Use of pig oocytes for training new professionals in human assisted reproduction laboratories

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Objective: To evaluate whether swine oocytes are useful for training new technicians in a human reproduction laboratory.
Design: Prospective study.
Setting: Graduate school in assisted reproductive techniques (ART) in Brazil.
Patient(s): Students in a human reproduction laboratory.
Intervention(s): Medium-sized follicles were aspirated from prepubertal gilts' ovaries and collected at a slaughterhouse. Oocytes were retrieved from the follicular fluid. Twenty-one students trained during four periods of 20 hours each were evaluated as to their ability to perform micromanipulation and were compared with a group of well-trained professionals (control group).
Main Outcome Measure(s): Students' ability in oocyte retrieval, oocyte manipulation, and intracytoplasmic sperm injection during and after the 80 hours of training.
Result(s): Students were able to retrieve, on average, 23.8 oocytes per ovary. Their micromanipulation skills substantially increased, reaching an oocyte retrieval rate of 77.2%, compared with 84.9% in the control group after the training period. Although the oocyte damage rate gradually decreased, from 52.0% after 20 hours of training to 5.4% after 80 hours, these figures were still above the control group oocyte damage rate by 0.3%, which was a statistically significant level. Regarding intracytoplasmic sperm injection, within 40 hours, no students were able to perform a single injection; and by the end of 80 hours, they achieved an average of 4.0 oocytes per hour, whereas the control group injected 20.6 oocytes per hour, a statistically significant difference.
Conclusion(s): Swine ovaries may be a useful tool in the spectrum of training techniques for unskilled assisted reproductive techniques laboratory professionals. (Fertil Steril® 2007;88:1408–12. ©2007 by American Society for Reproductive Medicine.)

Key Words: Pig oocytes, training new technicians, ICSI, micromanipulation

In 1992, Palermo and colleagues reported pregnancies and live births after intracytoplasmic injection (ICSI) of a single sperm into an oocyte (1). Over the last decade, ICSI has become the treatment of choice for many couples with untreatable infertility. Nevertheless, one of the most significant challenges in assisted reproduction technology (ART) is achieving technical proficiency in micromanipulation. According to Gvakharia et al. (2), fertilization rates of nonproficient personnel range from 30% to 40%, and oocyte damage rates could be $\leq$30%. However, ethical and moral concerns associated with the difficulty of obtaining oocytes renders impracticable the use of human oocytes for training purposes.

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Porcine oocytes aspirated from ovaries collected at slaughterhouses may be a suitable tool, because this process avoids the issues in the previous paragraph, allowing untrained personnel to manipulate oocytes and improve their ability. Pig ovaries contain an average number of 50 follicles of several different diameters, with a size range classified as small (<3 mm), medium (3 to 6 mm), or large (>6 mm) (3). In addition, ovaries of prepubertal gilts collected at slaughterhouses have been used for in vitro production of embryos in swine for a long time (4).

The aim in the present study was therefore to evaluate whether swine oocytes are useful for training new technicians in human reproduction laboratories.

MATERIALS AND METHODS

The study was performed at the Sapientiae Institute, a graduate school in ART, in Sao Paulo, Brazil. The program was

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designed to provide students with a coherent body of knowledge in the field of human reproductive technology, which included theoretical classes in biostatistics, epidemiology, bioethics and law, biotechnology, physiology of reproduction, embryology, assisted reproduction techniques, genetics, and others. The program also included practical sessions in which students were able to train in relevant aspects of ART, such as good laboratory practices, bioinsurance, oocyte manipulation, semen analysis, and sperm processing.

For this study, 21 students with a degree in biomedicine or biology were evaluated during an 80-hour training period in oocyte manipulation, and their performances were evaluated. The training was divided into four periods of 20 hours, split in five subsets, and ICSI was the main focus in 50% of the whole training time. The students' competency in oocyte retrieval from follicular fluid, oocyte denudation, and ICSI was assessed, and performances were compared with those obtained by a group of well-trained professionals in the same techniques (control group). Institutional review board approval was not required because the ovaries were obtained from a slaughterhouse.

Oocyte Collection and Removal of Cumulus and Corona Cells

Ovaries were obtained from prepubertal gilts at a local slaughterhouse, without any selection criteria, and transported to the laboratory in cold saline solution within 1 hour. Follicular fluid was aspirated from medium-sized follicles (3–6 mm in diameter) with an 18-G needle fixed to a 10-mL disposable syringe, and recovered cumulus–oocyte complexes were transferred to a 60- × 15-mm dish (Falcon, Sao Paulo, Brazil) with culture medium (G-1-V3-Plus; Vitrolife, Kungsbacka, Sweden). Removal of surrounding granulosa cells was performed enzymatically by using hyaluronidase (80 IU/mL, Irvine Scientific, Santa Ana). The remaining cumulus cells were removed in the absence of the enzyme by repeated pipetting with fine-bore glass Pasteur pipettes with different diameters.

Sperm Preparation and ICSI Training

Fresh human ejaculates from donors were prepared by discontinuous density-gradient centrifugation (90%–45% Isolate, Irvine Scientific). After liquefaction at room temperature, 1 mL of the semen sample was placed on a gradient consisting of a 1-mL layer of the 90% fraction at the bottom and a 1-mL layer of the 45% fraction at the top and was processed by centrifuge at 800 × *g* for 20 minutes. After centrifugation, the bottom fraction was aspirated and washed twice at 800 × *g* for 8 minutes. After washing, the concentration and motility of the final fraction were assessed. When necessary, the fraction was diluted or concentrated.

For the ICSI training, the oocytes were placed individually into 4-μL droplets of buffered medium (G-Mops-V3-Plus; Vitrolife), and sperm was placed in a central 4-μL droplet of a polyvinylpyrrolidone solution (Irvine Scientific) in a 60- × 15-mm dish (Falcon) covered with mineral oil. Intracytoplasmic sperm injection was performed with an Olympus inverted microscope (Olympus, Tokyo, Japan) that was equipped with Hoffman modulation contrast optics (Hoffman, Greenvale, NY).

Students' Performance Evaluation

Oocyte retrieval After follicle aspiration, the follicular fluid was placed into a 100- × 20-mm dish (Falcon), and the students checked the liquid for the presence of cumulus–oocyte complexes. The oocyte retrieval rate was calculated by dividing the number of recovered oocytes by the number of aspirated follicles.

Oocyte damage or loss After oocyte retrieval, students' competence during denudation and oocyte transport between dishes was evaluated by dividing the number of oocytes at the beginning of the procedure by the number of intact denuded oocytes in the end.

Time for ICSI performance Denuded oocytes assigned to each student were sufficient to ensure training during the subset time. Students' mechanical skills in performing ICSI included the ability to set up the micromanipulator, identify the best human spermatozoa available in the sample, aspirate it after immobilization, place the oocyte at the holding pipette, and perform sperm injection. Their mechanical skill was measured by the number of oocytes properly injected per hour, confirmed by the presence of spermatozoa in the ooplasm. Ruptured oocytes, oocytes injected with improperly immobilized or selected spermatozoa, and contamination in any step of the overall procedure were excluded from the final numbers.

Statistical Analysis

The data were analyzed by analysis of variance, Student's *t*-, and Mann-Whitney tests. Statistical significance was determined at $P \leq .05$.

RESULTS

The students' competence in oocyte retrieval from follicular fluid increased significantly, reaching 77.2% after 80 hours, compared with 84.9% in the control group ($P=.076$).

Although oocyte damage or loss rate gradually decreased during the study (52.0% after 20 hours, 41.1% after 40 hours, 19.7% after 60 hours, and 5.4% after 80 hours), it was still significantly higher than in the control group (0.3%, $P=.001$). Regarding ICSI procedure, within 40 hours of training, no student was able to perform an injection properly; after 60 hours, the students had improved to a level of 1.8 ± 1.4 oocytes per hour; and finally, at the end of 80 hours, they achieved an average of 4.0 ± 2.3 oocytes per hour, whereas the control group injected 20.6 ± 3.0 oocytes per hour ($P=.012$).

The average number of aspirated follicles, average number of oocytes recovered per student from follicular fluid,

recovery rate, average number of damaged or lost oocytes, the damaged or lost oocytes rate, and number of injected oocytes in 1 hour in relation to time of training are described in Table 1 and Figure 1.

DISCUSSION

Training new professionals to perform micromanipulation constitutes a major difficulty in the management of human ART laboratories because of ethical concerns associated with the use of human oocytes by unskilled technicians. A standardized training system using an animal model appears to be a good alternative to avoid these issues.

Since the first report of in vitro fertilization (IVF) with in vivo–matured oocytes (5) and successful fertilization after in vitro maturation reported by Marttioli et al. (6), improved methods have been developed for in vitro maturation, IVF, and in vitro production of pig embryos, and porcine oocytes currently are being widely used as a study model for animal reproduction techniques. However, the use of these oocytes for training professionals in human assisted reproduction micromanipulation had not yet been performed.

We have successfully established a standardized protocol for training new technicians by using swine oocytes. After 80 hours of training, it was observed that the students presented an oocyte retrieval rate close to that achieved by the well-trained professionals. Even though the students' oocyte damage or loss rate was higher than that in the control group, the rate decreased significantly during the sessions, and this issue deserves special attention, because in poor-responder women and patients with an unsynchronized cohort of follicles, the loss or damage of a single oocyte would have serious impact on the outcome of the cycle. In this manner, it may be suggested that a few more hours of practice would allow results very close to those of the well-trained professionals.

The best way to evaluate the success of the sperm injection technique is undoubtedly to assess fertilization rate, further embryonic development, and even implantation rate. Many studies reported the production of piglets after ICSI (7–9); however, the results in regard to fertilization, embryo cleavage, and implantation are very poor, and it would be difficult to discern whether the fertilization failure was a technical issue or a species characteristic. Also in the present study, because prepubertal porcine ovaries were collected, most of the punctioned follicles were <5 mm in diameter, and the retrieved cumulus–oocyte complexes had at least three complete cumulus cell layers, characterizing immature cumulus–oocyte complexes. Thus, to evaluate fertilization and embryo development after sperm injection in this group of immature oocytes, the oocytes would have to be matured in vitro before the injection, which would further reduce the number of eggs available for training.

It has been reported that inadequate sperm motility and morphology can be highly detrimental to embryo development and may lead to the production of potential defective embryos (10). Therefore, because sperm selection by the ICSI operator is one of the most important factors for the success of the technique, in the present study, human sperm were used.

The ICSI technique was the most critical part of the training, because we detected just a slight increase in the number of injected oocytes in 80 hours, reinforcing that this technique requires a lot of expertise by the professional. When sperm head decondensation was used as the main result to evaluate ICSI success, a 4-month period was needed to reach an acceptable level of competence in trainees (2). Thus, more training sessions focused just on ICSI would be necessary for the students in this study to achieve full competence to perform ICSI. In the human reproduction laboratory, an untrained professional uses only discarded or unfertilized oocytes to begin ICSI training and would probably take a longer time period to acquire proficiency. Because the students' oocyte damage or loss decreased and the number of injected

TABLE 1

Students' performance in relation to time of training.

Training time (h)	AF	OR	RR	DLO	DLR	IO
20	28.7 ± 7.0	21.0 ± 6.8	73.1	11 ± 3.1	52.3	0 ± 0.0
40	29.2 ± 7.1	20.4 ± 6.1	69.8	8.5 ± 2.1	41.6	0 ± 0.3
60	29.0 ± 7.0	21.3 ± 5.4	73.4	4.2 ± 1.4	19.7	1.8 ± 1.04
80	30.8 ± 5.2	23.8 ± 4.4	77.2[a]	1.3 ± 1.4	5.4[c]	4.0 ± 2.3[e]
Control	30.6 ± 1.52	26.0 ± 1.0	84.9[b]	0	0.3[d]	20.6 ± 3.06[f]

Note: Data are percentages or are mean ± SD. AF = average number of aspirated follicles; OR = average number of oocytes recovered per student from follicular fluid; RR = recovery rate; DLO = average number of damaged or lost oocytes; DLR = students' damaged or lost oocytes rate; IO = number of injected oocytes in 1 hour.

[a,b] $P=.076$.

[c,d] $P=.001$.

[e,f] $P=.012$.

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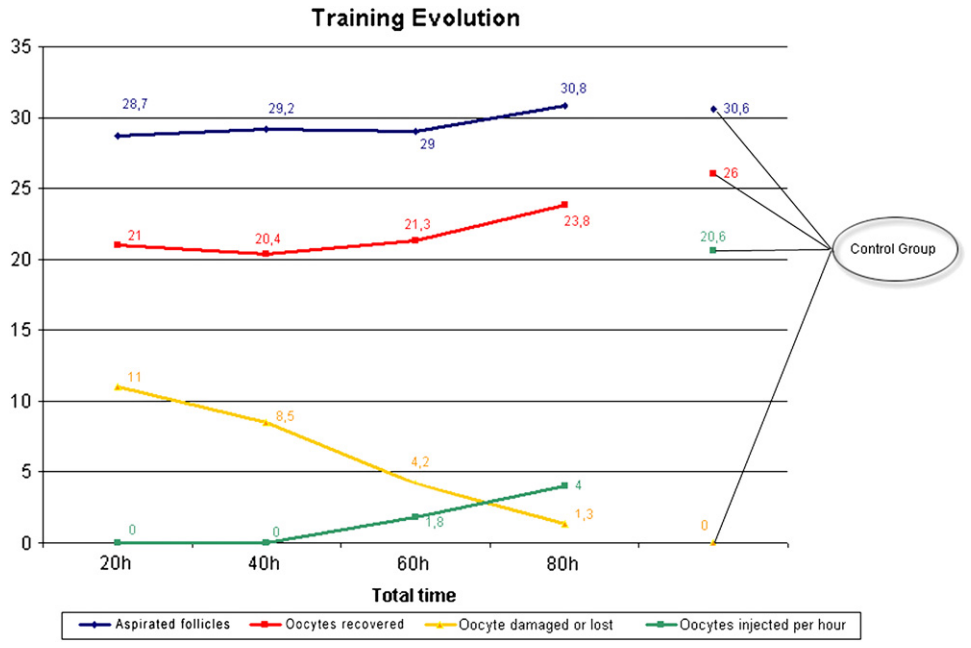
FIGURE 1

Students' performance evaluation throughout the time. Number of aspirated follicles, oocytes recovered, damaged or lost oocytes, and injected oocytes in 1 hour, per student, in relation to time of training.



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oocytes per hour increased over a period of time, we hypothesize that if the professional has previous training in porcine oocytes, the learning curve for ICSI in the human laboratory will be decreased.

In addition, we suggest that swine ovaries may be a good source of oocytes, because we observed during the study that it is possible to retrieve an average of 23.8 oocytes per ovary for a very affordable price (US$ 0.04 each ovary).

Other alternatives for training unskilled technicians would be the use of bovine oocytes aspirated from ovaries obtained at slaughterhouses or the use of mouse oocytes retrieved from the female mouse uterus.

Bovine oocytes and embryos have been studied as a model for ART for a long time. The first study in ICSI in bovines was conducted in 1984, when the formation of both male and female pronuclei was reported after the injection of a bovine spermatozoa into an in vitro–matured bovine oocyte (11). Recently, a bovine protocol for training professionals in preimplantation genetic diagnosis has been established to identify mistakes and difficulties, improving professionals' performance in different stages of preimplantation genetic diagnosis (12).

When compared with swine oocytes, bovine oocytes present a clearer cytoplasm and are much more similar to human oocytes. However, there are some inconveniences in obtaining those oocytes that may be avoided when swine oocytes are used. Most of the times, bovine slaughterhouses are placed far from human reproduction centers, which would bring some inconvenience related to transportation and storage of these ovaries.

Also, bovine estrous cycle, characterized by recurrent waves of follicular growth, produces a single dominant follicle in each wave that continues to develop, whereas the others undergo atresia (13). The dominant follicle remains dominant for few days, until it becomes atretic and regresses, to be replaced within approximately 5 days by the next dominant follicle from the next wave (14). If luteolysis occurs during the dominance phase, the dominant follicle will continue to develop and ovulate (15). In this way, independent of the moment that the bovine ovaries are collected, the number of antral follicles present in the ovarian cortex is much lower when compared with swine ovaries.

The other alternative would be the use of mouse or hamster oocytes. Gvakharia et al. (2) designed a system for teaching ICSI and provided a standardized method to assess technical competency, in which human sperm were microinjected into hamster ova and fertilization was observed. In fact, since 1976, when it was reported that zona-free hamsters' ova could be penetrated by heterologous sperm (16), hamster oocytes have been used to study reproduction techniques and to train professionals in micromanipulation. However, even though mice and hamsters are very small laboratory animals, require lower maintenance costs, and are easy to handle, their husbandry still requires specialized facilities and employees. Husbandry could be

avoided by the use of cryopreserved eggs, but the consequences of the freezing and thawing process may affect the quality of the material (17–19).

The use of swine oocytes for training also presents a bioethical advantage over the use of small laboratory animals, because pigs are primarily raised and slaughtered for food provision and ovaries are a byproduct, whereas mice or hamsters would be killed in this case, primarily for oocyte provision. Therefore, our results support the idea that swine ovaries may be a useful tool in the spectrum of training techniques for ART laboratory unskilled professionals.

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