Relationship of Follicular Size to the Development of Intracytoplasmic Sperm Injection-derived Human Embryos

Tsai-Fang Lee[1], Robert Kuo-Kuang Lee[2–4]*, Yuh-Ming Hwu[2], Yu-Fen Chih[2], Yi-Chun Tsai[2], Jin-Tsung Su[2]

[1]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taitung, [2]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, [3]Division of Reproduction and Endocrinology, Department of Medical Research, Mackay Memorial Hospital, Tamshui, and [4]Taipei Medical University, Taipei, Taiwan.

SUMMARY

Objective: To compare the embryonic development of oocytes obtained from follicles of different sizes.

Materials and Methods: Oocytes ($n=819$) were retrieved from women at 40 years of age or younger during 86 *in vitro* fertilization cycles and categorized as small, medium, or large based on the estimated volume of follicular fluid at the time of retrieval.

Results: The rates of good quality embryos from the large, medium, and small groups on days 2 and 3 were 76.85% and 66.20%, 74.00% and 61.33%, and 69.81% and 58.49%, respectively. There were no significant differences in the rates of good quality embryos between the three follicular volume groups.

Conclusion: Even though fewer oocytes completed maturation in the small follicle group than in the other two groups, the quality of the embryos in all three groups was the same on days 2 and 3. These findings suggest that follicles of all sizes should be aspirated during the intracytoplasmic sperm injection cycle as follicles of every size were a good source of embryos. [*Taiwan J Obstet Gynecol* 2010;49(3):302–305]

Key Words: assisted reproduction, embryo quality, follicular size, intracytoplasmic sperm injection, oocytes

Introduction

Collection of mature oocytes and selection of good quality embryos with the greatest likelihood of implantation are the most important issues in assisted reproduction. A parameter that can predict the presence of mature oocytes and embryos with greater implantation rates would greatly improve the outcomes of assisted reproduction technology. Dubey et al [1] showed that follicle size was a better predictor of successful *in vitro* fertilization (IVF) cycles than the morphological characteristics of the oocyte-cumulus-corona complex. During ovulation induction in IVF cycles, not all follicles develop in synchrony. Different sizes of follicles and maturities of oocytes are always present. A successful outcome of IVF is more likely to occur if ovulation induction results in an increased quantity of follicles, particularly if the follicles are also of a high mean volume [2]. Besides, individual oocytes and embryo quality can be traced back to the follicle size. This study evaluated the relationship between follicular size, as measured by the aspirated volume of the follicular fluid, and embryonic development.

Materials and Methods

Data were collected from women undergoing IVF by intracytoplasmic sperm injection (ICSI) due to male infertility between May 2003 and May 2006. Data from a total of 86 cycles from patients with 40 years of age or

*Correspondence to: Dr Robert Kuo-Kuang Lee, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan.
E-mail: janet818@ms35.hinet.net
Accepted: October 28, 2009

younger were analyzed. The following controlled ovarian hyperstimulation protocol was used in all patients. A daily subcutaneous injection of leuprolide acetate (Lupron; Abbott Laboratories, Chicago, IL, USA) for pituitary desensitization was started on the 20th day of the previous menstrual cycle and continued until the administration of human chorionic gonadotropin (hCG; Pregnyl; Organon, Oss, the Netherlands). Gonadotropins, a combination of human menopausal gonadotropins (Pergonal; Serono, Rome, Italy) and recombinant follicle stimulating hormone (Gonal-F; Serono, Aubonne, Switzerland), were administered intramuscularly from the third day of the menstrual cycle. The doses of recombinant follicle stimulating hormone and human menopausal gonadotropins used were adjusted depending on the ovarian response to stimulation. Beginning on the seventh day of the menstrual cycle, transvaginal ultrasonography was used to monitor follicular growth. When two or three dominant follicles reached a mean diameter greater than 18 mm, final maturation of the follicles was induced with 10,000 IU of hCG. Transvaginal ovum pick-up was performed under ultrasonographic guidance 34–36 hours later.

The fluid from each follicle was aspirated into a new test tube. During the study period, 819 oocytes were collected, rapidly isolated from the follicular fluid, rinsed, and placed in culture. The oocytes were categorized into three groups at the time of retrieval based on the estimated follicular fluid volume. Oocytes with less than 3 mL of follicular fluid were categorized as small, oocytes containing between 3–5 mL of follicular fluid were considered medium, and oocytes with more than 5 mL of follicular fluid were classified as large.

Oocytes were cultured in Quinn's advantage fertilization medium (SAGE BioPharma, Trumbull, CT, USA) supplemented with 10% serum protein substitute. Cumulus and granulosa cells were removed 2–3 hours after oocyte retrieval using hyaluronidase and mechanical pipetting, and then checked for the presence or absence of the first polar body. If the first polar body was extruded from the oocytes, they were defined as mature oocytes. ICSI was performed 4–6 hours after the retrieval of oocytes. The sperm-injected oocytes were then cultured in droplets of Quinn's advantage cleavage medium (SAGE BioPharma). Fertilization was assessed 16–18 hours after injection, and normal fertilization (the presence of two pronuclei) was recorded. Embryonic early cleavage was assessed 25–27 hours after ICSI. Embryonic cleavage and the morphologic appearance of the embryos were assessed 2 and 3 days after retrieval of oocytes. The quality of day 2 cleaved embryos was assessed based on the criteria of Veeck [3]. Embryos were graded based on the regularity of blastomere size, fragmentation, and the granularity of the blastomere cytoplasm as follows: grade 1, even-sized blastomeres with no cytoplasmic fragmentation; grade 2, even-sized blastomeres with minor cytoplasmic fragmentation covering ≤10% of the embryo surface; grade 3, uneven-sized blastomeres with variable fragmentation; grade 4, even or uneven-sized blastomeres with moderate to significant cytoplasmic fragmentation covering >10% of the embryo surface; and grade 5, few blastomeres of any size and severe fragmentation covering ≥50% of the embryo surface. The day 3 embryo quality grading system was modified from two previous reports [3,4]. Embryos were graded based on the regularity of blastomere size, fragmentation, and the granularity of the blastomere cytoplasm as follows: grade 1, even-sized blastomeres with no cytoplasmic fragmentation; grade 2, even-sized blastomeres with minor cytoplasmic fragmentation covering ≤20% of the embryo surface; grade 3, uneven-sized blastomeres with variable fragmentation; grade 4, even or uneven-sized blastomeres with moderate to significant cytoplasmic fragmentation covering >20% of the embryo surface; and grade 5, few blastomeres of any size and severe fragmentation covering ≥50% of the embryo surface. Grade 1–3 embryos were considered good quality embryos and grade 4–5 embryos as poor embryos. During all ensuing manipulations, the three groups were treated separately.

Statistical analysis

The data were analyzed using the χ^2 test. A p value of <0.05 was considered statistically significant.

Results

The average age of the patients was 33.45±4.44 years. The Table shows that a progressive and significant decrease in the rates of mature oocytes for the large, medium, and small follicular size groups (96.93%, 93.72% and 87.78%, respectively; $p<0.001$ for large *vs.* small size group, and $p<0.05$ for medium *vs.* small size group). After sperm injection into the oocytes presenting a first polar body, the percentages of fertilization and cleavage were not significantly different between the three groups (fertilization: 72.47%, 77.99%, and 72.15%; cleavage: 94.32%, 92.02%, and 92.98% in the large, medium, and small groups, respectively). The rates of early cleavage were 39.74%, 41.72%, and 30.99% in the large, medium, and small groups, respectively ($p<0.05$ for both the large *vs.* small group and the medium *vs.* small group). The maturation and early cleavage rates in the small group were significantly

Table. Correlation between follicular sizes and outcomes*

	Large	Medium	Small
No. of retrieved oocytes	326	223	270
No. mature oocytes	316 (96.93)†	209 (93.72)‡	237 (87.78)
No. of fertilized oocytes	229 (72.47)	163 (77.99)	171 (72.15)
No. of cleavage embryos	216 (94.32)	150 (92.02)	159 (92.98)
No. of early cleavage embryos	91 (39.74)‡	68 (41.72)‡	53 (30.99)
Day 2 embryo quality			
Good	166/216 (76.85)	111/150 (74.00)	111/159 (69.81)
Poor	50/216 (23.15)	39/150 (26.00)	48/159 (30.19)
Day 3 embryo quality			
Good	143/216 (66.20)	92/150 (61.33)	93/159 (58.49)
Poor	67/216 (31.02)	53/150 (35.33)	55/159 (34.59)
Arrest	6/216 (2.78)	5/150 (3.33)	11/159 (6.92)

**Data are presented as n (%). Values with different superscripts in the same row are significantly different (χ^2 test); †$p<0.001$ and ‡$p<0.05$ compared to small group.*

lower than in the other two groups. The rates of good quality embryos on days 2 and 3 in the large, medium, and small follicular size groups were 76.85% and 66.20%, 74.00% and 61.33%, and 69.81% and 58.49%, respectively. There were no significant differences in the rate of good quality embryos between the three groups.

Discussion

Oocytes obtained from small size follicles are generally believed to be of poorer quality than those obtained from large size follicles. Several studies have investigated the relationship between follicle size, oocyte developmental capacity, fertilization, cleavage, embryo quality, and pregnancy rates after ovarian stimulation for IVF [1,2,5–11]. In this study, we showed that the oocyte maturation rate from small size follicles was significantly lower than that from medium and large size follicles, and follicle size was positively correlated with the rate of mature oocytes. These findings are in agreement with previous studies [1,5–9].

Bergh et al [5] found that in IVF, the fertilization and pregnancy rates for large follicles (≥ 2 mL) were higher than for small follicles (< 2 mL) because small follicles were more likely to produce immature oocytes, leading to lower fertilization and hence lower pregnancy rates. However, because oocyte maturation is determined prior to ICSI and immature oocytes have already been eliminated, oocytes obtained from large and small size follicles have similar fertilization and pregnancy rates. Mitchell et al [9] proposed that mature oocytes are equally likely to be fertilized by ICSI regardless of follicular sizes. The present study also found no correlation between follicle size and fertilization or cleavage rates. However, the early cleavage rate of oocytes from small follicles was significantly lower than that of oocytes from median or large follicles. This may have been due to the worse quality of oocytes from small follicles than from large follicles.

Ectors et al [6] found that for ICSI there was no significant difference between the fertilization and development rates of embryos harvested from small (< 2 mL), medium (2–6 mL), and large (> 6 mL) follicles, but good embryo rates were significantly lower in the small follicle size group. Nogueira et al [10] also found that the outcome of ICSI with oocytes from small or large follicles from the same patient was not significantly different in two pro-nuclei fertilization rates, mean blastomere number, or embryo quality on days 2 or 3. On day 3 after ICSI in the present study, the number of embryos that had four cells or less generated by oocytes from small follicles was significantly greater than those generated by medium and large size groups. These findings indicate that mature oocytes retrieved from small follicles generate embryos of lower developmental potential than oocytes derived from larger follicles. A study of the relationship between follicular diameter, fertilization, and development after ISCI by Ectors et al [6] and data from the present study both support these findings.

Evidence suggests that follicles are developed in cohorts, wave after wave. Large, medium, and small size follicles are present in every cohort, and each of the small follicles could simply be the large follicles for the next wave that have not yet completed their development. The oocytes from these small follicles could

still become mature oocytes and good quality embryos. In the present study, there were no significant differences between the rates of good quality embryos on day 2 and day 3 between the three follicle size groups. Even though there was a positive trend towards a good embryo rate on day 2 and day 3 with follicular size and a trend toward an increased arrest rate on day 3 for smaller follicles, these differences were not significant. Further study is needed to test this observation using a larger sample size. Studies to determine the best methods to assess embryo quality are also needed to improve the success of IVF.

Ectors et al [6] suggested that follicular size might be positively correlated with the oocytes' ability to fertilize and to develop. Even though there was a lower percentage of embryo development for oocytes from small follicles, their use still increased the total available number of good quality and transferable embryos. This result suggests that all follicles should be aspirated independent of their size as they can all be sources of good embryos. This same strategy was also proposed by Salha et al [11]. In their study, embryo quality was similarly distributed in four follicular size groups (i.e. ≤ 1.0 mL, 1.5–3 mL, 3.5–5 mL, and > 5 mL) and did not vary with the aspirate volume. Their results suggest that in IVF cycles, oocytes aspirated from small follicles have the same developmental potential as those obtained from larger follicles. Triwitayakorn et al [7] also suggested that collecting oocytes from small follicles may increase the total number of good quality and transferable embryos.

In conclusion, this study demonstrated that even though fewer oocytes from the small follicle group completed maturation than in the medium and large size groups, the quality of the embryos in the three groups was not significantly different on days 2 and 3. Further studies are needed to establish whether aspirating all small size follicles in the ICSI cycle is warranted. The implantation potential of embryos from the three groups was not compared in this study. Taiwan has yet to reach a consensus on performing single embryo transfer. Once single embryo transfer becomes possible, the relationship between follicle size and implantation potential can be further examined.

References

1. Dubey AK, Wang HA, Duffy P, Penzias AS. The correlation between follicular measurements, oocytes morphology, and fertilization rates in an in vitro fertilization program. *Fertil Steril* 1995;64:787–90.
2. Arnot AM, Vandekerckhove P, DeBono MA, Rutherford AJ. Follicular volume and number during in-vitro fertilization: association with oocyte developmental capacity and pregnancy rate. *Human Reprod* 1995;10:256–61.
3. Lucinda L. Veeck. *An Atlas of Human Gametes and Conceptuses: An Illustrated Reference for Assisted Reproductive Technology*. New York: Parthenon, 1999.
4. Scholtes MC, Zeilmarker GH. A prospective, randomized study of embryo transfer results after 3 or 5 days of embryo culture in in vitro fertilization. *Fertil Steril* 1996;65:1245–48.
5. Bergh C, Broden H, Lundin K, Hamberger L. Comparison of fertilization, cleavage and pregnancy rates of oocytes from large and small follicles. *Hum Reprod* 1998;13:1912–15.
6. Ectors FJ, Vanderzwalmen P, Van Hoeck J, et al. Relationship of human follicular diameter with oocyte fertilization and development after in-vitro fertilization or intracytoplasmic sperm injection. *Hum Reprod* 1997;12:2002–5.
7. Triwitayakorn A, Suwajanakorn S, Pruksananonda K, Sereepapong W, Ahnonkitpanit V. Correlation between human follicular diameter and oocytes outcomes in an ICSI program. *J Assist Reprod Genet* 2003;20:143–7.
8. Miller KF, Goldberg JM, Falcone T. Follicular size and implantation of embryos from in vitro fertilization. *Obstet Gynecol* 1996;88:583–6.
9. Mitchell PR, Shen S, Anthony TD, Paolo FR, Charles EM, Marcelle IC. A quantitative assessment of follicle size on oocyte developmental competence. *Fertil Steril* 2008;90: 684–90.
10. Nogueira D, Friedler S, Schachter M, Raziel A, Ron-El R, Smitz J. Oocyte maturity and preimplantation development in relation to follicle diameter in gonadotropin-releasing hormone agonist or antagonist treatments. *Fertil Steril* 2006; 85:578–83.
11. Salha O, Nugent D, Dada T, et al. The relationship between follicular fluid aspirate volume and oocytes maturity in in-vitro fertilization cycles. *Hum Reprod* 1998;13:1901–6.