratory was not designed to compete with existing conventional ART facilities for patients willing to pay for all the bells and whistles. The concept is intended to make the expansion of new laboratories easier. The mobile IVF laboratory was designed to

be equivalent to a stationary conventional IVF laboratory that can be used for most patients (excluding cases where ICSI is required). The difference is that this laboratory is on wheels and can be shared by multiple facilities. This signifies that the mobile IVF laboratories can be used as an additional tool to extend ART services to locations where currently none is available.

Although the use of mobile IVF laboratories promises to improve access to ART, at present this is a practical academic exercise.[18] For the actual implementation of mobile IVF laboratories, several aspects should be considered. These include, but are not limited to, the necessary infrastructure requirements, the availability of disposables and consumables, as well as sufficiently trained staff. The day-to-day operations of the laboratory should be known, and skilled collaborators identified with clear roles and expectations. Team-members must have a shared vision and must be genuinely invested in the success of such a project. Patients and laboratory timelines should be synchronised, where a project coordinator occupies a central role to manage logistics, resources and troubleshoot with the team. Lastly, the legislative framework and regulatory differences between countries and areas need to be considered, prior to placement and operation of mobile IVF laboratories.

The manner wherein mobile IVF laboratories can be used is open to interpretation. As an example, two models describing the deployment of mobile IVF laboratories will be explored. The first is in the private sector in South Africa, and the second in the public sector. These examples will highlight some aspects of how mobile IVF laboratories can be used to improve ART services in South Africa, which could also be extrapolated to other LMICs.

Example 1: Sharing of a mobile IVF laboratory in the private sector

In the first scenario, the mobile IVF unit is envisioned to be used as a partnership between a number of fertility specialists without an IVF laboratory. The benefit to such a group of individuals would be that they can share the cost of acquiring a mobile IVF laboratory, while each having the freedom to practice at a preferred location. They would be able to set up their consultation rooms to attract potential patients, and perform the necessary diagnostic evaluations needed, as well as therapeutic procedures for which an IVF laboratory is not needed. The diagnostic evaluations for

female patients should not pose any problems in this scenario, while male patients can be directed to a specialized pathology laboratory to assess male reproductive health, or the enterprise could set up a basic andrology laboratory and employ an andrologist to perform basic semen analyses. The ART facility could also have an arrangement with a referral laboratory that can perform the semen analyses.

Furthermore, each clinician will need a procedure room to perform TVOA procedures or have an arrangement with a local day-clinic or hospital to perform the procedures in their theatre. Sufficient space should be available where the mobile IVF laboratory can be parked securely for an extended time, while embryo culture cycles are performed. A skilled embryologist should also travel with the mobile IVF laboratory and perform the IVF cycles. Additionally, a towing vehicle and driver with a towing licence would have to be available when the mobile IVF laboratory is moved. This can either be through obtaining a towing vehicle that complies with the necessary requirements to tow the mobile IVF laboratory. A dedicated driver with the required towing experience and licence should be available, or through an agreement with a towing company who can provide a towing vehicle and driver. The driver of the towing vehicle could also be taught to assist with the expansion of the mobile IVF laboratory's fold-out room. Lastly, the maintenance and insurance requirements for the mobile IVF laboratory would have to be provided for by the partners, which would not only include scheduled maintenance of the IVF laboratory equipment, but also scheduled maintenance of the trailer.

The logistics of the scenario where a number of fertility specialists share the mobile IVF laboratory would have to be carefully planned between their medical practices. Patients will be able to visit the clinicians' consultation rooms throughout the year for diagnostic and non-IVF procedures, and during this time patients can be identified who need ART. These procedures can be batched according to the availability of the mobile IVF laboratory. Urgent cases, that cannot wait for the mobile IVF laboratory, should be referred to conventional IVF laboratories that has the capacity to offer services immediately. Furthermore, patients with severe male factor infertility or known fertilization failure after IVF will have to be referred to conventional ART facilities to provide more advanced treatment options.[18,46]

With the batching of patients to await the arrival of the mobile IVF laboratory, the partners will have to calculate a balance between the patients' waiting time and a cost-effective schedule to rotate the mobile IVF laboratory. One suggestion would be to move the mobile IVF laboratory every four weeks to a different facility. Herewith the laboratory could be at each facility for four weeks with a 12-week interlude before returning to, for example one of four facilities.

As described in the above scenario, the MAR facilities which share the mobile IVF laboratory would have to refer some patients to conventional ART laboratories. A standard agreement with such a facility would be beneficial to have in place. Sharing the commissioning and operational cost of the mobile IVF laboratory by partners participating in the venture, will be more cost-effective than commissioning their own ART laboratory. This will enable them to develop their medical practice over time and increase the number of patients they assist. As their patient load and the consequent demand for ART procedures increases, they can then commission additional mobile IVF laboratories, or individual ART laboratories. The latter can provide simplified tWE IVF culture at the start; whereby supplementary ART equipment can be acquired over time to eventually upgrade the IVF laboratory to a conventional ART laboratory.

Example 2: Mobile IVF laboratories in the public sector

In the second scenario, a model is proposed for mobile IVF laboratories to be implemented in the public sector. The stratified manner of referral in the public sector will be used, as described in the South African National Clinical Guidelines for Safe Conception and Infertility.[46] According to these guidelines, all infertile individuals should receive a basic standard of care that includes information on infertility, an appropriate diagnostic workup, a discussion of relevant treatment options, and psychological and emotional support. The guidelines further state that the required standard of care should be provided over the four levels of the health system, namely Level 1: Primary health care clinics, Level 2: Regional hospitals, Level 3: Provincial tertiary hospitals, and Level 4: Specialised hospitals; with up referral from one level to the next as needed.[47] Table 6.3 outlines the proposed service available at each level and when patients should be referred from one level to the next.

Table 6.3: Infertility services according to levels of care in the public sector of South Africa (from: South African National Clinical Guidelines for Safe Conception and Infertility).[47]

Levels of care		Services provided	Comments and indications for referral to the next level
Level 1	Primary health care clinics, community health care centres, district hospitals, general practitioners (GP)	• Medical and fertility history • Screen and test for STIs on both partners, including syphilis serology • HIV, HBsAg, HCV Ab • Rubella IgG • Cervical cancer screening • TSH and prolactin levels if indicated	Nurses, general practitioners, and urologists may start an initial assessment for infertility and determine eligibility for referral to a gynaecologist. **Offer advice or refer if:** • >1 year of regular unprotected intercourse in women <35 years • >6 months of regular unprotected intercourse in women >35 years • Known cause of infertility • Irregular menstrual cycles
Level 2	Regional hospital (gynaecologist support)	In addition to all level 1 services: • Hormone profile (Day 2/3 FSH, LH, E2) • Ultrasound examination • Semen analysis • Hysterosalpingography (HSG) • Minor surgical procedures	**Refer if:** • Blocked tubes • Abnormal sperm analysis and hormone profile • Specialised surgery is required to improve fertility If patent tubes + normal sperm analysis – advise on the optimal time for sexual intercourse
Level 3	Provincial tertiary hospital (limited reproductive medicine sub-specialist support)	In addition to all level 1 and 2 services: • Major fertility enhancement surgery • Sperm processing • IUI / timed intercourse • Genetic screening	**Refer if:** • IVF/ICSI is required • Specialised infertility surgery is required • Specialised endocrine service are required
Level 4	Specialised hospitals with reproductive medicine sub-specialist support	In addition to all level 1, 2 and 3 services: • Anti-Mullerian hormone (AMH) test • Sperm processing • Sperm washing (PLHIV) • IUI/IVF/ICSI • Specialised reproductive surgery	These are specialised licensed and accredited fertility clinics that have highly specialised and accredited laboratories and personnel

The referral guide indicates the availability of a basic infertility diagnostic workup at level 2 of the health system, which are offered at regional hospitals. Limited reproductive medicine sub-specialist support should be provided at provincial tertiary hospitals, i.e. level 3 of the health system. The support by fertility specialists at level 3 centres does not include provision of ART services, and all ART patients should be referred to level 4 hospitals, where specialised ART services are available. These guidelines propose a treatment plan to identify infertile patients, where all patients in

need of ART are eventually referred to specialised hospitals.[47] Unfortunately, there are only three active public sector specialised hospitals available in South Africa that offers ART procedures, namely at SBAH, Tygerberg Hospital, and Grootte Schuur Hospital.[31]

Mobile IVF laboratories can be implemented at level 3 of the public health system, in conjunction with the limited reproductive medicine sub-specialist support already mentioned in the safe conception guidelines.[46] Patients with mild or moderate male factor and unexplained female infertility can be diagnosed at the level 2 and 3 hospitals, and receive basic ART treatments in a mobile IVF laboratory, without having to be referred up to the level 4 hospitals. Mobile IVF laboratories can be shared between two or three provinces. Patient procedures can be batched and scheduled according to the rotation of fertility specialists who provide “limited reproductive subspecialist support”. With the guidelines already making provision for procedure rooms where minor surgeries can be performed to improve fertility, ovarian aspiration cycles can be performed at these facilities. The only addition needed to provide ART services at level 3 hospitals would be a mobile IVF laboratory, with the necessary disposables and consumables, and a trained embryologist to perform the ART procedures. In this manner, the public sector can provide ART services in all the provinces in South Africa, while reducing the burden on the very few specialised hospitals with ART facilities. This is also aligned with the directive of the National Health Insurance (NHI) Act that was promulgated in 2024.[38]

These are only two examples of how mobile IVF laboratories can be applied within a resource poor environment. There are many more alternative options that remain, for mobile IVF laboratories to make ART more accessible. However, the lack of trained medical personnel to perform the ART procedures, especially embryologists to perform the IVF embryo culture cycles in the mobile laboratories, is a shared bottleneck in all the above scenarios.

Any large-scale roll-out of mobile IVF laboratories will have to go hand-in-hand with training of embryologists to work with the tWE simplified IVF culture system, careful consideration of the legal framework, and collaboration with existing ART facilities. The DoH and management of public sector healthcare providers should take cognisance of such opportunities to spearhead health reform of a constrained system.

6.4. Strengths of the study

The research project's strength originates from the uniqueness of the topic matter accomplished through a sequential approach, where the three components of the project addressed distinct, but interlinked objectives. The first study explored local data that has never been investigated and discussed before, i.e. patient demographics, socioeconomic profiles, and treatment progression within the context of a South African public sector ART unit.

The conceptualisation, design, and construction of a fully functional mobile IVF laboratory, using the tWE simplified embryo culture system, is a pioneering world-first achievement. No such mobile IVF laboratory had previously been constructed, and the study therefore contributes a high-impact, practical solution to the long-standing problem of ART inaccessibility in low-resource settings. The third study built on the second study's outcome and provided a proof-of-concept that the mobile IVF laboratory can be used to successfully culture and transfer of human embryos. The research yields immediately actionable insights, with evident contribution from theory into practice.

6.5. Limitations of the study

6.5.1. Study 1: Public sector ART

While the study provided valuable insight in the patient profiles in the public sector facility investigated, there are some limitations that should be acknowledged. The data gathered during the study was retrospectively obtained from patient information sheets that was completed by the patients themselves. The accuracy and reliability of the information is reliant on whether the patients provided a true reflection of their sociocultural situation when completing the forms. In addition, while the return rate of patients was considered, the follow-up of success after treatment was not included. The addition of whether ART treatments lead to successful pregnancies and live births would have been a worthwhile addition to the data. Further follow-up of patients whose first ART treatment was not successful could have flowed from the data, to comment on socio-culture profiles of patients who undergo a second attempt at ART. Another limitation was the impact of the global outbreak of the coronavirus on the return of patients for therapeutic services from 2020 onwards. Inclusion of patients during the

time of the COVID-19 pandemic would have skewed the data, especially on patients' return rate and TTP, due to limited ART cycles being performed and patients not being able to travel to the ART facility.

The information was gathered from the largest public sector ART facility in South Africa, whereby the conclusions made can only be applied to this facility. General commonalities such as expenses could be similar for the other three public sector Hospitals, but there will also be differences based on the location of the Hospitals and services rendered at these facilities.

6.5.2. Study 2 and 3: The mobile IVF laboratory

Although Study 2 successfully resulted in a functional prototype mobile IVF laboratory, several practical limitations were encountered. The building of the prototype faced constraints related to the size and weight of the trailer, which influenced mobility, storage, and ease of deployment. The quality of construction was also affected by the availability of only a few companies willing to undertake such a unique construction project. The development of the fold-out room required specialised engineering input, but assistance from technical experts was limited, which may have affected the optimisation of the final design. The pilot study performed as part of study 2 was also limited by a small sample size, affecting the statistical power.

The third study demonstrated successful real-world application of the mobile IVF laboratory. Due to the small sample size of the study, the outcome can only be seen as a proof-of-concept, and not a full validation of the mobile IVF laboratory. Procedures were also conducted at a single location, and the ability to move the IVF laboratory from one location to the next, and continue with ART cycles, has not been tested. The operational use and future roll-out of mobile IVF laboratories were outside the scope of this project and therefore remain unaddressed.

6.6. Recommendations

Drawing on the information obtained, recommendations can be made to provide ART treatment to patients via mobile IVF laboratories. Reducing the magnitude of cost barriers through simplified IVF cultures and mobile IVF laboratories should be

investigated further. Policy changes to include infertility treatment in national health benefits should be strongly advocated. The following are recommended for further research and application of the data:

6.6.1. Improving ART in the public sector

The use of the simplified tWE IVF culture system and mobile IVF laboratories can be a game-changing addition to public sector ART provision in South Africa. As highlighted in example 1 in the Discussion section, the current guidelines on how infertility should be managed in the public sector allows for a step-wise referral of patients from diagnostic to therapeutic ART.[46] This strategy directly aligns with the manner in which tWE NPO proposes a tWE laboratory should be designed, with diagnostic centres referring patients up to different levels of ART facilities.[47] Additionally, the use of mobile IVF laboratories in level 3 public health centres were described, where the use of a few mobile IVF laboratories would be able to alleviate the demand on specialised hospitals with ART centres. The availability of experienced medical personnel trained in assisted reproduction is limited.[31] Therefore, mobile IVF laboratories would be ideal to provide ART procedures to patients from different provinces, as a single team of fertility specialist and embryologist could travel with the mobile IVF laboratory to provide ART procedures where needed.

Research in the public sector must be encouraged, and the mandate of scientists to explore and question the *status quo* must be embraced. Private sector collaboration must be fostered to provide actionable assistance, and not just verbal encouragement for other stakeholders to provide solutions. Broader national analyses of ART access should be conducted across all public-sector ART units, to validate whether the findings at SBAH reflect national trends. Qualitative studies can be planned to contact and interview patients who do not return for therapeutic treatment, to understand decision-making, financial barriers, cultural concerns, and psychological factors. This information, in conjunction with the data obtained from study 1, should be showcased to the National Department of Health (NdoH) to encourage their participation on a national level. Renowned role players and stakeholders, such as SASREG and tWE NPO must pressurise the NDoH to take action and improve access to ART.

The rollout of affordable ART options and the use of mobile IVF laboratories will require collaboration from different levels of government. Local hospital management will have to take responsibility for the work performed on site, while provincial government ensure the mobile laboratories are supplied, stocked, equipped and manned with experienced personnel. The NDoH will be responsible for the overhead administration, for a cross-provincial project to be managed with care. Additionally, the NDoH is ultimately responsible to ensure the standard factors necessary to support ART services, whether low-cost or conventional, is provided for. These factors include having up to date and appropriate policies and legislation in place, and provide appropriate funding and good health service infrastructure.[49] Where the NDoH national budget fails to meet the required demands, Private-Public-Partnerships (PPP) should be considered for the industry to take hands with Government.[49] These types of partnerships have been shown to work successfully in improving healthcare dissemination in general through mobile healthcare services, in the form of the Phelophepa Healthcare Train,[50] as well as ART service delivery at the Tygerberg Hospital in Cape Town.[51]

Lastly, the potential impact of the NHI on MAR accessibility, comparing the cost of different scenarios, including the use of simplified IVF, should be investigated. The proposed use of mobile IVF laboratories, and PPP to provide support must be included in these investigations. The mobilization of resources towards ART and the inclusion of ART in the NHI benefits should be advocated for.

6.6.2. Future use of mobile IVF laboratories

Apart from the physical roll-out of mobile IVF laboratories to improve access to ART, either in the private or public sectors, further investigations are needed. By addressing concerns through the provision of evidence-based results and the championing of workable solutions, resistance to change by health-providers and the general public can be addressed. Negative perceptions can affect how a well-designed project is received. Therefore, acceptability and feasibility studies should be performed, to determine medical professionals' and patients' perceptions regarding mobile fertility services compared to static facilities.

Additionally, since the current mobile laboratory was designed and constructed as a prototype, the lessons learned during the process should be embraced and applied for future improvements. The functionality of the fold out room and the weight of trailer should be re-evaluated. The current design of the prototype mobile IVF laboratory can be improved upon with the assistance of experienced engineers or trailer designers. Proposed changed to the design that can be investigated is the use of different types of material for construction, that would still insulate the laboratory to ease temperature regulation, while reducing the total weight of the trailer. Additionally, the fold-out room for the embryo transfer room could be reconsidered. Alternative suggestions could be a modular or pop-up stand connected to the trailer, with canvas sides, eliminating hundreds of kilograms of weight by removing the necessary panels. A single panel to form a solid roof could be considered, to provide a structure for the temporary structure to attach to, and secure connections to prevent any water leakage. An alternative option would be to omit the additional room connected to the mobile laboratory, and to perform embryo transfers in the local facility's procedure room. Equipment required in the procedure room, as well as transport of embryos from the mobile IVF laboratory to the transfer room, could be considered.

Furthermore, the official incorporation of mobile IVF laboratories as a viable option to use must be encouraged. The SASREG accreditation process should be looked at, to accommodate the evaluation of mobile IVF laboratories into their current regulatory system. With the design of the prototype mobile IVF laboratory, care was taken to ensure the laboratory complies with the SASREG guidelines, and when mobile IVF laboratories are used in practice, there should be an option for ART facilities to be evaluated and certified by SASREG when such a laboratory is employed.

The application of mobile IVF laboratories must not be mistakenly approached as if happening in a vacuum, there are many associated aspects that should be considered. Some of these aspects are the use of the simplified tWE IVF culture system, mild ovarian stimulation, and training of medical professionals, to name a few. The simplified tWE IVF culture system has been validated as safe and effective, with favourable perinatal outcomes and significantly lower operational costs compared to conventional IVF.[18,44] Public sector facilities should consider adopting the tWE

simplified IVF culture system, especially for low-resource settings and for patients with mild male factor infertility. Since the simplified IVF culture system is used in the mobile IVF laboratories, the knowledge base would develop over time and more medical professionals would be experienced in the use of this system. At this point, the provincial hospitals can each establish a simplified IVF laboratory on site, and the mobile IVF laboratories can be used to provide services at a lower level of medical facility, such as regional hospitals, and provide training opportunities at these lower level facilities.

The use of mild stimulation has been advocated as one of the major cost saving initiatives for low-cost IVF and is supported by the tWE NPO.[18, 52] This approach has been encouraged to be used with the simplified IVF culture, and can be of benefit when rolling out the use of mobile IVF laboratories.[18] These mild ovarian stimulation protocols result in lower numbers of COCs recruited for development, but can also lower the chances of adverse outcomes due to ovarian hyperstimulation syndrome.[53] The mild stimulation protocols would result in safer practice when ART is performed in level 3 or even level 2 health facilities, when the infrastructure to respond to medical emergencies are not as easily available as at level 4 facilities.

Another recommendation that would not only proof beneficial to improving access to ART through the use of mobile IVF laboratories, but to the field of MAR in general would be a major investment in training of medical professionals in the field of MAR. Not only financial investment, but also time and other resources. The lack of sufficient fertility specialists and embryologists in South Africa is a major concern for the future.[31] This is especially a problem with the expectancy of embryologists to perform an increased number of technically advanced procedures.[54] All over Africa there are “fly-in-fly-out” professionals, who travel to a specific area for a short time period to provide service delivery while they are there.[31, 55, 56] South Africa’s well established MAR industry, in general, does not warrant the use of professionals flying in to perform ART procedures. However, with the field of MAR dominated by the private sector, very few training facilities and experienced trainers are available.[31] The use of travelling professionals might be something to keep in mind for training purposes. Since health professionals not registered with the Health Practitioners Council of South Africa may not practice and provide patient care in SA, institutional permission would have to be

obtained for them to provide training. Alternatively, *in lieu* of professionals traveling from abroad, retired professionals in South Africa could share their knowledge and experience with the younger generation. With the assistance from the NDoH and PPP, the establishment of more training facilities should be encouraged without delay. Such an endeavour would be well placed to incorporate the aid of the tWE NPO. This can be initiated through dedicated training for the operation of mobile IVF laboratories. The current embryologist training centres could incorporate mobile IVF laboratory and tWE simplified IVF culture system training in their standard curriculums, with the assistance from the tWE NPO. Additionally, dedicated tWE training centres can be established, to train embryologist to work on a local, as well as international level. Lessons learned during the COVID-19 pandemic can be of assistance in this instance, where it was realised that not all training and communication has to be in person.[57] Syllabi can be developed where trainees initially study theoretical principals and protocols with an online platform, followed by hands-on practical training at a later stage.

Lastly, the application of mobile IVF laboratories in South Africa, and the array of associated actions that is needed to get the mobile IVF laboratories' wheels rolling, should be seen as an opportunity for other LMICs to adopt similar principles. The use of mobile IVF laboratories should be investigated in all areas where access to ART is limited.

6.7. Conclusion

This thesis set out to investigate access to MAR in the public sector of South Africa and to explore an innovative, practical solution to expand the provision of ART to patients in the lower income brackets. The study substantiates with location specific data that access to ART must be improved in the coverage area investigated. This information can be applied to a major section of the South African landscape. The findings provide quantitative evidence for facts that has long been assumed but rarely demonstrated, i.e. that affordability and geographic accessibility remain major measurable determinants of ART access in South Africa.

In response to these barriers, the next component of the thesis reported on the successful development of a novel mobile IVF laboratory based on the simplified tWE

culture system. Building on this foundation, the project is completed by provided proof-of-concept data that IVF cycles, including oocyte insemination, embryo culture, embryo transfer, and cryopreservation, can be performed in a mobile IVF laboratory in a rural clinical setting. The pioneering project resulted in the first live births achieved from embryos cultured in a mobile IVF laboratory, marking an important milestone both locally and globally. Collectively, the outcome of the studies creates a coherent narrative. Limited ART availability and steep financial barriers exclude a substantial portion of the population from receiving fertility treatment. Simplified, low-cost mobile IVF laboratories offer a realistic, evidence-based strategy to improve access to ART.

Importantly, this thesis contributes a world-first demonstration of mobile IVF feasibility, positioning South Africa as a potential leader in developing context-appropriate ART solutions for low- and middle-income countries. Mobile IVF laboratories, when combined with simplified stimulation protocols, have the potential to transform MAR provision by reducing cost, decentralising laboratory services, and serving multiple clinical sites without duplicating infrastructure.

The work also lays foundational knowledge for policymakers and health systems planners seeking to incorporate innovative models of care delivery into national reproductive health strategies. The concept of mobile IVF laboratories creates new opportunities to provide improved access to ART, should there be the will and drive to take this innovative idea and build on it. Through collaboration of the public and private sectors, and the combination of low-cost IVF, mild ovarian stimulation and mobile IVF laboratories, access to ART in South Africa can be improved. Reflecting on the words by Neil Armstrong, “one small step for a man, one giant leap for mankind”,[58] the first prototype mobile IVF laboratory might just be a step in the right direction to provide global access to ART in the future.

6.8. References

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Chapter 7: Annexures

7.1. Annexure A: Co-authored articles

7.1.1. Multiyear outcomes of a simplified IVF culture system

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ELSEVIER

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
Accessible IVF
Assisted reproduction
ICSI
Infertility care
Low- and middle-income countries
Simplified IVF

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 (TWE), 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

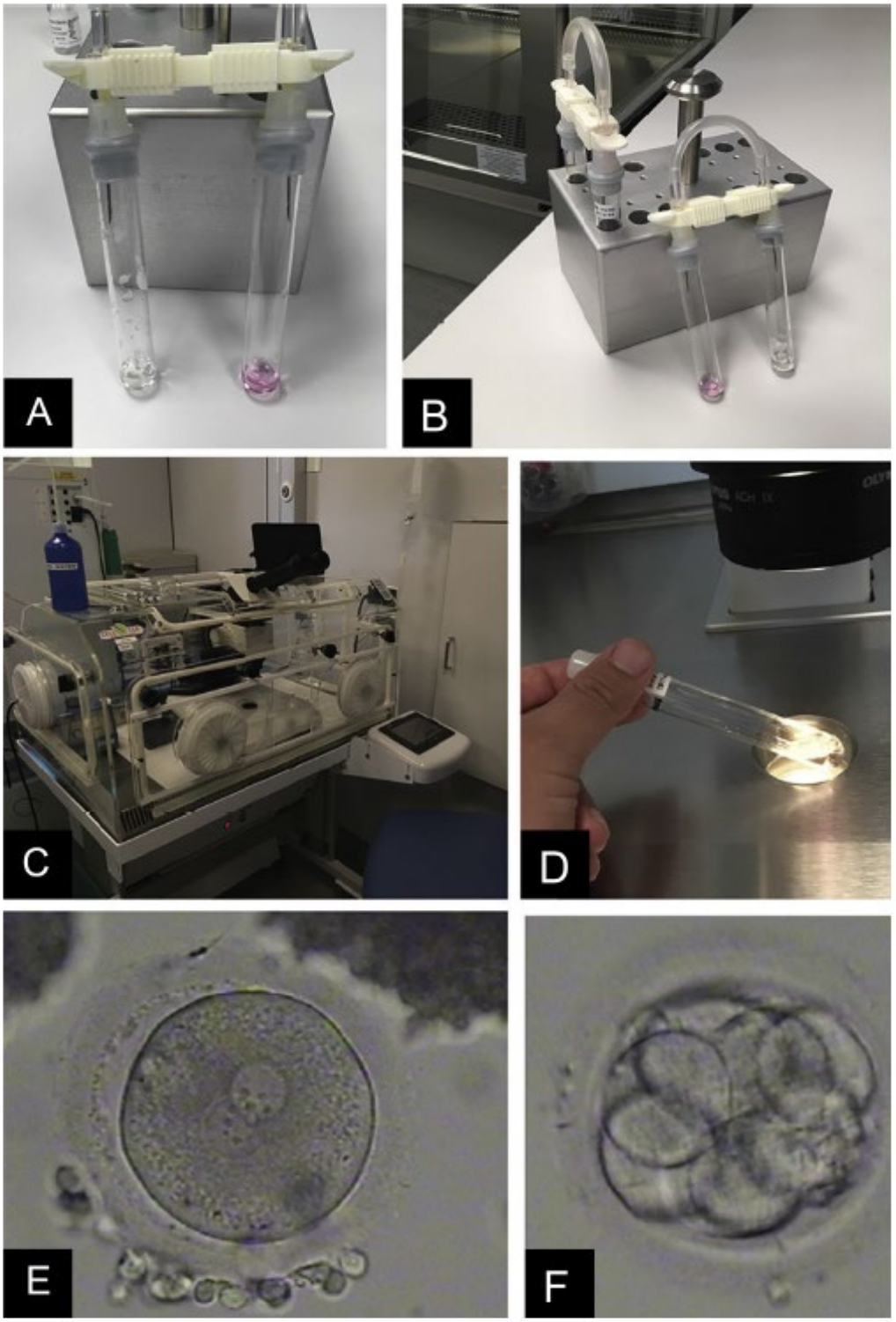

FIGURE 1 The 'WE lab or SCS system: to achieve media equilibration a connection is made between tube 1 with citric acid and sodium bicarbonate in water and tube 2 with IVF culture medium (A), incubated overnight at 37°C in a heating block (B). It transfers enough CO_2 gas produced by the chemical reaction 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 1WE 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.

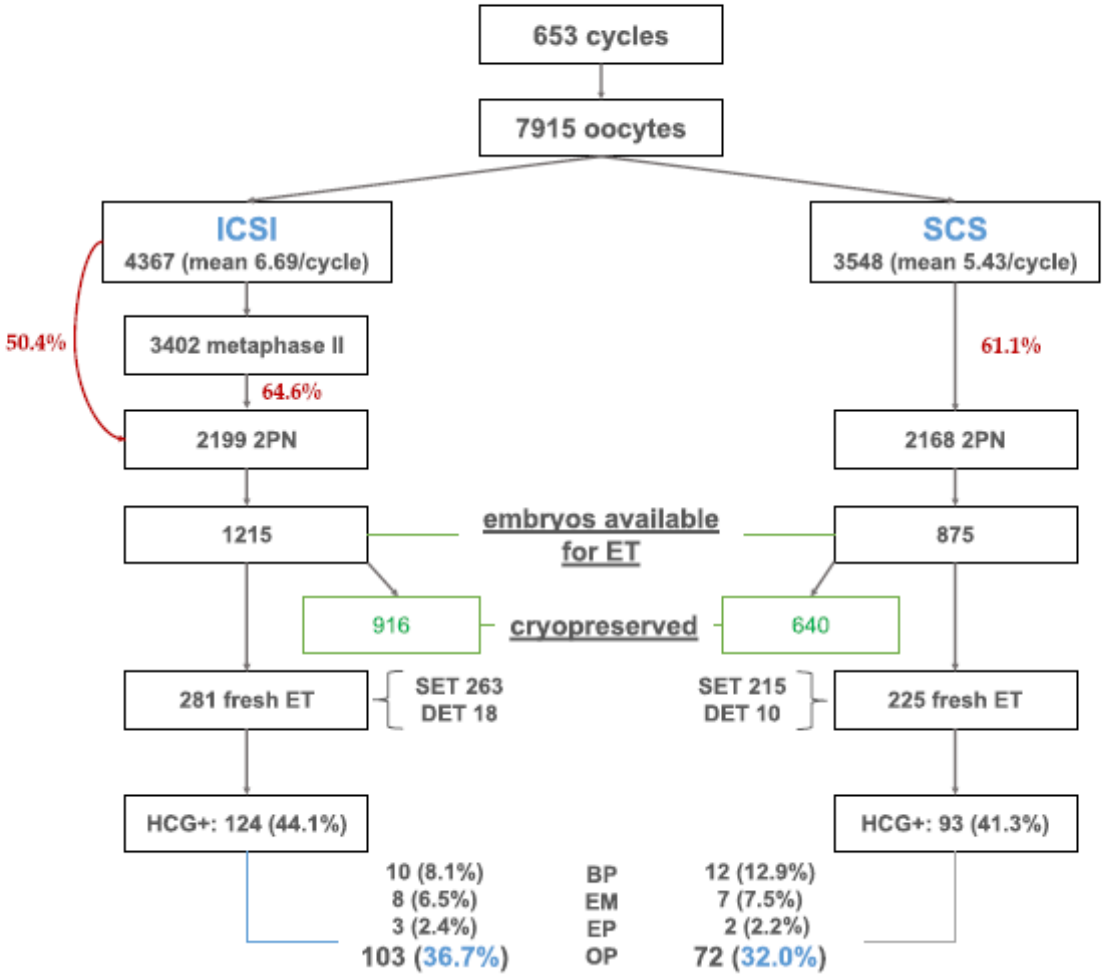

FIGURE 2 Flow chart of the study including 653 cycles. For BP, EM and EP the denominator was HCG+. 2PN = normal fertilization with two pronuclei; BP = biochemical pregnancy; DET = double embryo transfer; EM = early miscarriage (<12 weeks of gestation); EP = ectopic pregnancy; ET = embryo transfer; ICSI = 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 (^{5}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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7.1.2. Now is the time to introduce innovative ART in resource-poor countries

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Opinion

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

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ABSTRACT

Nearly 200 million people worldwide suffer from infertility. Disparities exist between developed and developing countries due to differences in the availability of infertility care, different reimbursement policies and socio-cultural differences surrounding procreation. In low- and middle-income countries, specialized infertility centres are either scarce or non-existent, mostly in private settings, and accessible only to the fortunate few who can afford them. The success and sustainability of ARTs will depend on our ability to optimize these techniques in terms of availability, affordability, and effectiveness. A low-cost, simplified IVF system has been developed and shown to be safe, cost-effective, and widely applicable to low-resource settings. Combined with inexpensive mild ovarian stimulation protocols, this could become a truly effective means of treating infertility and performing assisted reproduction at affordable prices, but only if such programmes are sincerely desired and supported by all relevant stakeholders. A receptive political, governmental, and clinical community is essential.

Keywords: accessible / affordable / assisted reproduction / infertility care / LMICs / low- and middle-income countries / simplified IVF

Introduction

Infertility is one of the most common chronic diseases in individuals of reproductive age, affecting roughly 8–12% of populations worldwide (Boivin et al., 2007). It is estimated that approximately one in every six individuals of reproductive age worldwide is affected by infertility (World Health Organization, 2023). Collective estimates from a comprehensive systematic review of lifetime and period prevalence of 12-month infertility were 17.5% and 12.6%, respectively (Cox et al., 2022). By using a different methodological approach, Mascarenhas et al. (2012) calculated that 70 million couples worldwide will require some degree of medical assistance to achieve pregnancy. The prevalence of infertility appears to be higher in low- and middle-income countries (LMICs), with rates as high as 30–40% reported in some regions of sub-Saharan Africa (Ombelet et al., 2008; Inhorn and Patrizio, 2015; Polis et al., 2017; Legese et al., 2023).

Infertility is often most prevalent in regions with high fertility rates, a demographic paradox known as 'barrenness amid plenty'. The United States Agency for International Development (USAID) estimated that there are more than 200 million women and girls with unmet needs for contraception each year. Unmet need is defined for women who want to delay or stop childbearing. Particular challenges regarding contraception include lack of access due to the absence of appropriate health services, fear of side effects, fewer method options, and 'stock-outs' of contraceptive supplies (Schivone and Blumenthal, 2016). For adolescent African girls in particular, this partly explains why fertility levels are high, especially in rural areas.

Although primary infertility is generally known to have the highest burden of disease, secondary infertility also applies to many who have become pregnant but have subsequently experienced a pregnancy loss or death of a child (Inhorn and Patrizio, 2015). Some studies have reported significantly higher rates of secondary infertility, compared with primary infertility, in certain regions such as Africa, where rates of infection-related infertility from postpartum infections or unsafe abortions are high (Larsen, 2000; Sharma et al., 2009).

The impact of infertility and unintended childlessness in LMICs tends to be much more pronounced than in Western societies, particularly for women. In these fundamentally pronatalist contexts, childless women are often stigmatized, isolated,

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ostracized, disinherited, and neglected by the whole family and not infrequently, by their local community. This can lead to polygamy, divorce, intimate partner violence (IPV), isolation, economic instability, banishment, and even suicide in some cases (Dyer et al., 2002, 2004, 2005; Ombelet et al., 2008; Inhorn and Patrizio, 2015; Chiware et al., 2021; Wang et al., 2022). In many LMIC societies, women are completely dependent on children for economic survival. The birth of a child can secure a marriage, guarantee property and inheritance rights, and/or provide social security in old age (Dyer and Patel, 2012; Editorial, The Lancet Global Health, 2022). Childlessness must therefore be seen as a social and public health issue, additional to the individual medical problem, and treated as such with compassion and dignity.

Despite the severe burden associated with childlessness in most LMICs and notwithstanding the recognition, concerns, and often recommendations expressed by national and international health organizations and private humanitarian and philanthropic programs, barriers to infertility care persist and thus it remains a low priority for local politicians, community leaders, and healthcare providers (Ombelet et al., 2008; Chiware et al., 2021; Gerrits et al., 2023; Whittaker et al., 2024). Typically, the absence of affordable fertility services in LMICs has been justified by arguments of overpopulation and limited resources, resulting in inequitable access to infertility treatment compared to developed countries (Ombelet et al., 2008).

Many articles in the most influential journals that address the problems of imbalanced access to ART in LMICs focus mainly on 'awareness campaigns' to prevent infertility in susceptible individuals and destigmatising of infertility. Policymakers are coaxed to increase societal funding and to create sustainable multidisciplinary and multi-stakeholder consortia to improve equity of access (Starrs et al., 2018; Afferri et al., 2022; Fauser et al., 2024). Strangely, almost all of these articles barely discuss the essential reason for inequalities in access to ART in LMICs: the high cost. Advanced ART methods require highly skilled personnel, expensive equipment and consumables, and rigorous maintenance. Once the logistical, pecuniary competitive interests, and costs to build and operate ART programs to high-resource country standards are realized, the good intentions, apparent enthusiasm and verbal support by the aforementioned groups and local governing bodies dissipate. Although it seems self-evident, the obvious solution to eliminating these barriers and rekindle enthusiasm for expanding access to treatment for those under-served is to reduce logistical and operating costs. While this solution sounds simplistic, the implementation of it is complex, although entirely feasible, as discussed in the following segment.

According to a WHO-initiated systematic landscape analysis, one of the most important limitations when considering implementation options for low-cost ART in LMICs is the lack of high-quality outcome-based trials (Chiware et al., 2021). They concluded that affordable ART initiatives should be evaluated for efficacy and safety through robust research and further adapted to local infrastructure. Despite the fact that a number of high-quality studies have been published reporting on the efficacy, safety, cost-effectiveness, and successful outcomes of using a simplified, low-cost IVF system (Ombelet et al., 2022a,b, 2023a,b), interest in this approach remains remarkably low among infertility specialists, societies, NGOs, foundations, and industry and healthcare managers. A 2022 Editorial in The Lancet Global Health mentioned that ESHRE called for action to give adequate attention to the issue of infertility in developing countries (Editorial, The Lancet Global Health, 2022). Indeed, in 2008, the ESHRE special task force on 'Developing Countries and Infertility' held an expert meeting in Arusha, Tanzania, resulting in a 'Human Reproduction Monograph' stating: 'After a fascinating period of almost 30 years of IVF and 15 years of ICSI, we must admit that only a small part of the world population benefits from these new reproductive technologies. Time has come to give adequate attention to the issue of infertility in developing countries' (Ombelet, 2008). The editor questioned why this call for action has not led to a wake-up call and a better and more effective approach to this problem over the next 15 years.

An international call for affordable infertility care in LMICs

The right of persons to access infertility treatment is a human dignity that was confirmed in consecutive UN international statements (United Nations 1948, 2014, 2000). Article 12 of the International Covenant on Economic, Social and Cultural Rights also acknowledges the right of everyone to the highest attainable standard of physical and mental health (United Nations, 1966).

In a fact sheet published in 2021, the World Health Organization (WHO) recognizes that the provision of quality family planning services, including fertility services, is one of the core elements of reproductive health (World Health Organization, 2021, 2024). This very important message includes a necessary requirement and desire to work with interested and relevant stakeholders to provide fertility services globally and to provide technical assistance at a country level to Member States in order to develop or strengthen the implementation of national fertility policies and services.

Although addressing infertility is fundamental to realizing the right of individuals and couples to establish a family (Mburu et al., 2023), political declarations and commitments need to be followed by action, and progress towards these goals still remains virtually non-existent. Important international non-profit organizations (NPOs) including Family Health International, International Planned Parenthood Federation and The Population Council still focus primarily on the prevention of unwanted pregnancies, safe motherhood, and the reduction of unsafe abortions, as well as the prevention of sexually transmitted infections (STIs) and HIV/AIDS. Even today, the implementation of affordable infertility care in LMICs is not a priority for these organizations despite the fact that this message is increasingly cited in the most influential scientific journals (Vayena et al., 2009; Murage et al., 2011; Hammarberg and Kirkman, 2013; Inhorn and Patrizio, 2015; Starrs et al., 2018; Chiware et al., 2021; Fauser et al., 2024).

Causes of infertility in LMICs

Infertility may be caused by female and/or male factors or may remain unexplained. Female factors include advanced reproductive age and the resultant diminished ovarian reserve, chronic anovulation, and tubal factor infertility or other pelvic pathologies such as endometriosis, adenomyosis, and uterine congenital anomalies. Male infertility can result from impaired sperm production due to a variety of underlying conditions including hormonal, infectious, genetic, and environmental aetiologies (World Health Organization, 2023).

The causes of infertility vary according to country/region. In many LMICs, from Asia to Latin America and Africa, infection-related tubal blockages are an important cause of female infertility as a result of poor obstetric and postpartum care, untreated

STIs, unsafe abortions, and harmful cultural practices (Ombelet et al., 2008; Ombelet and Onofre, 2019).

Particularly in sub-Saharan Africa, STIs are the most common cause of female and male infertility. As tubal factor infertility and severe male infertility are best treated with expensive IVF-related procedures, we should be aware that the most expensive form of treatment is usually what is needed in the majority of cases in the poorest countries.

Lack of affordable infertility care in LMICs

Treatment of infertility is generally not prioritized in national population and development policies or reproductive health strategies of LMICs and is rarely covered by public health financing (Afferri et al., 2022). Severe or life-threatening conditions rightly take precedence over expensive fertility treatments. However, these priorities fail to recognize the severe psychosocial and economic burden of infertility in LMICs (Inhorn and Patrizio, 2015; Chiware et al., 2021) and the options for providing such care at an affordable cost.

Access to healthcare is disease-specific and is determined by both the demand for and supply of such services. Affordability to consumers is a strong driver for access. Affordability can be changed by: (1) reducing the cost and complexity of infertility interventions, (2) providing reimbursement policies, and (3) increasing the disposable income of individuals. Currently, it is apparent that while only the cost of treatment and reimbursement policies are amenable to policy intervention, the implementation of innovations that can expand access and reduce inherent costs remains inadequate.

On average, ART costs range between 10 000 and 20 000 USD in the USA and between 3000 and 6000 USD in most LMICs, with substantial variations between and within countries (Njagi et al., 2023). These costs may be direct or indirect; according to Huyser and Boyd (2013), one-third of these direct costs are linked to laboratory expenses and almost one-third are explained by medication costs. Indirect cost includes costs due to complications (multiple pregnancies, thrombo-embolic diseases, ovarian hyperstimulation syndrome, etc.) and can be considerable as they are typically borne by the community as a whole.

To meet a population's need for ART, it has been estimated that, annually, at least 1500 couples per million inhabitants should have access to IVF (Fauser et al., 2002). This is only possible if adequate ART centres are available, and the costs associated with ART treatment are within reasonable limits, which is surely not the case in LMICs (Makuch and Bahamondes, 2012; Ombelet and Onofre, 2019; Chiware et al., 2021; Afferri et al., 2022).

Introduction of low-cost initiatives for LMICs

To achieve universal access to ART, it is crucial to develop low-cost ART models with simplified diagnosis and treatment protocols, while maintaining quality of care. What are the barriers to making ART less expensive? ART is a booming business; the global market was valued $34.7 billion in 2023 and is expected to reach $62.8 billion in just 10 years (Editorial, The Lancet, 2024). This growth is driven by increasing demand for fertility treatments, 'add-on' treatments and advancements in reproductive technologies such as preimplantation genetic testing for aneuploidy and monogenic mutations. However, while the 'fertility enterprise' continues to grow, access to IVF remains limited or non-existent for most of the world's population because of high treatment costs. In more than half of LMICs, the direct cost for one ART cycle is higher than the average annual gross domestic product per capita (Njagi et al., 2023). Better access to effective and safe patient-centred and evidence-based treatments is needed. A profit-driven fertility industry cannot continue to exploit the vulnerability of people desperate to have children (Editorial, The Lancet, 2024). Therefore, it is evident that low-cost initiatives are urgently needed to meet the demand.

Non-IVF treatment options

In the era of IVF, we sometimes overlook many other infertility treatment strategies. Ovulatory dysfunction represents almost 20% of female infertility and can be treated effectively with a low-dose ovarian stimulation regimen using clomiphene citrate and/or gonadotrophins combined with timed intercourse. In subfertile women with anovulatory polycystic ovary syndrome (PCOS), letrozole or laparoscopic ovarian drilling is recommended (Franik et al., 2022). For women younger than 40 years with unexplained infertility or moderate male infertility, three to six cycles of IUI with mild ovarian stimulation should be recommended as a first-line therapy, provided tubal patency has been documented and a strict cancellation strategy is followed to avoid multiple pregnancies (Cohlen et al., 2018). Endoscopic surgery can be a valuable and cost-effective solution in case of moderate and severe endometriosis, uterine malformations, PCOS, intrauterine adhesions, etc. Although these treatment options are very valuable, a large proportion of the infertile population will ultimately only be assisted through IVF-related techniques. For these patients, low-cost ART services are needed and can be largely achieved with a lower price tag achieved by using mild ovarian stimulation protocols and simplified IVF laboratory design and procedures.

Implementation of simplified low-cost ART services

Mild ovarian stimulation

The value and effectiveness of less expensive and less intensive mild ovarian stimulation protocols in ART settings have been proven (Nargund and Fauser, 2020; Nargund et al., 2022). The use of clomiphene citrate or tamoxifen with or without low-dose recombinant FSH or hMG (gonadotrophins) can be an affordable alternative with acceptable results in all categories of women compared to conventional stimulation IVF, with the added benefit of minimal side effects and a very low complication rate (Ferraretti et al., 2015; Datta et al., 2020, 2021; Gianaroli et al., 2022; Nargund et al., 2022). Beside harm minimization, this approach also reduces the cost for the public health system.

Simplified IVF laboratory procedures

In 2014, we published the results of using a novel simplified IVF culture system (SCS) (Van Blerkom et al., 2014). This system avoids logistical issues common in high-complexity programs (e.g. supply chain disruptions and on-time delivery of disposables such as culture vessels and media), the high costs of medical gases, and the complex incubation equipment and infrastructure typical of IVF laboratories in high-resource settings (Fig. 1). IVF insemination was also addressed whereby only 2000–5000 motile washed spermatozoa showed successful outcomes with virtually no fertilization abnormalities such as dispermic penetration, which means this system can be used in cases of mild and moderate male infertility in lieu of ICSI with similar outcomes (Van Blerkom et al., 2014; Ombelet et al., 2022b). In the case of severe

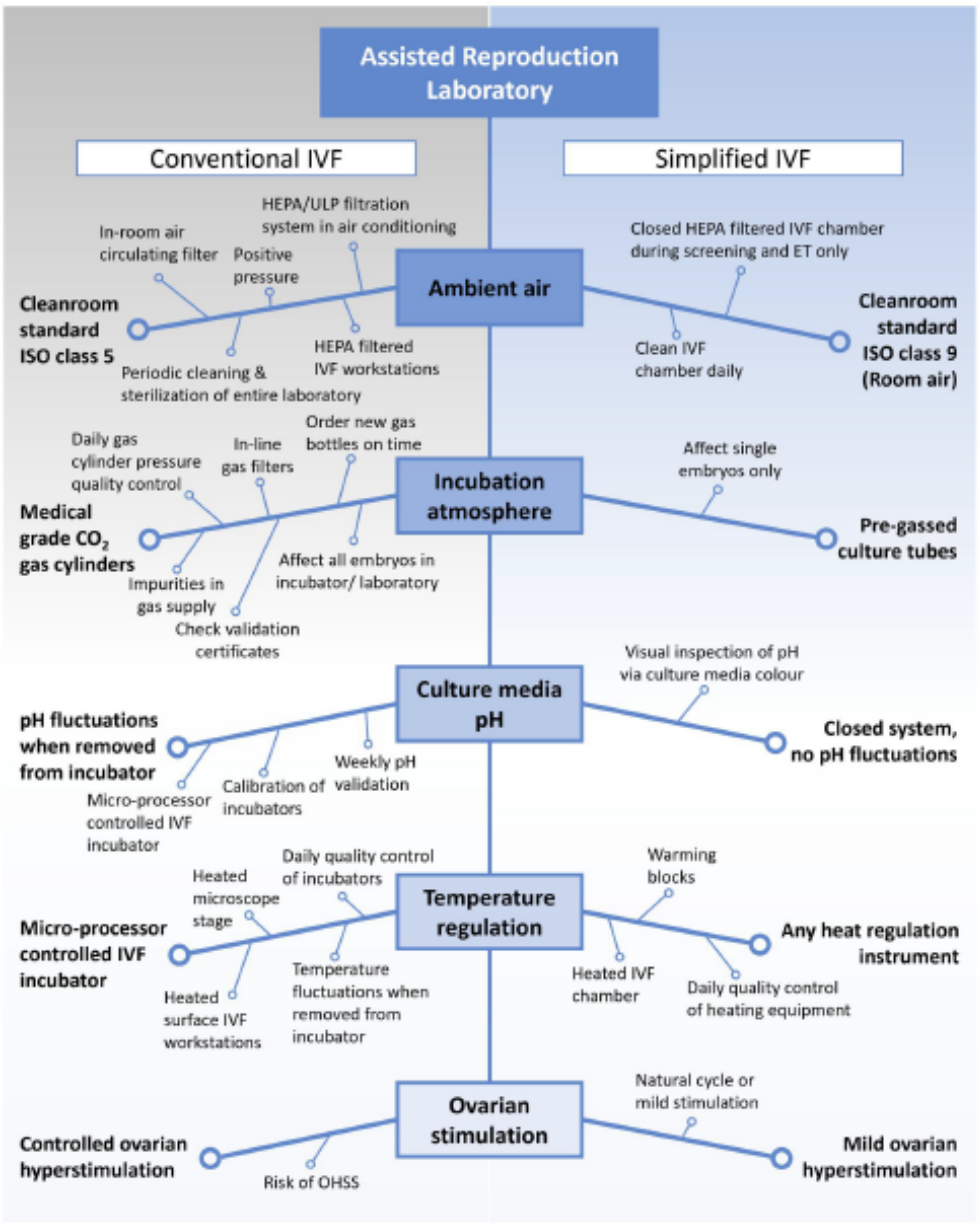

Figure 1. A comparison of the simplified culture system with conventional IVF culture, showing various required parameters that need to be controlled.

male infertility where too few motile and morphologically normal spermatozoa are obtained after processing for conventional IVF, ICSI would likely be appropriate in these specific instances. In the simplified system, preimplantation embryogenesis from insemination to transfer or for cryopreservation, is undisturbed and occurs within the same culture tube. This avoids many of the problems often encountered in regular IVF laboratories, such as unwanted temperature changes, air quality problems, as well as prolonged examination for fertilization and stage-appropriate developmental progression, especially where costly time-lapse incubation systems are unavailable or affordable.

With the combination of mild ovarian stimulation, fewer embryos are expected, but even so, there would be excess embryos from time to time. Cryopreservation of embryos has become both commonplace and a cost-effective necessity that can improve the likelihood of a successful outcome in infertility treatments. While we have demonstrated that successful outcomes from cryopreservation can be achieved using the SCS approach (Ombelet et al., 2014), its use in an SCS/WE program might seem counterintuitive for low costs and a significant negative factor for adoption. This would be a relevant concern if the controlled rate freezing with expensive programmable instrumentation was

still in use. By contrast, vitrification with demonstrably higher success rates has significantly simplified the cryopreservation process in terms of materials, laboratory personal training, time and effort, and with respect to outcomes, it is a cost-effective option when a fresh embryo transfer fails.

We recently reported the results of a prospective non-inferiority study comparing ICSI and SCS using sibling oocytes. No differences in ongoing pregnancy rate, implantation rate, and miscarriage rate between SCS and ICSI were detected in investigating 653 SCS/ICSI cycles (Ombelet et al., 2022b). In the same patient cohort, we observed similar perinatal outcomes for babies born after SCS and ICSI, both in fresh and frozen-thawed cycles (Ombelet et al., 2022a). In another study, it was shown that for SCS singletons, the preterm birth (PTB) and low birthweight (LBW) rates were significantly lower compared to a large cohort of all babies born after conventional IVF in Belgium during the same study period (Ombelet et al., 2023b). When comparing the PTB and LBW rates of the SCS singletons with all 553 865 singletons conceived and born in Flanders, the PTB and LBW rates were found to be similar to those of singletons born after natural conception. One potentially important finding was that compared to babies born after ovarian stimulation and IVF/ICSI, SCS singletons had lower PTB and LBW rates (Ombelet et al., 2023a). These studies provide sufficient evidence that the SCS technology is a safe, effective, and successful low-cost method and well-suited for the purpose for which it was designed: implementation in low- and moderate-resource settings where most infertile couples reside.

From a cost–benefit point of view, the differences in costs when setting-up, managing and maintaining a conventional high-tech IVF laboratory versus a SCS laboratory were examined. The discounted cash flow method (DCF) was chosen to evaluate the investments. The results showed that the SCS laboratory clearly presented the highest net present value (NPV) and was identified as the most attractive investment for its purpose (Christiaens, 2018).

In the Science Museum in London, and on the occasion of the celebration of 40 years of IVF in 2018, an exhibition called 'IVF: *Six Million Babies Later*' was opened where visitors can see the equipment used in the first human IVF lab alongside the new 'shoebox' or SCS culture and incubator equipment 'designed to dramatically reduce the cost and improve the accessibility of IVF'. The two culture systems are fundamentally similar, as the new low-cost system uses the combination of a simplistic back-to-basics approach in regards to disposables and protocols, and 40 years of experience and improvements in culture media and knowledge of important steps and quality control measures. The results with the SCS system are similar to those reported by contemporary high-resource IVF programs, indicating that costly and complex instrumentation is not always required to achieve successful outcomes; nor does the location of the IVF laboratory need to be fixed.

In October 2023, the first mobile unit for the Walking Egg Project (Dhont, 2011; Ombelet, 2014) was unveiled in Pretoria, South Africa (Fig. 2). The Walking Egg non-profit organization was founded in 2010 with the aim of raising awareness of childlessness in resource-poor countries and making infertility treatment in all its aspects, including ARTs, available and accessible to a much larger portion of the population. The mobile unit, as it was shown in Pretoria, contains all the equipment needed for simplified IVF, including an embryo transfer room. The mobile unit was designed to comply with all necessary quality requirements of an IVF laboratory, ensuring conventional IVF standard culture provided in a different manner (Fig. 1). In combination with a facility providing a procedure room for oocyte retrieval, the mobile unit can perform most ART procedures, including semen analyses and processing with cryopreservation, IUIs, oocyte retrievals with cryopreservation or IVF insemination, and embryo culture followed by embryo transfer or cryopreservation, as well as subsequent embryo thawing and transfer.

The conversion to mobile laboratories would enable a larger population to access ART services and provide these services to economically disadvantaged infertile couples in resource-poor communities or regions without specialized infertility centres. Prospective studies investigating mobile IVF outcomes are currently on-going.

Training and data collection

Training, quality control, regular audit, and systems of accreditation and registration need to be in place to maintain appropriate standards of care and external verification of outcomes, as is generally practiced in IVF programs, as noted below. Training will need to be supported by experts in the field who are to deliver appropriate courses at the highest level in a very short period of time. While the levels of experience of trainees, the quality of facilities, and health policies and regulations can be country-specific, we do not consider this to be a barrier to adoption as much can be effectively done in a preliminary manner online with in-person follow-up prior to patient implementation of the SCS. In this context, while the implementation of low-cost infertility treatment in LMICs may sound daunting, we have found, for the SCS, that with a reasonable training program specifically designed for intended embryologists, the necessary experience and expertise can be attained. Training will not only involve laboratory personnel but also medical, paramedical, and administrative members of the IVF team. An effective online capacity can be available such that issues associated with oocyte quality, fertilization, or embryo development can be viewed and discussed remotely with members of the Walking Egg Program (Van Blerkom et al, 2019), so no centre implementing this system can be without external expertise if and when needed.

Given the lack of access to national ART data in most LMICs, we suggest that for centres providing low-cost infertility treatment, registration of all cycles in a national or international registry, preferably using an online system, is required from the outset. Regular audits and systems of accreditation and registration should be implemented to maintain appropriate standards of care.

Declining global fertility rates and family building

The GBD 2021 Fertility and Forecasting Collaborators (2024) recently published a comprehensive demographic analysis with forecasts to 2100. They concluded that 'by 2100, the largest concentrations of livebirths will shift to low-income settings, particularly a subset of countries and territories in sub-Saharan Africa, which are among the most vulnerable to economic and environmental challenges. Extreme shifts in the global distribution of livebirths can be partially ameliorated by improved female education and met need for modern contraception'.

This indirectly underlines the fact that infertile couples in these countries can expect little help in the future and that their problem is completely unaddressed, despite the undeniable need for reproductive justice for people suffering from infertility in LMICs. The number of babies that would be added to this

Figure 2. First performance and show of The Walking Egg mobile IVF unit in Pretoria, South Africa on 28 October 2023.

population as a result of ART is minuscule, yet the suffering of people is being completely ignored.

The need for funding and support of all main stakeholders

Infertility is a reproductive health concern deserving attention, as confirmed by the 2018 report of the Guttmacher-Lancet Commission on Sexual and Reproductive Health and Rights (Starrs et al., 2018). Fathalla et al. (2006) have already argued that sexual and reproductive health for all is an achievable goal if cost-effective interventions can be properly scaled up, if political commitments are revitalized, and if financial resources are mobilized, allocated rationally and used more effectively.

All international fertility societies and organizations are nowadays appealing for action to increase access to infertility care and ART in LMICs, but governments and sexual and reproductive health rights (SRHR) organizations tend to neglect infertility. We still observe a lack of commitment or willingness to take advantage of low-cost IVF options even from the very same sources that champion women's 'reproductive rights' based on 'social justice' (Van Blerkom et al., 2019). Despite the undeniable value of prevention programmes and awareness campaigns, we believe that the most obvious and timely approach to increase accessibility to proper infertility management in LMICs is to reduce costs significantly. Soon, one cannot expect that insurance companies and governments will reimburse ART in LMICs unless we can prove that we can provide high-quality and successful infertility care at a reasonable price, with special attention to avoid complications such as multiple pregnancies and OHSS which contribute very high societal costs.

This also means that we urgently need funding to perform research on the effectiveness, safety, and costs associated with the implementation of low-cost fertility centres in LMICs.

The need for funding is crucial and is likely to require input and collaboration from various private, public, and governmental participants who, by necessity, have a central role in the implementation of this endeavour. Funding is needed for: (1) the fixed costs of establishing and operating new fertility centres and, where appropriate, mobile units, (2) training the medical, paramedical, and administrative staff, and (3) educating the public, which implies contacts with schools, politicians, traditional healers, and the media, as appropriate for each country. The most important recommendations for setting up low-cost IVF centres in LMICs are summarized in Table 1.

We sincerely hope and expect that the highly profitable medical and pharmaceutical industries that have supported IVF in high-resource countries will continue to do so by making relevant contributions, (such as providing drugs at low cost, producing basic ultrasound and laboratory equipment at low cost) and participate in the expansion of the novel mobile units described above. As the fertility industry in LMICs evolves, there is a risk that the focus will shift from evidence-based and patient-centred practice to shareholder returns and business growth. However, a for-profit fertility industry cannot continue to ignore the vulnerability of people who desperately want to have children (Editorial, The Lancet, 2024).

According to an article in the 22 July 2023 issue of The Economist, developing technologies to make IVF more affordable could potentially increase the number of IVF babies worldwide from the current 64 000 per month to more than one million per month, which would clearly be a boon to those it is intended to serve (The Economist, 2023). In May of 2024, the WHO published another factsheet on infertility. Although they mention that 'ART technologies are still largely unavailable, inaccessible, and unaffordable in many parts of the world, particularly in LMICs', the crucial issue of lowering the costs associated with ARTs is not mentioned at all (World Health Organization, 2024).

Conclusions

In the majority of LMICs, access to well-organized, high-quality fertility centres is woefully lacking and, when available, is too expensive for the vast majority of the population. We need to stop complaining and lamenting about the lack of attention, interest, and action to alleviate infertility where it is desperately needed, and test and use the currently available solutions that can substantially increase access to ART by making diagnosis and treatment affordable without loss of quality. Non-IVF ART treatment options should be the first choice in selected cases, and if IVF-related procedures are required, the combination of inexpensive mild ovarian stimulation protocols and simplified IVF systems will greatly increase access.

Effective and accessible low-cost ART can only be successfully introduced if there is political will and economic and community

Table 1. Overview of the most important recommendations to consider when starting low-cost IVF centres in low- and middle-income countries.

Community
The community/region should be empowered to support the program.
Information to the community must be discrete and applicable, taking into account socio-cultural and religious differences.
The integration of family planning, safe motherhood care, and infertility services should be pursued.
Low-cost IVF centres
Locations for pilot-projects need to be decided.
A business plan with clear cost structures must be formulated.
Personnel and support services
Sufficient trained personnel must be available to reliably offer ART services without interruption.
A training programme for medical and paramedical staff should be designed if needed. This implies a careful and strict follow-up and regular audits,
Support for low-cost IVF centres must be available to have a ready supply of appropriate disposable items and medication, along with advice from experienced experts in the relevant fields.
The implementation and roll-out of a low-cost IVF centre must be planned to fall within the local framework of healthcare providers, with access to referral services for more advanced cases.
ART protocols, equipment, and disposables
The application of ART should be designed to be practical, repeatable, and efficient.
Equipment should be basic, purpose-made, and robust.
IVF laboratory products should be ready-to-use and should have a long shelf-life.

support. Particularly in LMICs, strengthening infertility services and integrating them with contraceptive and maternal health services within public health structures is essential. Now is the time for action to begin implementation where it is most needed, as it is important to recognize that the choice to have children, even when it requires assistance, is encompassed in the concept of reproductive rights.

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Authors' roles

All authors made substantial contributions and have read and approved the final version of the manuscript. W.O. and J.V.B. had the initial idea for this commentary and are responsible for conception, visualization, writing the original draft, and revising the final manuscript. G.B., C.H., F.L., G.N., H.S., K.V., and R.C. revised the manuscript critically and added additional information and references. G.B. and C.H. prepared the tables and figures. All authors approved the final version of the article to be published.

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Opinion

7.2. Annexure B: Study information and informed consent documents

7.2.1. Study 2 Pilot study informed consent

INFORMATION LEAFLET AND INFORMED CONSENT FOR NON-CLINICAL RESEARCH

TITLE OF STUDY

Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

Setup and implementation of tWE laboratory: Quality control through the culture of non-viable embryos

1) INTRODUCTION

You are invited to participate in the research study for a Doctoral degree (PhD). This information will help you to decide if you would like to participate. Before you agree to take part in the study you should fully understand what is involved. If you have questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator Mr G. Boshoff. You should not agree to take part unless you are completely satisfied about all the procedures involved.

2) THE NATURE AND PURPOSE OF THE STUDY

The objective of this study is to confirm the use of an *in vitro* fertilization (IVF) laboratory, using an easier and cheaper system of growing human embryos, called the tWE laboratory. This laboratory system has been tested in Belgium since 2013, and used in Ghana since 2018, to help couples become pregnant. The Belgian group has reported results from this testing to be very favourable, with pregnancy rates being the same as standard IVF. *This laboratory system will be used in South Africa to have a more affordable IVF option available to patients.*

You as a participating couple would provide the cells needed to perform this study. These cells will be eggs that have abnormally fertilized during your IVF attempt, that cannot be used to form a healthy baby.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study involves using the tWE laboratory to grow embryos. Since you are already undergoing an IVF procedure, you will go ahead with this procedure without any change. As part of this process, eggs and sperm will be taken from you as a couple. After the eggs have been removed and the sperm added to them, the normal fertilized eggs will be grown to form embryos for your use, in the standard IVF laboratory.

Should there be eggs with abnormal fertilization (where more than one sperm has entered the egg), making them genetically abnormal and *not usable for transfer back into you*, these eggs will be used to test the tWE laboratory. These abnormal fertilized eggs will be grown in the tWE laboratory for five days.

4) RISK INVOLVED

There is no risk in participating in the study. You will be asked to give permission that abnormally fertilized eggs, *that cannot be used further*, become available for use in the tWE laboratory. These embryos grown in the tWE laboratory are abnormal and cannot be used to make a baby and will be thrown away after the study is completed, according to standard protocol (RBL SOP F2.4.1 Day 0-5 Embryo development evaluation).

5) POSSIBLE BENEFITS OF THE STUDY

The investigation of the tWE laboratory will give information on the way embryos grow in this laboratory. This information will be used to motivate this laboratory to be permanently placed at Steve Biko Academic Hospital. Other hospitals in South Africa can also use this type of embryo culture system in the future. This will help patients that cannot afford standard IVF to also attempt to fall pregnant with the help from a laboratory using this system.

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Your participation in this study is entirely voluntary and **you can refuse to participate or stop at any time without stating any reason.** Your withdrawal will not affect you in anyway.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This study has received written approval from the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria. A copy may be obtained from the investigator should you wish to view it.

8) INFORMATION AND CONTACT PERSON

The contact person for the study is Mr G. Boshoff. If you have any questions about the study, please contact him at Tel: 012 354 2061.
Alternatively, you may contact his supervisor Prof. C. Huyser at Tel: 012 354 2208.

9) COMPENSATION

Your participation is voluntary. No compensation will be given for your participation.

10) CONFIDENTIALITY

All information that you give will be kept strictly confidential. Once we have analysed the information no one will be able to identify you. Research reports and articles in scientific journals will not include information that may identify you.

CONSENT TO PARTICIPATE IN STUDY

Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

Setup and implementation of tWE laboratory: Quality control through the culture of non-viable embryos

We confirm that the person asking our consent to take part in this study has told us about the nature, procedure, risk, discomfort, and benefits of the study. We have also received, read, and understood the above written information (Information Leaflet and Informed Consent) regarding the study. We are aware that the results of the study, including personal details, will be anonymously processed into research reports. We are participating willingly. We have had time to ask questions and have no objection to participating in the study. We understand that there is no penalty should we wish to discontinue with the study and our withdrawal will not affect us in any way.

Volunteering couple:
Partner 1 Name: _____ (Please print)

Partner 1 Signature: _____ Date: _____

Partner 2 Name: _____ (Please print)

Partner 2 Signature: _____ Date: _____

Investigator's name: _____ (Please print)

Investigator's Signature:_____ Date: _____

Witness's name: _____(Please print)

Witness's signature:_____ Date: _____

7.2.2. Study 3 Informed consent

INFORMATION LEAFLET AND INFORMED CONSENT FOR NON-CLINICAL RESEARCH

TITLE OF STUDY

Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

Setup and implementation of tWE laboratory: Embryo culture in the mobile tWE laboratory

1) INTRODUCTION

You are invited to participate in the research study for a Doctoral degree (PhD). This information will help you to decide if you would like to participate. Before you agree to take part in the study you should fully understand what is involved. If you have questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator Mr G. Boshoff. You should not agree to take part unless you are completely satisfied about all the procedures involved.

2) THE NATURE AND PURPOSE OF THE STUDY

The objective of this study is to confirm the use of an *in vitro* fertilization (IVF) laboratory, using an easier and cheaper system of growing human embryos, called the tWE laboratory. This laboratory system has been tested in Belgium since 2013, and used in Ghana since 2018, to help couples become pregnant. The Belgian group has reported results from this testing to be very favourable, with pregnancy rates being the same as standard IVF. *This laboratory system will be placed in a mobile laboratory to be used in South Africa to have a more affordable IVF option available to patients.*

You as a participating couple would provide the cells needed to perform this study. These cells will be eggs and sperm, obtained for IVF.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

This study involves using the mobile tWE laboratory to grow embryos. Since you requested to undergo an IVF procedure, you will be prescribed medication for a mild ovarian stimulation protocol to help your body to make more eggs than usual. This is the same treatment used in preparation for a standard IVF procedure and will be done under the control of a doctor who has specialized as a Gynaecologist and subspecialized after that in the treatment of infertility. After the stimulation is completed, eggs and sperm will be taken from you as a couple. This will happen in the doctor's practice in the procedure room always used for this. After removing of the eggs, these eggs and sperm will be taken to the mobile laboratory and used to grow embryos. At the correct time, you will also come to the mobile laboratory for the embryo transfer procedure.

The tWE laboratory uses an embryo culture system with less equipment than a standard IVF laboratory. This is because the tubes that the eggs and sperm will be placed in, are prepared and closed beforehand and does not need new air to be blown in all the time, as is required in a standard IVF laboratory. Because of the less equipment used, the laboratory can be installed in a mobile room (a large trailer designed for this purpose) and the cost of this laboratory is cheaper than a standard laboratory.

The eggs and sperm obtained from you as a couple will be placed inside the tubes, to grow embryos, and these embryos will be checked regularly. Based on the way the embryos grow, the ones that looks the best will be used for embryo transfer and, if there are any left that can be used later, for freezing. This is the same as what would have happened if the embryos were grown in the standard IVF laboratory.

You will be expected to visit the clinic on the agreed upon dates (at least three or four visits), for the stimulation process and to remove the eggs. You will be given medication for ovarian stimulation and explained how to take this medication. You should take the medicine as described and the way the eggs grow inside you will be looked at when you visit the clinic. When the eggs have grown large enough to be taken out of your body, you will be scheduled for an egg retrieval procedure. On the day of egg retrieval, you will visit the clinic for this procedure and after the eggs are removed from your body, the researcher will place the eggs in culture tubes in the mobile laboratory outside of the hospital building. You will also be expected to provide a sperm sample, which will be taken to the mobile laboratory to place with the eggs to make embryos.

Once the embryos are cultured, and if an embryo transfer is planned, you will be informed at least one day in advance to come to the clinic. On the day of embryo transfer, you will have to walk to the mobile laboratory outside of the hospital building. The embryo transfer will take place inside this mobile laboratory and after the embryo transfers, you have to let us know within three weeks if you are pregnant or not. If there are embryos remaining after the embryo transfer, that can be cryopreserved according to the laboratory's standard protocols, these will be frozen.

Once frozen, these embryos will be kept in the storage dewars of the standard IVF laboratory. The storage of these frozen embryos is the responsibility of the standard IVF laboratory and they would be in contact with you regarding any costs associated with the storage. You can then schedule a frozen embryo transfer with the clinic at a later stage at your own cost.

Please note, should less than 3 eggs develop during the stimulation treatment, the stimulation prescribed for the study will end and you will exit the research group, after a debriefing meeting with the doctor overseeing the stimulation. If you wish to continue with IVF with the eggs already developing, you can discuss this with the clinic staff, to continue with standard IVF at your own cost.

4) RISK INVOLVED

There is no added risk to you as a person in participating in the study, other than the standard risks associated with assisted reproduction treatment. Similar to standard IVF treatment, there is no guarantee that you will be pregnant after the treatment. Your eggs and sperm will be used to culture embryos in the tWE laboratory, rather than the hospital's current IVF laboratory. The embryo growing system used in this laboratory has been tested at the Ziekenhuis Oos Limburg, Belgium, and by 2020 there has been more than 190 babies born, and more ongoing pregnancies, after using this system (www.thewalkingegg.com). More information and the research articles published on this culture system can be given to you at request. The embryos grown in the tWE laboratory will be available for embryo transfer or freezing, to be used in the same way as embryos grown in the standard laboratory.

5) POSSIBLE BENEFITS OF THE STUDY

The investigation of the mobile tWE laboratory will give information on the way embryos grow in this laboratory. The stimulation medication and laboratory fee for this system is subsidised by the research and provided to you at no cost. Please note, there may still be some fees payable to the hospital.

Other hospitals in South Africa can also use this type of embryo culture system in the future. This will help patients that cannot afford standard IVF to also attempt to fall pregnant with the help from a laboratory using this system.

6) WHAT ARE YOUR RIGHTS AS A PARTICIPANT?

Your participation in this study is entirely voluntary and **you can refuse to participate or stop at any time without stating any reason.** Your withdrawal will not affect you in anyway.

7) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This study has received written approval from the Research Ethics Committee of the Faculty of Health Sciences at the University of Pretoria. A copy may be obtained from the investigator should you wish to view it.

8) INFORMATION AND CONTACT PERSON

The contact person for the study is Mr G. Boshoff. If you have any questions about the study, please contact him at Tel: 012 354 2061. Alternatively, you may contact his supervisor Prof. C. Huyser at Tel: 012 354 2208.

9) COMPENSATION

Your participation is voluntary. No compensation will be given for your participation.

10) CONFIDENTIALITY

All information that you give will be kept strictly confidential. Once we have analysed the information no one will be able to identify you. Research reports and articles in scientific journals will not include information that may identify you.

CONSENT TO PARTICIPATE IN STUDY

Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

Setup and implementation of tWE laboratory: Embryo culture in the tWE laboratory

We confirm that the person asking our consent to take part in this study has told us about the nature, procedure, risk, discomfort, and benefits of the study. We have also received, read, and understood the above written information (Information Leaflet and Informed Consent) regarding the study. We are aware that the results of the study, including personal details, will be anonymously processed into research reports. We are participating willingly. We have had time to ask questions and have no objection to participating in the study. We understand that there is no penalty should we wish to discontinue with the study and our withdrawal will not affect us in any way.

Volunteering couple:
Partner 1 Name: _____ (Please print)

Partner 1 Signature: _____ Date: _____

Partner 2 Name: _____ (Please print)

Partner 2 Signature: _____ Date: _____

Investigator's name: _____ (Please print)

Investigator's Signature:_____ Date: _____

Witness's name: _____(Please print)

Witness's signature:_____ Date: _____

7.3. Annexure C: PhD Committee approvals

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

29 March 2021

Prof Carin Huyser
Department of Reproductive Biology
Faculty of Health Sciences

Dear Prof Huyser

STUDENT: BOSHOFF G (PhD REPRODUCTIVE BIOLOGY)
TITLE: Validation of the Walking Egg *in vitro* embryo culture system with application in a mobile laboratory setup

The above-mentioned student's protocol has been approved by the PhD committee.

We wish the student all the best with his studies.

Kind regards

PROF V STEENKAMP
CHAIR: PhD COMMITTEE

Deputy Dean: Teaching and Learning
Room 5-20.1, Level 5, Health Sciences Building
University of Pretoria, Private Bag X323
Gezina 0031, South Africa
Tel +27 (0)12 319 2174
Email vanessa.steenkamp@up.ac.za
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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

22 March 2023

Prof C Huyser
Department of Obstetrics and Gynaecology
Faculty of Health Sciences

Dear Prof Huyser

Student: G Boshoff (PhD: Reproductive Biology)
Title: Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup

RE: AMENDMENT

The amendment requested (letter dated 27 February) for the above student have been approved by the PhD Committee.

With kind regards,

Martin Brand
Chair: PhD Committee

Email: martin.brand@up.ac.za
Tel +27 (0)12 354 2097

Department of Surgery
Room 71107, Level 7, Bridge E, Steve Biko Academic Hospital
Steve Biko Academic Hospital, Private Bag X169
Pretoria, 0001, South Africa

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

7.4. Annexure D: Ethics Committee approvals

www.uhasselt.be
Campus Hasselt | Martelarenlaan 42 | BE-3500 Hasselt
Campus Diepenbeek | Agoralaan gebouw D | BE-3590 Diepenbeek
T + 32(0)11 26 81 11 | E-mail: info@uhasselt.be

UHASSELT
KNOWLEDGE IN ACTION

Definitief gunstig advies

Faculteit Geneeskunde en Levenswetenschappen
Comité voor Medische Ethiek
Voorzitter: prof. dr. Ivo Lambrichts
Secretariaat: Marleen Missotten
Tel.: 011 26 85 02
Fax: 011 26 85 99
E-mail: cme@uhasselt.be

ons kenmerk	uw kenmerk	Diepenbeek
CME2023/046		07/09/2023

Titel protocol	**Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup**
Nummer protocol	
Opdrachtgever	Universiteit Hasselt
Eudractnummer	nvt
Belgisch nummer	nvt
Onderzoeker	*Prof. dr. Willem Ombelet*

Geachte collega,

Het hierboven vermeld dossier werd besproken en goedgekeurd. Deze studie valt niet onder de wet van 7 mei 2004, inzake experimenten op de menselijke persoon.

Het comité bevestigt dat de onderzoeker en zijn medewerkers voldoende bekwaamheid bezitten om deze studie uit te voeren.

Het instituut beschikt over voldoende faciliteiten om deel te nemen aan deze studie.

Na inzage van de informatie en documenten met betrekking tot dit dossier is het Comité van oordeel dat deze studie, zoals beschreven in het protocol, wetenschappelijk relevant en ethisch verantwoord is. Het comité verwacht dat de privacy te allen tijde wordt verzekerd.

Het Comité voor Medische Ethiek van UHasselt handelt volgens de geldende richtlijnen van de 'International Conference of Harmonization (ICH) Good Clinical Practice (GCP)' en volgens alle geldende en van toepassing zijnde wetten en reglementen.

Met oprechte hoogachting,

Prof. dr. Ivo Lambrichts
Voorzitter Comité voor Medische Ethiek

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

Faculty of Health Sciences **Research Ethics Committee**

27 May 2021

Approval Certificate
New Application

Dear Mr GM Boshoff

Ethics Reference No.: 149/2021
Title: Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup

The **New Application** as supported by documents received between 2021-04-20 and 2021-05-26 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-05-26 as resolved by its quorate meeting.

Please note the following about your ethics approval:

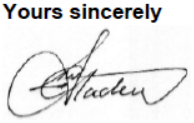
- Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-05-27.
- Please remember to use your protocol number (149/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely



On behalf of the FHS REC, Professor Werdie (CW) Van Staden
MBChB, MMed(Psych), MD, FCPsych(SA), FTCL, UPLM
***Chairperson**: Faculty of Health Sciences Research Ethics Committee*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
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Gezina 0031, South Africa
Tel +27 (0)12 356 3084
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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

12 August 2022

Approval Certificate
Annual Renewal

Dear Mr GM Boshoff,

Ethics Reference No.: 149/2021 – Line 1
Title: Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup

The **Annual Renewal** as supported by documents received between 2022-07-15 and 2022-08-10 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-08-10 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2023-08-12.
- Please remember to use your protocol number (149/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

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Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

1 April 2023

Approval Certificate
Amendment

Dear Mr GM Boshoff,

Ethics Reference No.: 149/2021 – Line 2
Title: Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup

The **Amendment** as supported by documents received between 2023-03-01 and 2023-03-29 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-03-29 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Please remember to use your protocol number (149/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Professor C Kotzé
MBChB, DMH, MMed(Psych), FCPsych, Phd
***Acting Chairperson**: Faculty of Health Sciences Research Ethics Committee*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
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Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through June 30, 2025 and Expires 07/28/2026.

Faculty of Health Sciences **Research Ethics Committee**

12 September 2024

Approval Certificate
Amendment

Dear Mr GM Boshoff,

Ethics Reference No.: 149/2021 – Line 3
Title: Validation of the Walking Egg in vitro embryo culture system with application in a mobile laboratory setup

The **Amendment** as supported by documents received between 2024-08-27 and 2024-09-11 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2024-09-11 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- The Research Ethics Committee (REC) must monitor your research continuously. To this end, you must submit as may be applicable for your kind of research:
  a) annual reports;
  b) reports requested *ad hoc* by the REC;
  c) all visitation and audit reports by a regulatory body (e.g. the HPCSA, FDA, SAHPRA) within 10 days of receiving one;
  d) all routine monitoring reports compiled by the Clinical Research Associate or Site Manager within 10 days of receiving one.
- The REC may select your research study for an audit or a site visitation by the REC.
- The REC may require that you make amendments and take corrective actions.
- The REC may suspend or withdraw approval.
- Please remember to use your protocol number (149/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

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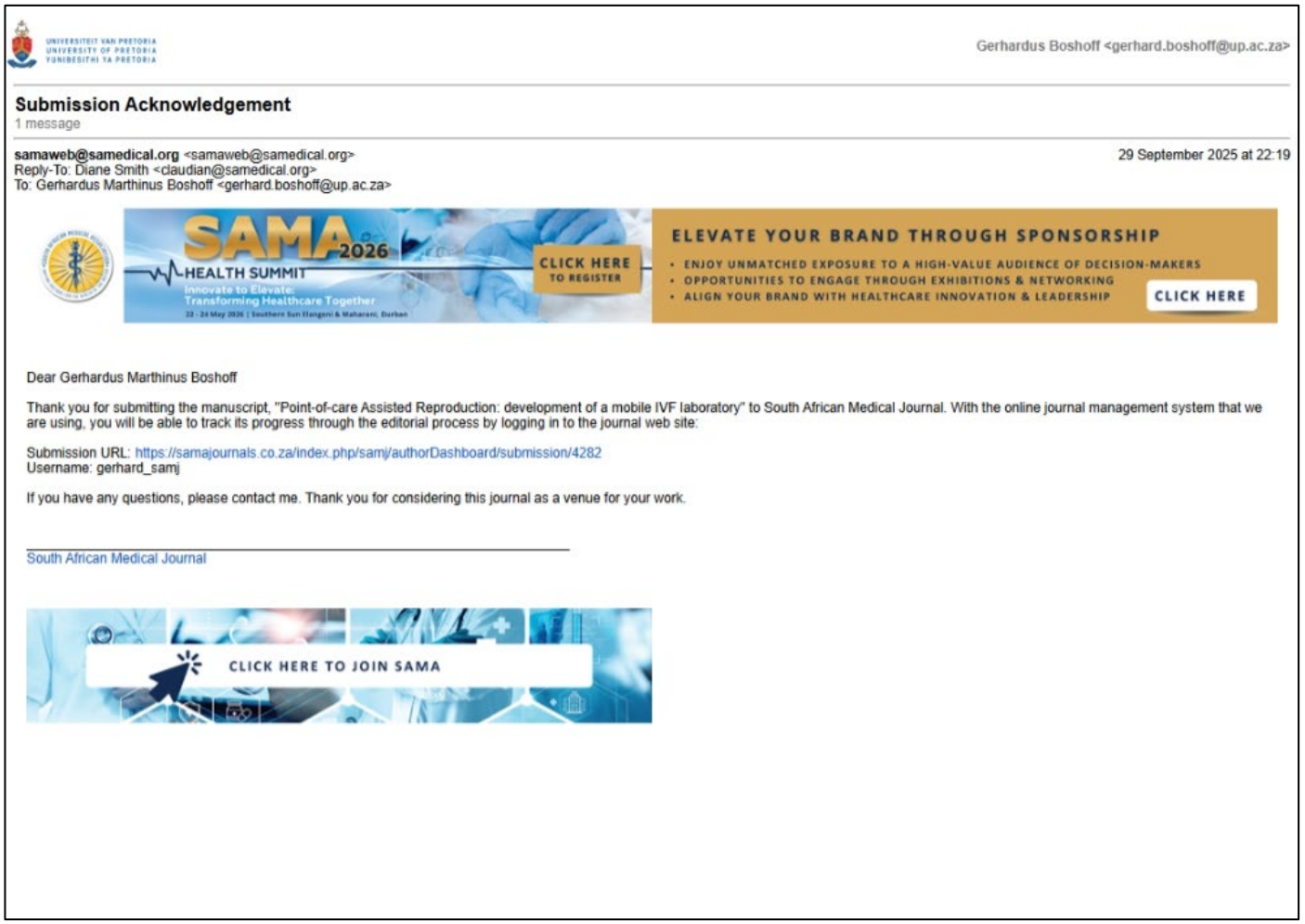
7.5. Annexure E: Proof of submission to journal



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Gerhardus Boshoff <gerhard.boshoff@up.ac.za>

Submission Acknowledgement
1 message

samaweb@samedical.org <samaweb@samedical.org> 29 September 2025 at 22:19
Reply-To: Diane Smith <claudian@samedical.org>
To: Gerhardus Marthinus Boshoff <gerhard.boshoff@up.ac.za>

Dear Gerhardus Marthinus Boshoff

Thank you for submitting the manuscript, "Point-of-care Assisted Reproduction: development of a mobile IVF laboratory" to South African Medical Journal. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: https://samajournals.co.za/index.php/samj/authorDashboard/submission/4282
Username: gerhard_samj

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

South African Medical Journal