Are we ready to inject? Individualized LC-CUSUM training in ICSI

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Abstract

Purpose The objective of this prospective, single center study was to develop a personalized training scheme for intracytoplasmic sperm injection (ICSI) through the use of learning curve-cumulative summation (LC-CUSUM), which allows to tailor training to the trainee performance, and to validate it against the performance of experienced embryologists.

Methods Five trainees microinjected latex microspheres (LM) into vitro matured oocytes. A microinjection was considered successful when the oocyte did not lyse in the 24 h following the injection.

Results Each trainee became proficient at ICSI after a variable number of injections, ranging from 35 to 80. Trainees that achieved proficiency went on to perform ICSI with human gametes in a clinical setting with proficiency comparable to that of experienced embryologists.

Conclusions We show that LC-CUSUM based personalized ICSI training is feasible and allows trainees to be as proficient as trained embryologists when treating actual patients.

Keywords ICSI · LC-CUSUM · Learning curve · Training

Capsule A personalized training scheme for intracytoplasmic sperm injection (ICSI) through the use of learning curve-cumulative summation (LC-CUSUM) has been developed and evaluated to ensure that trainees exiting the program are as proficient at ICSI as trained embryologists.

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Introduction

ICSI, the introduction of a sperm into the cytoplasm of oocyte using a micropipette, is a widespread assisted technology of growing popularity, used in 115,529 ICSI cycles initiated in 2011 in the USA alone, [1]. In Europe, ICSI is also used frequently with 271,711 ART cycles initiated during 2010, which corresponds to 68.40 % of all ART cycles reported in the region [2].

Much like other laboratory procedures, ICSI is operator-dependent and requires training to be performed successfully. Most laboratories establish internal training schemes for junior embryologists in order to learn ICSI. Nevertheless, painfully few ICSI training protocols have been reported in scientific literature [3]. Moreover, commonly used training schemes are not tailored to the trainee and do not allow for individualized assessment of proficiency. In addition, the vast majority of training schemes are not validated against clinical results, so much so that it is considered acceptable for a recently trained junior embryologist to have lower fertilization rates than senior colleagues. Finally, the difficulties encountered by clinics and hospitals to procure and operate with gametes and embryos of animal origin, both from the legal and practical point of view, might push ICSI to be learned on viable human gametes, opening ethical issues of great relevance [4].

The cumulative summation (CUSUM) test is a mathematical model originally developed for the production industry with the aim to detect whether a process was “in control” or “out of control.” The aim of CUSUM test is to identify the need to suspend a process when “out of control,” that is, out of a predefined level of acceptance. LC-CUSUM (learning curves) curves are a modification of the CUSUM method and are designed to determine when a preset level of competence is achieved, thus allowing to know, for example, when a trainee has achieved proficiency at a certain task [5, 6]. Learning

curves (LC)-CUSUM have been used to evaluate training in ART-related tasks, such as, folliclemetry during ovarian stimulation [7] oocyte retrieval [8], oocyte vitrification [9], and embryo transfer [10, 11].

The aim of the study was to design, implement, and evaluate an LC-CUSUM based, individualized training program in ICSI that is clinically effective, avoids the use of animal gametes, and minimizes the ethical issues related to the manipulation of human gametes, specifically avoiding fertilizing oocytes for training purposes and then discarding the resulting embryos.

Materials and methods

Study population

This is a prospective study analyzing ICSI training in five trainees. At the time of enrollment in the study, trainees were proficient in semen processing and handle oocytes using plastic capillaries for cumulus cell removal, but did not have any experience with micromanipulation.

Study setting and procedure

The training for ICSI is divided in two main parts, a standard one identical for all trainees, followed by an individualized and LC-CUSUM based one.

The standard portion of the training is carried out as follows: a 1-h theoretical lecture is given describing micromanipulation equipment and consumables for microinjection, how to use an inverted microscope, how to place micropipettes on injectors, how to move the micropipettes, and how to set up an ICSI dish. Then, the trainee practices for about 8 h positioning the oocyte and immobilization and capture of sperm with the ICSI micropipette. In the last hour, the trainees inject in vitro matured oocytes under the supervision of a senior embryologist. No spermatozoa are present, and the injection is done with a PVP filled capillary. Afterwards, each trainee injects with medium and PVP 20 discarded oocytes.

At this point, the trainee moves on to the individualized part of the training, which consists of performing ICSI using latex microsphere (LM; accu-beads[®], HAMILTON THORNE INC.), similar in size to the head of a sperm, instead of spermatozoa and resuspended in PureSperm[®] Wash (Fig. 1). Injections are performed, whenever possible, according to laboratory schedule and material availability, typically in batches of 2–4 oocytes each time, until proficiency is achieved (see below).

LC-CUSUM curves and statistical analysis

Injected oocytes were cultured (G1[™] PLUS, Vitrolife) at 37 °C, 6 % CO₂. The following day, an embryologist

assesses the outcome of each oocyte (lysed vs. not lysed, Fig. 1) in the order of injection. In case the LM was found outside of the plasmalemma, i.e., ICSI was not performed correctly, the oocyte was considered as lysed. Outcomes were recorded by the main investigator, and LC-CUSUM curves were constructed. The CUSUM method was originally developed by the production industry and detects whether a process is “out of control,” that is, out of a predefined level of acceptance; outside of these instances, the performance is assumed to be “in control” by the model. LC-CUSUM is a modification of the CUSUM method designed to determine when a preset level of competence is achieved, thus reducing the risk of incomplete training when new operators perform complex procedures (Biau et al. 2008). When an operator begins a new procedure, by definition, the process is considered “out of control” until the trainee reaches the level of competence. LC-CUSUM monitors each subsequent procedure and identifies when the process becomes “in control.” We performed all analyses according to the most recent recommendations (Biau and Porcher 2010); the LC-CUSUM score was calculated at each procedure (t) as: $St = \min(0, St-1 + Wt)$. In case of success, $W = \log((1-p_0)/(1-p_0-d))$; in case of failure, $W = \log(p_0/p_0+d)$, and p_0 = acceptable failure rate; d = acceptable deviance from acceptable performance to be detected. St was plotted on the y-axis against the successive procedures on the x-axis.

In order to interpret the LC-CUSUM graph, one needs to be mindful of the fact that each success will cause the curve to move downward towards the decision limit (h_{LC}), while each failure will cause the curve to move back up towards the x-axis. Performance cannot be considered acceptable (i.e., the trainee is not proficient) while the score line remains in the area between the x-axis and the decision limit (h_{LC}), the so-called continuation region. Monitoring therefore continues as the trainee line move downwards until it crosses the h_{LC} , when competence is achieved. Of note, there is a holding barrier at the x-axis, which cannot be crossed, making the test able to detect the early progresses of the “out of control” trainee. The acceptable and unacceptable failure rates (lysis rate of injected oocytes) for “in control” and “out of control” processes, respectively, were a priori set at 5 and 10 % by a panel of experts. The acceptable deviance from acceptable performance to be detected (d) was set at 0.025. These resulted in a decrease of 0.027 units for each successful measurement and an increase of 0.406 for each failure. Under both scenarios (“in” and “out” of control), a simulation of 10,000 replicates of 100 procedures was carried out to select the h_{LC} value optimizing error I and II: an h value of -0.92 resulted in a risk of 15 % of declaring competency in an “out of control” scenario (false discovery rate [FDR])

Fig. 1 ICSI training with LM. **a** LM in a drop of medium, with one LM loaded in the injection pipette. **b** ICSI initiated, the LM is visible close to the tip of the injection pipette. **c** An oocyte 24 h after injection; the LM is clearly visible in the cytoplasm of the oocyte (success for LC-CUSUM). **d** An oocyte 24 h after injection; the oocyte is lysed and degenerated (failure for LC-CUSUM)

and of not declaring competency in a “in control” scenario (true discovery rate [TDR]).

Follow-up phase

Once trainees achieved proficiency at ICSI based on their LC-CUSUM curves, they moved on to performing ICSI from actual ART cases. For the first 50 oocytes injected, they performed ICSI on a portion of MII oocytes, while a senior embryologist performed ICSI on the remainder oocytes from the same patient. This allows safeguarding of the patients: on one hand, extending the supervision of the trainee to real cases before s/he works completely independently, and also ensuring that, in case the training had failed, patients would still have embryos available for transfer. If the fertilization rate obtained by the former trainee was comparable to that of the embryologist, the trainee continued to perform ICSI independently. If the fertilization rate remains significantly lower, then the trainee underwent retraining by the most experienced embryologist in the team, and continues performing ICSI paired up with a senior embryologist for another 50 oocytes. The results of the follow-up phase are presented in Table 1, including fertilization rate, lysis rate, rate of developed embryos and mean embryo quality, for the former trainees and for the senior embryologists.

Ethical approval

Before inclusion in the study, candidates were informed about the study purpose and protocol, and a written informed

consent was obtained from all trainees included. This study received the approval of the local IRB.

Results

Demographic characteristics

The trainees included in this study were trained as ART laboratory technicians and had on average 5 years of experience working in an ART laboratory. They were all women and the average age of the trainees was 32.5 (range 29–39).

Individualized ICSI training

The competence in ICSI using LM was achieved after microinjection of 35, 35, 70, and 80 oocytes with LM (Fig. 2). One trainee did not achieve competence in ICSI after 80 oocytes and was removed from the training program. One on one training under continuous supervision by a senior embryologist was initiated for this trainee, which is still ongoing.

During the follow-up period after competence was achieved, fertilization rates were comparable for all trainees to that of the senior embryologist performing ICSI on part of the gametes of the same patients ($p > 0.05$, Table 1). Moreover, lysis rate after ICSI and embryo development to day 3 were comparable between recently trained personnel and senior embryologists.

Table 1 Comparison of laboratory outcomes between recently trained personnel and senior embryologists after performing ICSI on 50 MII from actual cases. The senior embryologist performed ICSI on a portion of the oocytes, and the recently trained person on the rest. Mean embryo quality at day 2–3 was assessed on a scale 1 to 10 where 10 is the highest possible level of quality in terms of number of blastomeres, fragmentation, and blastomere size. A *p* value ≤ 0.05 has been set as statistically significant

	Trainee	Embryologist	<i>p</i> ^c
Fertilization rate ^a			
TRAINEE 1	74 %	72.3 %	0.94
TRAINEE 2	72 %	74.1 %	0.90
TRAINEE 3 ^d	48 %	72 %	0.15
TRAINEE 4	60 %	71.4 %	0.55
Lysis rate ^a			
TRAINEE 1	6.1 %	10.1 %	0.60
TRAINEE 2	10 %	10.2 %	0.97
TRAINEE 3 ^d	8 %	11.3 %	0.72
TRAINEE 4	8 %	7.1 %	0.86
Evolving embryos rate ^b			
TRAINEE 1	100 %	100.0 %	1
TRAINEE 2	100 %	89.09 %	0.039
TRAINEE 3 ^d	88.0 %	96.39 %	0.14
TRAINEE 4	73.3 %	84.00 %	0.75
Mean embryo quality ^c			
TRAINEE 1	6.7	7.0	0.44
TRAINEE 2	6.9	7.2	0.26
TRAINEE 3 ^d	7.0	6.8	0.68
TRAINEE 4	6.8	6.5	0.57

^a Chi² test with Yates correction

^b Fisher exact test

^c Student's *t* test

^d The trainee injected mostly vitrified/thawed oocytes, while the senior embryologist injected mostly fresh oocytes

Discussion

Training of laboratory personnel in ART is an issue of urgent relevance; it was recently reported that in 23 out of the 27 countries analyzed, ART personnel work without attending any officially recognized educational program [12], with training mostly offered on the job. ICSI is a widely used laboratory technique, whose correct execution impacts greatly on the number of embryos available for transfer, affecting both the initial and cumulative pregnancy rate of the cycle. In spite of the centrality of ICSI to more than several hundred thousand ART cycles performed each year, no standardized training, personalized follow-up, or proficiency assessment post training has been reported.

Examples of ICSI training and assessment are scarce at best in the scientific literature. One such example assessed competence by injecting human sperm into hamster oocytes [3]. The training protocol required the injection of at

least 150 hamster eggs for each trainee, and acceptable fertilization rates and low damage rate, by comparison with an expert operator, were set as markers of proficiency. Another option reported for ICSI training involves the use of oocytes from prepuberal gilts [13]. Although the use of animal rather than human gametes might seem a convenient option, several drawbacks make it less than ideal. First of all, many ART facilities find it challenging to procure animal material, especially farm animals' gametes. Secondly, guidelines for the handling of human gametes and embryos might prohibit the presence of animal cells in an ART laboratory. Moreover, species specific differences in zona hardness, plasmalemma elasticity, and gamete size, might cause an additional and unacknowledged learning curve during the transition to the human species. Finally, in several countries, the production of interspecies constructs with human gametes is not allowed, effectively limiting training options.

More than 20 years ago, Van de Bergh and colleagues proposed to perform ICSI training on in vitro aged, unfertilized human oocytes donated from patients undergoing conventional IVF [14]. One hundred fifty-one unfertilized oocytes were gathered for training and resulting embryos were replaced in 48 transfers, obtaining 8 ongoing pregnancies. Ethical considerations regarding the risk of polyspermy when IVF failures were due to failed oocyte activation would likely make this option not feasible today. An alternative option would be to inject discarded oocytes but never transfer the resulting embryos. A similar approach was proposed to train reproductive endocrine fellows [15], without providing details on how ICSI proficiency was assessed. Importantly, in many countries, the generation of a human embryo in vitro is only permitted in the context of assisted reproductive care, i.e., to produce embryos for a patient embryo transfer. Apart from the important legal and technical issues highlighted, the described training schemes are not tailored to the trainee, that is to say, the number of repetitions carried out to learn ICSI is preset, and there is no control on whether the trainee has learned after, for instance, 30 or 80 procedures. In some cases, trainees would be over-trained, thus wasting time and money, and in some cases, the trainees would move to the patient without being ready, thus causing a lowering of patient care and cycle efficiency. Furthermore, the vast majority of training schemes are not validated against clinical results; their assumption is that, upon completing whatever training is in place, the embryologist will be trained. In reality, recently trained embryologists will very often achieve fertilization rates lower than experienced embryologists and will, by necessity, finish learning on patients. Since ICSI is a technique requiring precision, good hand-eye coordination, and a certain level of skill and speed to be successful, we

Fig. 2 LC-CUSUM curves of the trainees included in the study. To interpret the LC-CUSUM graph, one needs to remember that each success (no lysis) will cause the curve to move downward towards the decision limit ($h_{LC} = -0.92$), while each failure will cause the curve to move back up towards the x-axis. Performance cannot be considered acceptable (i.e., the trainee is not proficient) while the score line

remains in the area between the x-axis and the decision limit (h_{LC}), the so-called continuation region. Monitoring therefore continues as the line move downwards until it crosses the h_{LC} , when competence is achieved. **a–d** Trainees achieved competence after 35, 35, 70, and 80 procedure, respectively. **e** The trainee did not achieve competency after 80 procedures and was removed from LC-CUSUM

reasoned that an individualized monitoring of training should best fit the expected variability in learning curve among trainees. LC-CUSUM curves afford this

possibility, and, accordingly, we found that a significant range of procedures is necessary to learn ICSI. Moreover, LC-CUSUM curves can be tailored to adapt to a

particular clinic or program, varying, for instance, the level of accepted lysis percentage depending on the expected outcome of the training.

The current work aimed at developing a training scheme tackling the ethical and legal issues as well as being individualized and validated. The training is based on two assumptions: first of all, we assumed that substituting the sperm with a LM would constitute an appropriate proxy for the ICSI procedure performed with sperm. The second assumption is that a high proficiency in performing ICSI with LM, corresponding to an oocyte lysis rate of 5 % or lower, would constitute an appropriate proxy for the ability to perform ICSI and obtain fertilization when using sperm. None of these assumptions have been tested previously; however, we show here than after performing the suggested training with LM and achieving low lysis rates, all trainees performed as well as senior embryologists during their first 50 “real” ICSIs.

We have also found that some trainees (1 of 5 in our case) will not learn to be proficient at ICSI even after a substantial number of attempts (80 in our case). This is an important finding, since the same trainee would be allowed to treat patients in most fixed procedure-based training. The options for these trainees are twofold. They could be excluded from this task, or they could undergo further training. In this second case, each laboratory should decide how many oocytes are too many to continue the training, and when a one on one retraining with the assistance of a senior embryology is in order instead. We have placed this limit in 80 oocytes, but this could be different for other facilities and training programs.

We recognize that the need for a relatively high number of discarded oocytes for training is a weak point of the proposed study, especially in smaller facilities; however, we consider this a self limiting issue, since smaller ART facilities should by definition train and incorporate fewer embryologists, and discarded oocytes could be vitrified and accumulated for later use. Moreover, all trainees were ART technicians, which are, collectively, exact and detailed oriented. Nonetheless, the differences in training time indicate that a significant interpersonal variability might be expected even among trainees already proficient in lower complexity ART tasks. The number of trainees, 5, included in the study, is equal or higher than what presented in most ART LC-CUSUM studies [8, 11] and should allow for adequate generalizability of results and training expectations.

In conclusion, we show here an easy to implement program for individualized ICSI training that, unlike most current training schemes, is both individualized and validated against post training proficiency. ART facilities should adopt individualized training for ICSI and allow for sufficient time to adjust to different trainee’s training paces.

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Compliance with ethical standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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