A Simplified IVF Laboratory Method

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Introduction

Reproductive care as a universal right has been neglected for a long time. Whilst conventional countries developed state of the art equipment to perform assisted reproductive technologies (ART) with high success rates, developing countries or so-called 'resource-poor countries' were left in the dark (Boivin et al., 2007; Cates et al., 1985; Rutstein and Iqbal, 2004). Estimates on worldwide infertility prevalence show that between 52.6 to 72.4 million couples are affected by the lack of medical assistance in resource-poor countries to achieve a pregnancy (Mascarenhas et al., 2012). The prevalence of infertility is directly related to the high number of sexually transmitted diseases and pregnancy-related infections in developing countries (Ombelet et al., 2008). Consequently, most cases are only treatable by using IVF/ICSI treatments, which are too costly or unavailable in large parts of the world. If infertility is not caused by a severe male factor, conventional IVF treatment can be sufficient. However, fertilizing and culturing embryos in an IVF program can represent over 50% of the total financial cost (Aleyamma et al., 2011). Some attempts have been made to develop a system to perform an IVF treatment without a large investment in a laboratory installation. As part of the Walking Egg Project (Dhont, 2011; Ombelet, 2013; 2014) and based on previous findings and experience (Van Blerkom and Manes, 1974; Swain, 2011) we developed an IVF methodology that is simple, safe and effective in treating infertile patients in a low-resource setting without loss of success rates compared to a conventional IVF environment. We estimate the cost of the simplified laboratory method, which we named tWE-lab IVF culture system, between 10-15% of the current cost in a high-resource setting. Regular IVF programs are not suitable for couples with moderate or severe male factor because the number of spermatozoa for insemination is too low. In the tWE-lab IVF culture system, only 1000-5000 good motile spermatozoa are needed to achieve good fertilization results, making it suitable for a large part of the patients requiring IVF/ICSI, including those with moderate male factor (Van Blerkom et al., 2014).

The tWE-lab system is based on a simple chemical reaction in a closed environment which removes the need for an expensive IVF laboratory with CO_2 incubators, medical gas supply and air purification systems. CO_2 is necessary to equilibrate the pH of the IVF culture medium to a value between 7.25-7.40, optimal for embryo development. Continuous culture at 37 degrees Celsius is necessary for viability of the embryo and can be achieved by an incubator, heated box or warm water bath. The simplified IVF method uses 2 chemicals, citric acid and sodium bicarbonate, to produce the CO_2 necessary to adjust the pH. The use of these chemicals for pH equilibration in a closed environment had been described (Swain, 2011) and Van Blerkom used the idea to transport animal oocytes already 30 years ago (Van Blerkom and Manes, 1974). The tWE-lab system was designed in collaboration with Dr. Van Blerkom and is now a standardized procedure to perform an IVF treatment, for which all materials are available in a compact ready-to-use kit.

The simplified IVF method uses 2 glass tubes in which the first tube serves as the generator of CO_2 by a chemical reaction between citric acid and sodium bicarbonate to produce the CO_2 for equilibration of the culture medium in the second tube (Fig. 1). A connection between the tubes is established by needles and tubing to transport the produced gas from the generator tube to the tube with culture medium. The connection can easily be

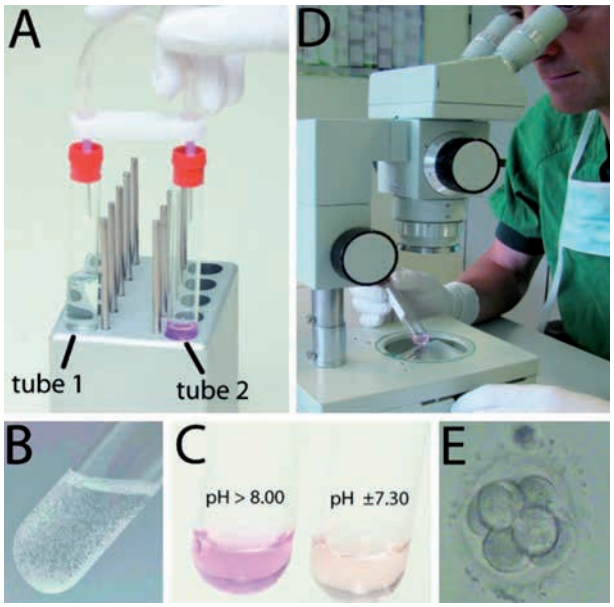

Fig. 1. — tWE-lab method: (A) Equilibration of the culture medium in tube 2 by the CO_2 produced in tube 1, (B) production of CO_2 in the generator tube, (C) phenol red shift from dark pink (pH>8.00) to salmon pink (pH around 7.30), (D) embryo evaluation through the glass walls of the closed tubes, (E) an embryo visualized in the tWE-lab tube.

removed after the 24-hour equilibration of the culture medium has completed. The tubes with culture medium can be used immediately or they can be stored in a cold (2-8 degrees Celsius) environment. To keep warm, the tubes are placed in a heated block (alternatively water bath or simple incubator without the need for a gas supply) at 37 degrees Celsius. After oocytes have been harvested from the follicles, they are inserted individually in the equilibrated tubes using a 1 ml syringe and needle, without opening the tube or disturbing the air environment inside the tube. The tWE lab system provides a closed environment to ensure stable culture conditions and protect the gametes and embryos from possible adverse external effects. Between 1000 and 5000 washed sperm cells are injected in a similar way to the tubes to inseminate the oocytes. After 24 hours, fertilization is assessed by keeping the tubes at a slant and looking through the glass walls at the oocytes to see the 2 pronuclei that are a sign of sperm protrusion. Fertilization and embryo development are assessed through the glass walls of the tube. This simplified method removes the need for expensive air control systems and gives a very stable and safe environment for embryos to develop. The search for the materials needed in the kit has been extensive and all items have important specifications that ensure good operation of the low-cost IVF method. Changing as little as the tube or the needle can result in a complete loss of function of the tWE-lab system as all components are strictly chosen to match each other.

Outcomes from the low-cost culture method were compared with those from a conventional IVF culture system in a study conducted at the IVF unit in Genk Belgium and were shown to be identical (Van Blerkom et al., 2014). As the study was performed in routine IVF cycles, surplus tWE-lab embryos were frozen and embryos that originated from the tWE-lab system that were thawed and transferred have also led to the birth of healthy children (Ombelet et al., 2014). These studies prove the tWE-lab system to be an alternative low-cost system for a routine IVF procedure. Next step is to implement the tWE-lab IVF system in a centre that is limited in resources. Our goal is to open the first low-cost tWE-lab centre in Ghana in 2015. Preparations for the set-up of the centre are ongoing. If our method can be reproduced in other centres we believe this will reach a large part of the worlds' infertile population and finally provide equity and social justice for many childless couples currently still left without resources and help (Ombelet, 2011).

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