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Performing ICSI with commercial microinjection pipettes enhanced pregnancy rates

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Background/aim: Many technical factors can affect intracytoplasmic sperm injection (ICSI) outcomes. The role of the injection micropipette could be of vital importance in ICSI programs. The main goal was to compare ICSI pregnancy outcomes between commercial and home-made injection micropipettes in a large population with male factor infertility.

Materials and methods: Five-hundred and eleven ICSI cycles with severe male factor were included in this retrospective study. ICSI cycles were divided into two groups: A (home-made micropipettes, n = 267) and B (commercial micropipettes, n = 244). Rates of fertilization, embryo formation, and chemical and clinical pregnancies were compared between the groups. The independent samples t-test, chi-square test, and Fisher's exact test were used, whenever appropriate, for statistical analysis.

Results: A total of 3621 MII oocytes were retrieved, of which 2003 were fertilized. The rate of normal fertilization was significantly higher in group A (57.9%) compared to group B (52.5%). However, the rate of embryo formation showed an increase in group B compared to group A (90.4% and 85.9%, respectively, P = 0.002). In addition, the clinical pregnancy outcomes improved in group B.

Conclusion: Our findings indicate that clinical pregnancy improves when commercial injection micropipettes are used in ICSI programs.

Key words: Commercial micropipette, home-made micropipette, male infertility, ICSI outcome

1. Introduction

Micromanipulation technology has become one of the principle techniques in new fields of biomedical sciences, including chimera production, nuclear transfer, and cytoplasmic transfer in order to improve embryo development. It also establishes transgenic animals through injection of pronuclear DNA content, preimplantation embryo genetic diagnosis using blastomere biopsy, embryo splitting, and intracytoplasmic sperm injection (ICSI) (1–4).

Introduction of ICSI was the most effective treatment program in order to give the opportunity of having babies for couples with male factor infertility; also it can improve the fertilization rate and embryo quality in patients with nonmale factor infertility (5,6). The valuable laboratory outcomes of ICSI could be oocyte survival rate, fertilization rate, and formation of good quality embryos. Many technical factors may affect ICSI outcomes, such as sperm immobilization prior to ICSI (7), operator skill (8), polyvinylpyrrolidone (PVP) (9), orientation of the first polar body (10), injection micropipette (11), aspiration of ooplasm (12), sperm direction inside the micropipette

(13), excessive manipulation inside the oocyte (14), effect of sperm entry (15), and the ICSI technique itself (16). Undoubtedly, one of the most important goals in assisted reproductive technologies (ARTs) is to improve the formation of high quality embryos to increase the chance of pregnancy among infertile couples. Despite impressive improvement in the ICSI technique, the rate of fertilization in the majority of ART programs (60%–70%) is far from ideal and needs further improvement (17).

One way to improve fertilization rate in ICSI programs is to avoid or minimize oocyte degeneration. ICSI is an invasive technique in which one spermatozoon is directly injected into the ooplasm of a mature oocyte using a fine glass micropipette. A well-designed micropipette with passing mouse embryo assay (MEA) threshold may cause less damage to the oocyte and reduces degeneration, while increasing the normal fertilization rate. The vital role of the injection micropipette in ICSI outcome has been shown in only one previous study (11). The present retrospective analysis was designed to compare ICSI outcomes using commercial versus home-made injection micropipettes in a large population with severe male factor infertility.

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2. Materials and methods

2.1. Patients

A total of 511 ICSI cycles of severe male factor infertility that were referred to the research and clinical center for infertility in Yazd, Iran, were reviewed retrospectively from November 2014 to November 2015. Cycles were divided into two groups: A (home-made micropipettes, n = 267) and B (commercial micropipettes, n = 244). Semen samples were collected by masturbation. Nonejaculated spermatozoa, egg donation, and in-vitro matured oocytes were excluded from the study. This study was approved by our institute's ethics committee (Ref. No: 2458) and all patients signed informed consents.

2.2. Ovarian hyperstimulation and sperm preparation

GnRH agonist (Sanofi-Aventis, Germany) downregulation followed by rFSH (Merck-Serono, Switzerland) was used for ovarian hyperstimulation. Then 10,000 IU of human chorionic gonadotrophin (hCG, i.m.; IBSA, Switzerland) was administered. The ovarian response was checked by transvaginal ultrasonography and evaluating the level of serum estradiol. Approximately, 36 h after hCG injection, oocyte pickup was done under transvaginal ultrasound guidance (18).

Semen analysis was done according to WHO guidelines (19). All sperm preparations were performed using the swim-up or density gradient centrifugation techniques (20,21). The ICSI procedure was conducted using fresh samples as described previously (22).

2.3. Micropipette preparation

After washing the borosilicate capillaries (Drummond, USA) with milli-Q water, they were pulled by horizontal pipette puller (Sutter Instrument Co., USA). For making the injection micropipette, the pipette was ground. The grinder (EG4, Narishige, Japan) whetstone was rotated at 1500 rpm with continuous dripping of milli-Q water during the rotation. The angle of the pipette with the whetstone was 45°. The micropipette was washed with hydrofluoric acid 0.1% for getting rid of any remaining glass debris in the micropipette. Then the clean micropipette was washed in deionized water several times. For bending the micropipette, with aid of a heating microforge (MF9, Narishige, Japan), an angle of 35°–40° was formed with an inner diameter of 4–5 µm. For preparation of the holding pipette, it was heated by microforge after pulling. The bending was the same as above. The inner and opening diameters were 12–15 and 3–5 µm, respectively. After preparation, the micropipettes were sterilized by dry heat at 120 °C for 6 h. The inner diameter of commercial injection micropipette (Vitrolife, Sweden) was 4–5.5 µm with a bending angle of 30°. Each commercial micropipette

had already been tested with MEA by the manufacturing company.

2.4. Fertilization and embryo evaluation

After ICSI, the injected oocytes were washed in equilibrated culture medium and placed in 50-µL drops of G1 medium (Vitrolife, Sweden) covered with mineral oil (Ovoil, Vitrolife, Sweden). The injected oocytes were checked 16–18 h post-ICSI for normal fertilization (presence of two pronuclei and two polar bodies). Embryo transfer (ET) was done on day 2. Only high-grade embryos with no or minimal cytoplasmic fragmentation were transferred.

2.5. Pregnancy outcomes

Chemical pregnancy was determined by a positive βhCG level 14 days after ET. Clinical pregnancy was determined by visualization of the gestational sac in ultrasonographic images or by detection of fetal heartbeat. Once chemical pregnancy was confirmed at the end of the second week of gestation, the patients were followed for 4 weeks for clinical pregnancy assessment.

2.6. Statistical analysis

The data are presented as mean ± SD. The Mann-Whitney test was used for comparison of quantitative data between the two groups. The chi-square and Fisher's exact tests were used whenever appropriate using SPSS 16 (Chicago, MI, USA). The odds ratio with 95% confidence interval is also presented. The odds ratios were referred to the rates of fertilized oocytes, embryo formation, and chemical and clinical pregnancy. All tests were hypothesized two tailed and P-values less than 0.05 were considered statistically significant.

3. Results

A total of 3621 MII oocytes were retrieved, of which 2003 were fertilized normally. Patient characteristics are shown in the Table. In groups A and B, 15 and 17 cycles were missed to follow their pregnancy outcomes, respectively. The rate of normal fertilization was significantly higher in group A compared to group B (57.9% and 52.5%, respectively, P = 0.001). However, the rate of embryo formation showed a significant increase in group B compared to group A (90.4% and 85.9%, respectively, P = 0.002). Higher pregnancy rates were reported in ICSI cases with commercial injection micropipettes (Table).

4. Discussion

The design and quality of the microinjection needle is one of the most important factors in successful ICSI programs (23). There are several studies in this field and some of the characteristics of micropipettes, e.g., sharpness (24,25), inner diameter (11), and multiple pipette use

Table. Intracytoplasmic sperm injection outcomes in groups A (home-made micropipette) and B (commercial micropipette).

Variables	Group A	Group B	P	Odds ratio (95% CI)
No. of cycles	267	244		
Female age ^a	31 (18–46)	30 (18–48)	0.009	
Metaphase II oocytes ^a	6 (1–37)	6 (1–20)	0.08	
Transferred embryos ^a	2 (1–4)	2 (1–4)	0.6	
2 PN %	(1061/1830) 57.9	(942/1791) 52.5	0.001	1.2 (1–1.4)
Embryo formation %	(912/1061) 85.9	(852/942) 90.4	0.002	0.6(0.4–0.8)
Chemical pregnancy %	(72/252) 28.5	(71/227) 31.2	0.5	0.8 (0.5–1.3)
Clinical pregnancy %	(52/252) 20.6	(50/227) 22.0	0.7	0.9 (0.5–1.4)

a: data are shown as median (min–max)

PN: pronuclear

CI: confidence interval

(26), have been evaluated previously. In this retrospective investigation, we assessed ICSI outcomes between two different injection micropipettes, namely commercial and home-made. We tried to omit some confounding factors, such as source of sperm, as only ejaculated spermatozoa were included in the study. One of the important factors that may affect the rate of fertilization in ICSI is the operator's skill (8). In our study, ICSI was performed by two expert embryologists with at least 7 years of experience. Moreover, ICSI by one embryologist would be ideal in order to minimize interindividual variability in the ICSI technique.

Another common factor in the two groups was the holding micropipette, which was also home-made in all cases. The inner diameter of the injection pipette can affect the fertilization rate, and a smaller inner diameter can improve the fertilization rate and embryo development (11). It seems that there were no considerable differences between the inner diameters of the commercial and home-made micropipettes in the present study. The inner diameters of both commercial and home-made micropipettes were similar (4.5–5 µm vs. 4–5 µm). However, both the sizes and diameters of the commercial pipettes were more consistent compared to the home-made micropipettes. This factor may be one of the advantages of using commercial micropipettes. The other characteristics of the commercial micropipettes are the sterilization method. One of the major differences with the home-made ones is that the commercial micropipettes importantly passed quality control tests (e.g., MEA, endotoxin). The commercial pipettes seem to be more cost-effective, as in-house preparations of micropipettes

require expensive instruments and expert operators. One of the variable factors between the two groups is the internal angle; the internal angles in groups A and B were 35°–40° and 30°, respectively. This factor also may not affect the ICSI outcomes, because the angle for the injection micropipette can vary from 30° (27) to 45° (28). As shown in Figure 1a, the distal arm in the home-made micropipettes is 5–6 times higher when compared to the commercial ones. In addition, the spike's length in the home-made micropipettes is about twice the length of the spike in the commercial ones (Figure 1b). It may have some detrimental impacts on the oocyte during ICSI, such as rupture of the opposite side of the oolemma. Another difference is related to the angle between the distal and proximal arms, which is slightly higher in the home-made micropipettes.

To the best of our knowledge, this is the first study to compare the outcomes between home-made and commercial microinjection needles for ICSI, which is widely practiced in the treatment of male infertility. However, there are some shortcomings of the study, such as: 1) lack of comparison of oocyte degeneration rates following ICSI between the two groups, and 2) lack of comparison of nonfertilized and abnormally fertilized (3PN) rates between the groups. We suggest conducting a study comparing the role of commercial with home-made micropipettes for immobilization of spermatozoa using nonejaculated samples in the ICSI setting. In conclusion, in comparison with home-made injection micropipettes, it seems that the commercial injection micropipettes can improve the rate of clinical pregnancy in ICSI programs in large populations of male factor infertility.

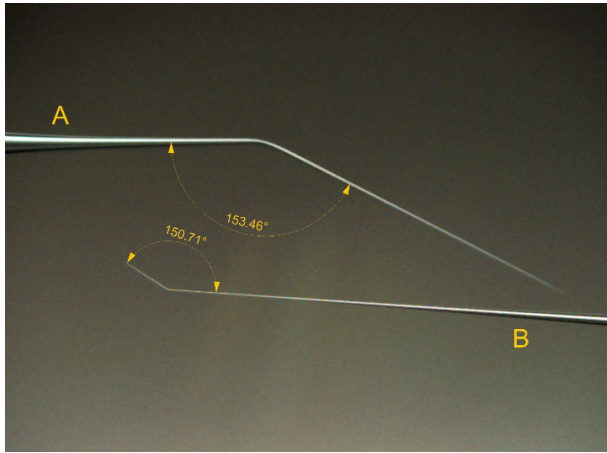


Figure 1a. Comparison of distal and proximal arm and internal angle between home-made (A) and commercial micropipettes (B).

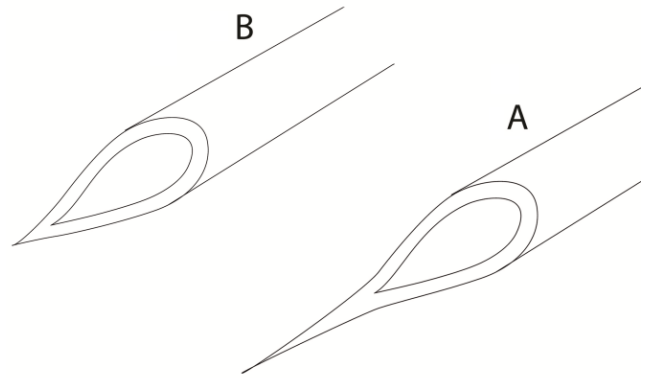


Figure 1b. Comparison of spike length between home-made (A) and commercial micropipettes (B).

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