The Walking Egg Project: an example of medical education and training

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Introduction

Infertility is a universal health issue and it has been estimated that 8 to 12% of the couples worldwide are infertile. The large majority of childless couples are residents of developing countries. WHO estimates that childlessness affects around 186 million married couples in the developing world alone (excluding China). However, in some parts of the world, the burden of childlessness can be so severe that 1 in 4 couples are affected (Rutstein & Iqbal, 2004; Boivin et al., 2007).

Consequences of involuntary childlessness are usually much more dramatic in developing countries, particularly for women. Negative psychosocial consequences are often severe and childless women are frequently stigmatised, isolated, ostracized, disinherited and neglected by the entire family and even the local community. This may result in physical and psychological violence, polygamy, even suicide (Papreen et al., 2000; Van Balen & Gerrits, 2001; Daar & Merali, 2002; Dyer et al., 2004, 2005; Van Balen & Bos, 2009; Gerrits & Shaw, 2010). Because many families in developing countries completely depend on children for economic survival, childlessness has to be regarded as a social and public health issue, and not only as an individual medical problem (Ombelet et al., 2008; Dhont, 2011b; Ombelet, 2011).

The most important reason for childlessness in developing countries is the high incidence of sexually transmitted diseases (STDs), pregnancy-related infections due to unsafe abortions and home deliveries in unhygienic circumstances, mainly in rural areas. The high prevalence of genital infections in developing countries is commonly compounded by a complete lack of diagnosis together with incomplete, inappropriate or no intervention at all. Severe male infertility due to STDs and female infertility due to tubal block can only be treated by "expensive" assisted reproductive technologies (ART) which are either not available or only within reach of those (the happy few) who can afford it, mostly in a private setting (Ombelet et al., 2008).

Despite its relatively high prevalence and the cultural values associated with childbearing, infertility care remains a low priority area for local health care providers and community leaders. A surprisingly low interest is shown on the issue of infertility and childlessness in developing countries, not only on a national, but also on an international level (Fathalla et al., 2006, Ombelet, 2011).

The Walking Egg non-profit organization was founded in 2010 by scientists and an artist to realize the idea of implementing accessible infertility programmes in resource-poor countries (Dhont, 2011c). Right from the start The Walking Egg has opted for a multidisciplinary and global approach

towards the problem of infertility. In cooperation with the Special Task Force (STF) on "Developing countries and infertility" of the European Society of Human reproduction and Embryology (ESHRE) and the WHO, The Walking Egg npo gathers medical, social and economical experts to discuss and work together towards its goal (Dhont, 2011). **The Walking Egg Project** aims to raise global awareness surrounding childlessness, and to make infertility care in all its aspects, including assisted reproductive technologies, available and accessible for a much larger part of the population in resource-poor countries. It is obvious that implementation of ART will depend on our ability to optimise these techniques in terms of availability, affordability and effectiveness (Ombelet & Campo, 2007). Our ***first objective*** is the establishment of a low-cost "one-stop clinic" for the diagnosis of infertility. Simplification of the IVF procedures without loss of quality is our ***second objective***. Our ***final goal*** is the implementation of "accessible" infertility services integrated within health care facilities, providing quality family planning, reproductive health education, maternity- and child care. Urbanization is rapid, particularly in low-income countries and emerging economies, which allows for centralization of reproductive health services.

Methods to organize **medical education and training for the one-day diagnostic phase** of the Walking Egg Project are described in the current paper. **These methods** will be used for pilot-centres who want to be part of The Walking Egg Project.

The one-day diagnostic phase

Standardized investigation of the couple at minimal costs will enhance the likelihood that infertile couples, both men and woman, will come to the centre. The best option would be to develop **specialized primary health care facilities,** where infertile couples can obtain sexual and reproductive health care and education (prevention of cancer, STDs, diagnostic procedures for infertility, family-planning, etc.).

Existing reproductive health care centres are mostly located in big hospitals with long waiting lists and far away from villages. The advantage of a "specialized primary health care facility" is that it can become a place for both medical and educational activities, as well as a place where women can discuss their problems.

Since tubal obstruction associated with previous pelvic infections is the most important reason for infertility in some developing regions, hysterosalpingography and/or hystero-salpingo-contrast-sonography are simple and accessible techniques to detect this problem without major costs. Combining these techniques with an accurate anamnesis will identify the majority of women's infertility causes, such as ovulatory disorders and tubal infertility. Male factor infertility can easily be evaluated by a simple semen analysis. Semen analyses can also be performed by well-trained paramedicals, another important advantage for developing countries.

Office mini-hysteroscopy to investigate intrauterine abnormalities has been simplified in its instrumentation and technique, so that it can become a non-expensive diagnostic technique accessible for every gynaecologist, provided there has been appropriate training (Ombelet & Campo, 2007).

Moreover, all of these procedures can be performed by a small team of health care providers within a short period of time in an inexpensive setting (Ombelet and Campo, 2007).

Our objective is to organize a one week course for all members of the team who are involved in the set-up of a pilot-centre, part of the Walking Egg Project. This training will need the support of experts in the field, who are capable to tutor the training courses at the highest level in a very short time, taking into account the experience of the trainees and the quality of facilities that can be expected in the new pilot-centres.

The main topics to be included in the training courses are described in Table I.

1. *Reproductive health care education basic course*

Local midwifes and nurses have to be trained in the basic knowledge of human fertility from menarche to menopause, with special attention to menstrual cycle physiology and reproductive endocrinology. Trainers have to be experienced in fertility-awareness methods not only to increase the knowledge of having intercourse during the most appropriate period, but also to inform couples how to prevent pregnancy if needed as a natural method of family planning. The trainees will be counseled about the methods and interpretation of basal body temperature and cervical mucus recordings.

Awareness and prevention of sexual transmitted infections and HIV are also part of this course.

2. *A general and medical history of both partners and basic clinical examination*

A questionnaire will be provided for both partners. This questionnaire can be adapted to the local situation in the specific locations and countries. A protocol of how to perform a basic clinical examination of both partners will be discussed. Case discussions can be used to teach the relevant elements of an infertility work-up.

Table I. — Key categories of the training courses.

- Reproductive health care education basic course
  → Target group: **nurses, midwifes**
- A general and medical anamnesis of both partners and basic clinical examination
  → **clinician (medical)**
- Screening for infections and STDs
  → **clinician (medical, paramedical)**
- How to perform and evaluate a hysterosalpingography and/or hystero-salpingo-contrast-sonography
  → **clinician (medical, paramedical)**
- Standard Operational Procedures for the gynaecological and fertility ultrasound scan
  → **clinician (medical, paramedical)**
- Basic semenology training course according to WHO 2010 manual
  → **laboratory staff, (paramedical)**
- Sperm washing procedures
  → **laboratory staff, (paramedical)**
- Mini-hysteroscopy
  → **clinician (medical)**
- Documentation and registration
  → **administrative staff (clerical)**

3. *Screening for infections and STDs*

A short list of affordable screening tests (with costs) is listed in Table II. Training the laboratory staff to perform these tests and interpret the results will be offered to all team members. The prevention of the transmission of infections, as a result of assisted reproduction, is the cornerstone of this part of the course.

4. *How to perform and interpret a hysterosalpingography and/or hystero-salpingo-contrast-sonography*

How to perform a hysterosalpingograph and evaluate the results can be learned by showing a large number of different pictures associated with different pathologies. The impact of the different uterine and tubal abnormalities will be discussed by experts in the field.

Patency of the tubes can also be investigated by hystero-salpingo-contrast sonography. Fluid filled dilatation of the fallopian tubes (i.e. hydrosalpinges) can be identified by the demonstration of elongated fluid filled swellings with incomplete septae on one or both sides of the uterus. The identification of hydrosalpinges by ultrasound is important, as studies have shown that if they are not surgically removed or clipped, they are associated with reduced pregnancy rates following IVF.

5. *Standard Operational Procedures for the gynaecological and fertility ultrasound scan*

Table III gives an overview of the different aspects considering training in gynaecological and fertility ultrasound scanning. With the help of experienced experts, this training course can easily be organized during a two-day course. After the course, a CD-ROM can be given to the trainees to ensure prolonged training and quality control.

6. *Basic semenology training course according to WHO 2010 manual*

Semen analysis remains a cornerstone in the evaluation of the male's fertility potential on condition that it is performed by trained technologists. During the suggested training sessions, the main goal is to enhance the technical skills of the trainees as far as the following aspects are concerned; (i) staining techniques (ii) sperm morphology (iii) sperm concentration (iv) cellular elements and (v) sperm motility and vitality (Björndahl et al., 2010; WHO, 2010).

The format of this training course will consist of **3 sessions**:

1. Pre-training evaluation

During this session each trainee will receive a fresh semen sample to record the sperm concentration, motility and vitality, cellular elements and percentage normal sperm on pre-stained slides.

2. Training session

Following the pre-training session, the proper evaluation technique of each sperm parameter will be discussed and explained in detail. This training will include PowerPoint presentations of motility, vitality and morphology, video clips and by using pre-stained slides.

Table II. — Description and cost summary of selected rapid tests for infectious screening in ART laboratories (estimated prices, Huyser C, 2012).

Description	Quantity per pack	Cost per pack (€)	Cost per item / test (€)
Determine Rapid HIV 1 & 2 Combo (with accessories)*	100	150.16	1.50
Determine Rapid HBsAg (with accessories)*	100	112.36	1.12
HBsAg Dipstick (serum/plasma)	30	11.43	0.38
HBsAg Cassette (serum/plasma)	30	33.74	1.12
HCV Cassette (serum/plasma)	30	48.10	1.60
Rapid Chlamydia Cassette (swab/urine)	20	64.25	3.21
Determine Rapid Syphilis (with accessories)*	100	109.80	1.10
Syphilis Cassette*	30	35.83	1.19
Gonorrhoea Rapid Cassette (swab)	25	94.00	3.76

*serum/plasma/whole blood.

2.1. Sperm concentration

The counting procedure will be explained using PowerPoint projections of the Neubauer counting chamber containing sperm images (Figure 1a). Estimation of the sperm concentration using a standardized wet preparation (10 µL semen drop covered with 22 × 22 mm cover slip) will be performed on a Neubauer counting chamber by each trainee.

2.2. Motility

High quality video clips will be projected containing a low number of sperm exhibiting motility values according to the categories; progressive motile, non progressive and immotile. Using increasing sperm numbers on consecutive video clips, the trainees will be requested to record the 3 motile categories.

2.3. Vitality

Pre-stained eosin/nigrosin slides will be evaluated and discussed.

2.4. Morphology

The morphology training session initiates with a detailed explaining of the configuration of a

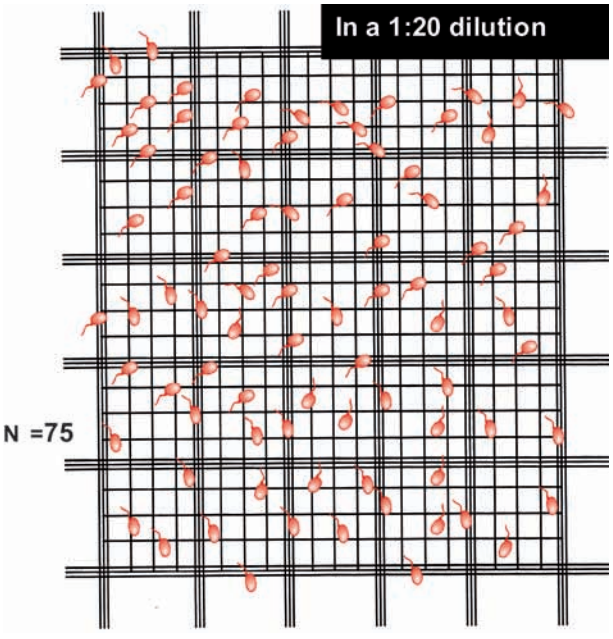


Fig. 1a. — Neubauer counting chamber containing sperm

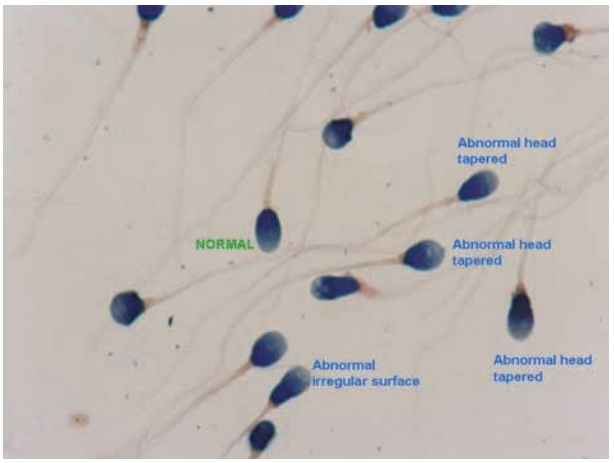


Fig. 1b. — The morphology training session with detailed explanation of staining methods and the configuration of normal / abnormal spermatozoa according to the WHO (2010).

Table III. — An overview of the different aspects considering the basic aspects of training in gynaecological and fertility ultrasound scanning.

Prior to scan

- An empty bladder is needed for intravaginal ultrasound.
- Check the regularity and usual length of the menstrual cycle and the particular day of the cycle.
- Prepare the patient on the scan couch in the lithotomy position, explain the procedure and check the volunteer does not have a Latex allergy. Clean and prepare the transducer with a condom.

Scanning technique

- A systematic approach to scanning is important.
- The probe should be introduced gently. The bladder is a key indicator of the orientation of the scan.

The uterus

- Note whether the uterus is anteverted or retroverted.
- The full length of the uterus should be measured sagittally from the external os of the cervix to the fundus and also transversely (antero-posterior (A-P) and transverse diameters).
- The endometrial thickness should be measured as the maximal thickness in the longitudinal uterine axis. Polyps should be measured separate from the endometrium in two dimensions at 90 degrees in the sagittal plane. The morphology of the endometrium ("triple layer" or "secretory").
- Previous Caesarean section scars can be identified as an echogenic line in the myometrium just above the cervix. If the scar is full thickness the length should be measured.
- The probe is then moved laterally to each side to examine the myometrium for fibroids or areas of adenomyosis (diffusely thickened uterus with areas of heterogeneous ultrasound signals).

The ovaries

- Measure the longitudinal, A-P and transverse diameters in mm. The ovarian volume (in ml) should then be calculated (in cm) using the formula for the volume of an ellipsoid i.e. the 3 diameters (in cm) × 0.5233. This will give the ovarian volume in ml. This is a valuable measurement when assessing ovarian reserve and polycystic ovaries (PCO).
- Assessment of the morphology of the ovary taking into account the time of the cycle. If the mean diameter of the dominant follicle is greater than 25 mm mid-cycle, it should be regarded as an ovarian cyst. Complex cysts are measured in 3 dimensions. The wall thickness, septal thickness, loculations and solid elements should be noted and papillations should be measured. The internal wall of the cyst should be described as being „smooth" or „irregular".
- A common cyst found in women of reproductive age is the endometriotic cyst. Typically these have relatively thick walls with contents of uniform echogenicity (frequently called "ground glass").
- To assess ovarian reserve, we measure the Antral Follicle Count (AFC). The small growing follicles (2-6 mm in diameter) are an accurate reflection of the ovarian reserve and correlate with the response to stimulation, the count is the sum from both ovaries. A count less than 5 indicates a low ovarian reserve. A high AFC may be an indication of PCO and an increased response to stimulation.
- After day 7, a dominant follicle can be identified and it is then customary to take the mean of 3 diameters as the follicle is rarely symmetrical. Serial monitoring of follicular growth will predict an expected time for ovulation which is when the mean diameter is approximately between 18-22mm.
- Following ovulation a corpus luteum can easily be identified. It is thick walled, usually cystic and very vascular. Small solid corpora lutea are associated with low progesterone levels and poor implantation.

normal/abnormal spermatozoon according the WHO 2010. Following this session, high quality micrographs containing numbered spermatozoa will be discussed and illustrations of the normal sperm cell will be emphasized. The trainees will then be requested to partake in an initial pre-training session using projected images of numbered sperm (Figure 1b).

Following this group session pre-stained slides will be supplied to each trainee for evaluation to ensure, once again, prolonged training and quality control.

7. *Sperm washing procedures*

In developing countries, the selection of a sperm preparation technique is dictated by the blood-borne virus status (HIV) of the male patient and the nature of the semen sample (Huyser et al., 2008; Huyser & Fourie, 2010).

- The *HIV-seropositive* status of a patient, as well as the initial microscopic observation of a high number of "round cells" (leukocytes/inflammatory and germ cells) in the semen sample necessitates

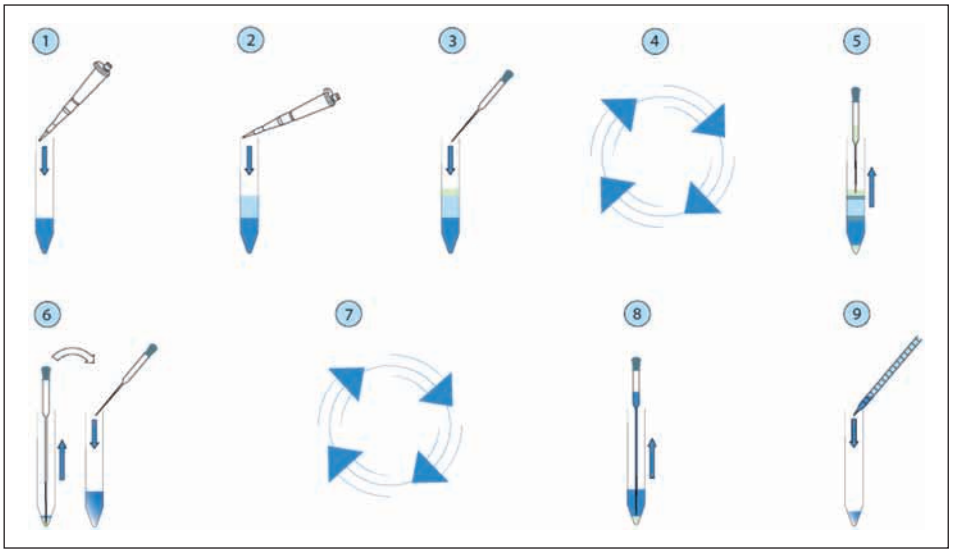
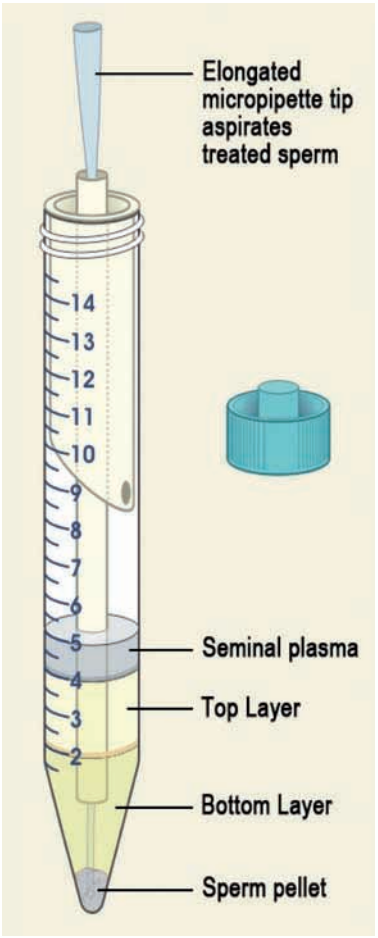


Fig. 2a. — Stepwise illustration of sperm processing: Density gradient centrifugation (obtained with permission Nidacon International AB).



Procedure:

1) A polypropylene insert (ProInsert™) is placed in a 15ml sterile conical centrifugation tube, the 80% and 40% (bottom and top). PureSperm® gradients together with the liquefied semen (≤ 1.5 ml semen per 2 ml of each gradient) are layered sequential and aseptically through the insert.

2) A clear interface should be noticeable between the layers. Individually wrapped sterile plastic Pasteur pipettes are used to layer each gradient and semen. A bench top centrifuge with a swing out rotor is used to centrifuge the sample at 300 *g* for 20 minutes.

3) An elongated micropipette is used to aspirate the sperm pellet (0.2 mL) from the bottom of the tube. The design of the insert facilitates precise layering of density gradients and semen, allowing access to the treated sperm pellet post-centrifugation directly, without exposure to the upper gradient layers with possible pathogens and cell debris.

4) The sperm pellet is transferred aseptically to a new conical tube containing 5 ml of PureSperm® Wash/embryo culture media and centrifuged at 500 *g* for 10 minutes. The supernatant is aspirated with a new plastic Pasteur pipette using circular movements, till the beginning of the tapered section of the tube. Using a digital pipette with sterile micro-tip, 0.2 ml of the purified sperm fraction is pipetted from the bottom of the tube.

5) Resuspend the sperm sample in a suitable volume of media for insemination (calculate IMC).

Fig. 2b. — Schematic illustration of sperm processing: Density gradient centrifugation

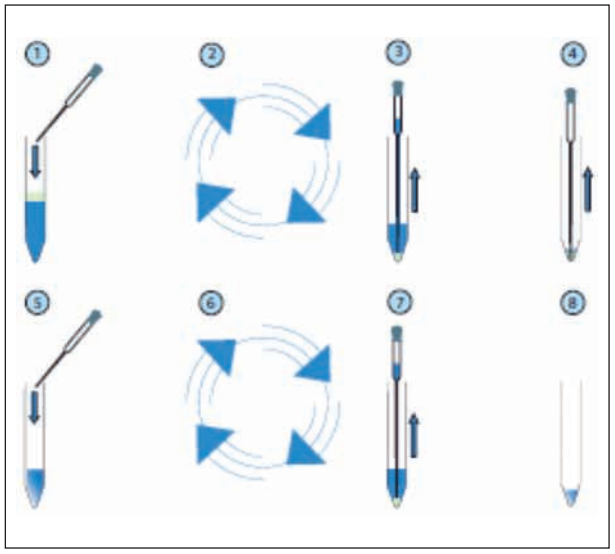


Fig. 3. — Stepwise illustration of sperm processing: Uni-layer density gradient centrifugation (obtained with permission from Nidacon International AB).

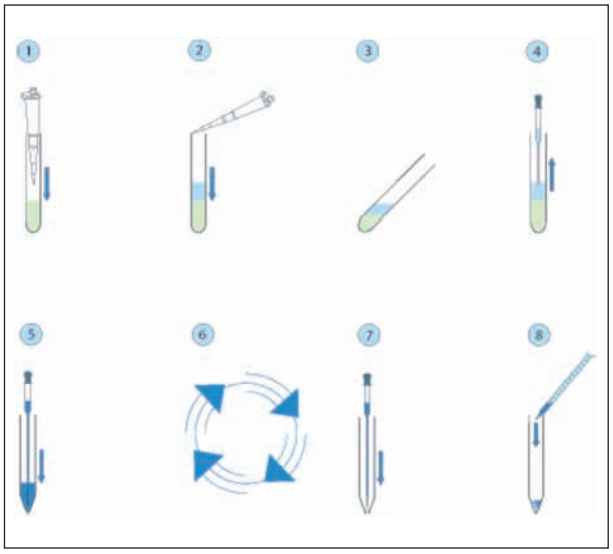


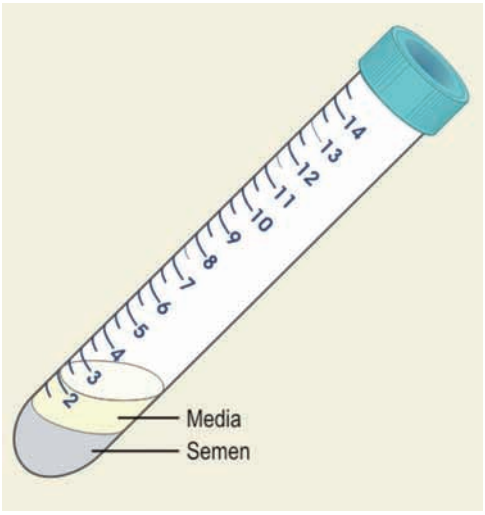
Fig. 4a. — Stepwise illustration of sperm swim-up and wash procedure (obtained with permission from Nidacon International AB).

the use of discontinuous density gradient centrifugation with subsequent washing.

- *HIV-seronegative* patients can be processed using a
  - uni-layer gradient (80% density gradient) when oligozoopermia, and/or teratozoospermia or asthenozoospermia or leukospermia are observed; or a
  - direct swim-up method if a sample is considered largely normal.

7.1. Density gradient centrifugation: Two-step (patients with blood-borne viruses)

HEPES buffered two step PureSperm® gradients, sperm preparation products and an insert (ProInsert™ from Nidacon Int. AB), will be used to process semen samples for insemination and assisted reproductive technologies. All solutions for sperm processing should be brought to room temperature before use. Opened bottles should be stored at 2-8°C



Procedure:

1) Pipette 1 ml liquefied semen into a sterile round bottomed tube. Layer 1.5 mL embryo culture media (supplemented with 100 U/mL Penicillin) or PureSperm Wash®, aseptically over the semen.

2) The round bottomed tube is then carefully placed at a 45° angle at 37°C. After 60 minutes, remove tube and position the tube upright in a test-tube rack. Using a new sterile (unused) pipette, carefully remove 0.5-1.0 ml of media containing the motile sperm fraction. Pipette the fluid then into a sterile conical centrifuge tube containing 5ml embryo culture media or PureSperm Wash®.

3) The conical tube is centrifuged at 500 *g* for 10 minutes. Continue according to steps 4 and 5 as describe previously for the density gradient procedure.

Fig. 4b. — Schematic illustration of sperm swim-up and wash procedure

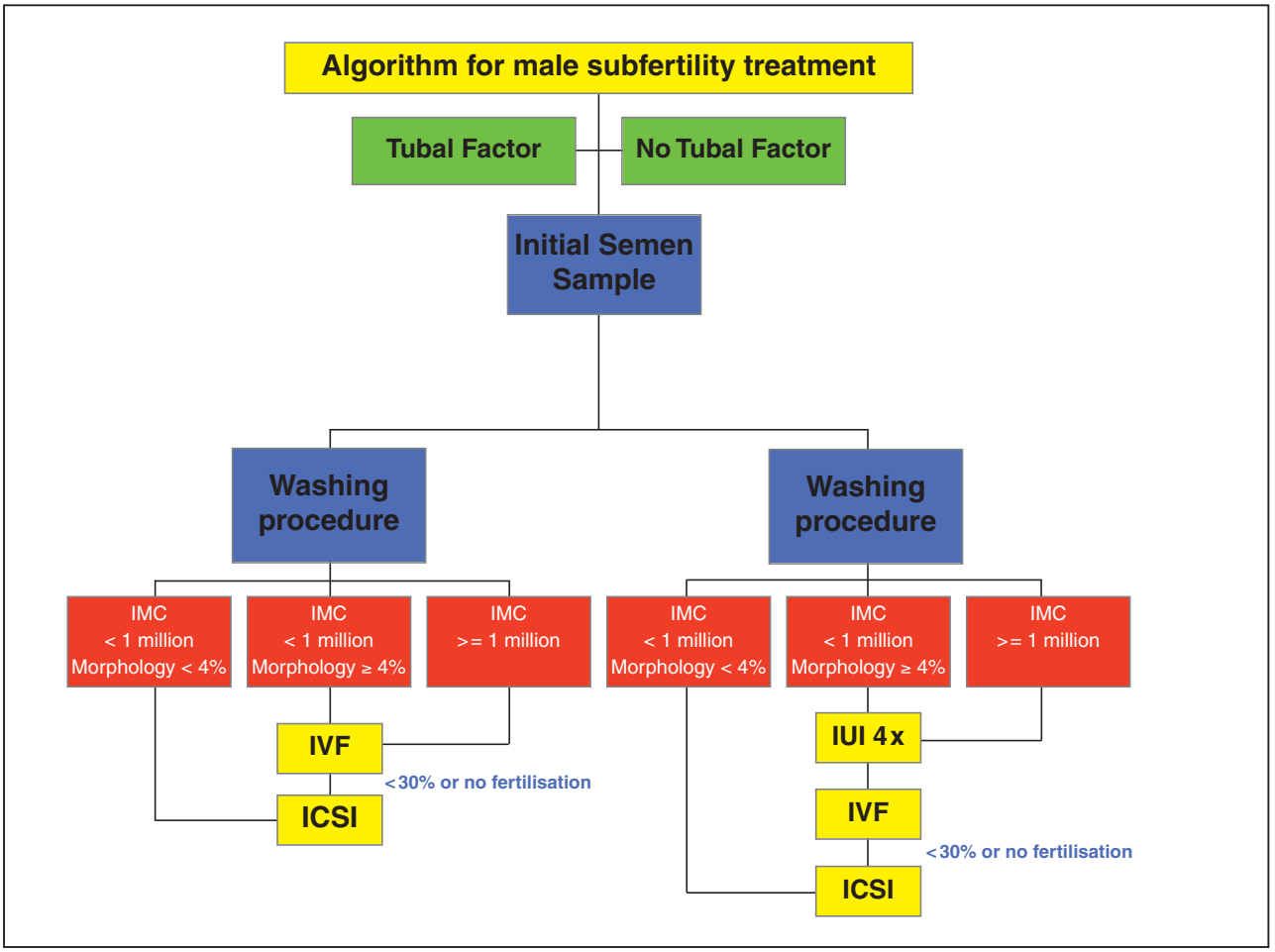


Fig. 5. — Proposed algorithm of male subfertility treatment (IMC = inseminating motile count or the number of motile spermatozoa after washing procedure, IUI = intra-uterine insemination, IVF = in-vitro fertilization, ICSI = Intra-Cytoplasmic Sperm Injection).

after re-sealing, opening and resealing always under sterile conditions. All items and solutions needed for the procedure are listed below.

The step-wise sperm processing using 40% and 80% ready-to-use gradient solutions is illustrated in Figures 2a and 2b.

7.2. Uni-layer density gradient

The step-wise sperm processing using an 80% ready-to-use gradient solution is illustrated in Figure 3. The method is similar to the previously described procedure, albeit without the 40% gradient and ProInsert™.

7.3. Swim-up and wash procedure

The step-wise sperm washing using media overlay with centrifugation is illustrated in Figure 5. The method is simplistic and schematically depicted in Figure 4a and 4b.

7.4. Semen quality after wash procedure

Whatever method has been used, it is very important to calculate the IMC (inseminating motile count). The IMC is the total number of motile spermatozoa after sperm wash procedure. The IMC is very important in selecting patients for either IUI (intrauterine inseminations), IVF (in-vitro fertilization) or ICSI (Intra-Cytoplasmic Sperm Injection). IUI seems to be the first line treatment providing at least one tube is patent and the IMC is more than 1 million (Ombelet et al., 1997). Furthermore, in cases with less than 1 million motile spermatozoa, IUI remains successful provided the sperm morphology score is 4 % or more (Figure 5).

8. *Mini-hysteroscopy*

The hysteroscopic evaluation of the female reproductive tract has also been simplified in its instrumentation and technique so that it can be offered in a one stop ambulatory approach, even in developing countries (Campo, 1999).

Office mini-hysteroscopy has become a non-expensive diagnostic technique accessible for every gynaecologist when using a small diameter optic, saline as distension medium and an atraumatic insertion technique (Campo et al., 2005). The

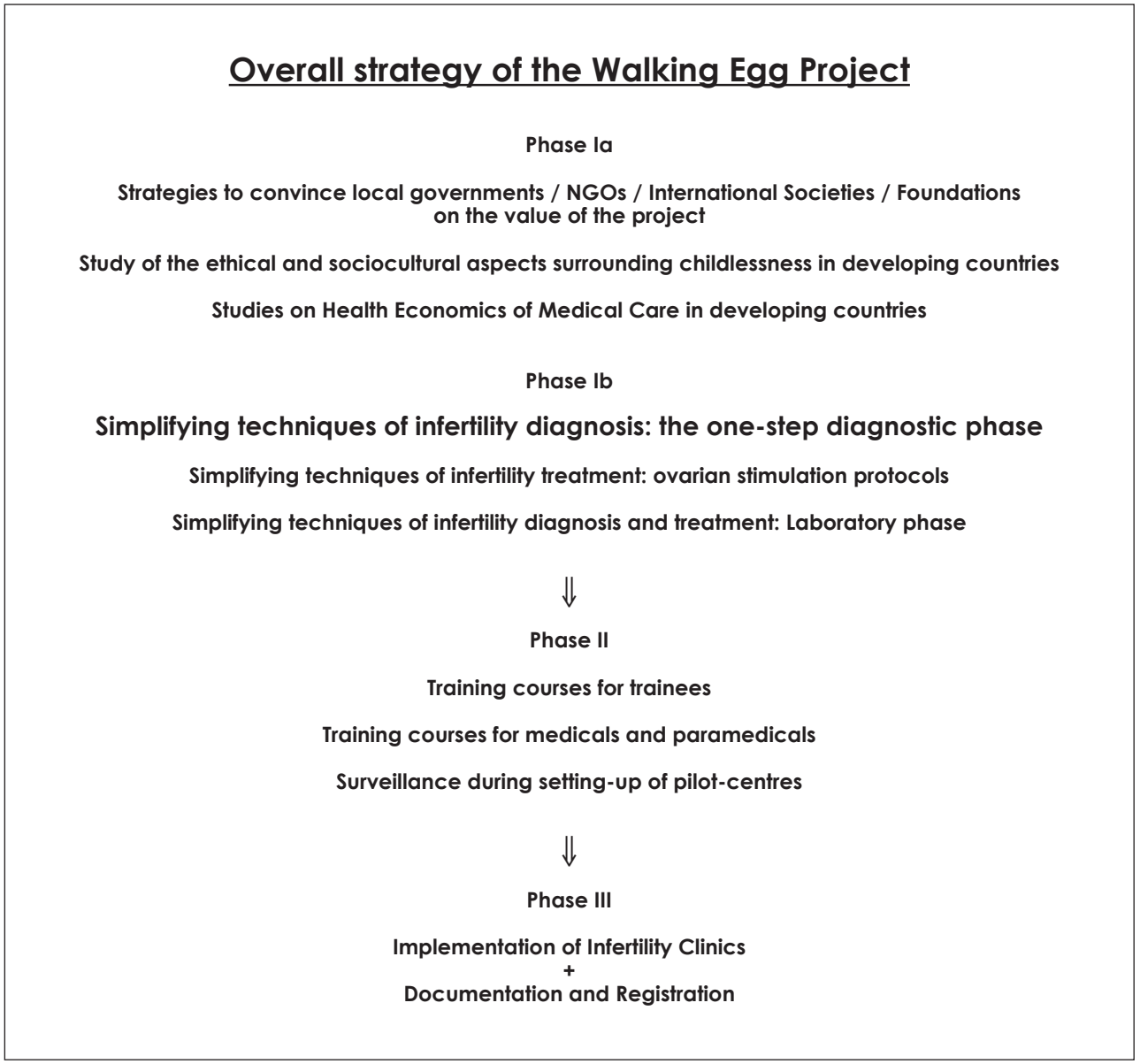


Fig. 6. — Overall strategy of the Walking Egg Project

European Academy of Gynecological Surgery is currently in the final phase of validating a structured programme for training and certification of hysteroscopic diagnostic and surgical procedures. The methodology followed to measure and validate the different hysteroscopic skills and knowledge of an individual is similar as described for laparoscopy in a paper of Campo et al. in this Monograph.

Because the methodology used is accessible, affordable and followed by a subsequent scientific validation, we believe that – in the near future – training, certification and clinical implementation of hysteroscopic procedures will be performed in a much larger number of infertility centres worldwide.

9. *Documentation and registration*

We believe that within each pilot-centre on-line data registration of all ART activities is mandatory. Administrative staff and (para) medicals have to be aware of the importance of correct and trustable data registration. The ultimate goal is to offer all pilot-centres a similar registration programme, which should be customer-friendly with a limited but sufficient number of items (increased personnel compliancy). Continuous monitoring of service activities will be centralized, and provide feed-back to clinics for clinical and laboratory policy adjustments, information to couples on clinic performance,

and information to society. Confidence can then be built and maintained.

Conclusion

Simplifying the diagnostic work-up in an infertility care programme allows accessibility for a much larger part of the population in developing countries.. Taking a history through questionnaires is easy to perform. A clinical examination and infectious screening of both partners can be performed at a reasonable price. Office mini-hysteroscopy, hystero-salpingography and/or hystero-salpingo-contrast-sonography are simple and accessible techniques to detect tube abnormalities and uterine malformations without major costs. Combining these techniques with an accurate anamnesis will identify the majority of women's infertility causes such as ovulatory disorders and tubal infertility. A basic semen analysis, before and after semen preparation, can provide information about the diagnosis and expected treatment procedure to follow. Semen analyses can also be performed by well-trained paramedicals, another important advantage for developing countries.

The organization of training courses for trainees, medicals and paramedicals will be extremely important and these courses have to be goal-oriented, with minimal theoretical and maximum practical guidance.

To conclude, infertility will likely become one of the more predominant components of future reproductive health care practice. Taking advantage of information and communication technologies will increase the effectiveness and accessibility of health care services, as well as change patient behaviors to seek timely treatment. As evidence-based, affordable solutions begin to drive global guidance within both public and private health care system solutions, access to care for the infertile couple will become one of the largest emerging fields in global medicine.

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