Journal of Assisted Reproduction and Genetics, Vol. 20, No. 4, April 2003 (© 2003)

Clinical IVF

Correlation Between Human Follicular Diameter and Oocyte Outcomes in an ICSI Program

Anusorn Triwitayakorn,[1,2] Somchai Suwajanakorn,[1] Kamthorn Pruksananonda,[1] Wisan Sereepapong,[1] and Vichuda Ahnonkitpanit[1]

Submitted January 10, 2002; accepted December 2, 2002

***Purpose*:** To determine the correlation between the follicular sizes and oocyte recovery, metaphase II oocyte recovery, fertilization rate and good embryo quality from mature and immature oocytes in an intracytoplasmic sperm injection (ICSI) program.

***Methods*:** 991 follicles obtained from 72 ICSI cycles were classified into three groups according to their diameters as measured by transvaginal ultrasound including group A (<10 mm), group B (10–14 mm), and group C (>14 mm). All obtained oocytes were classified according to their nuclear maturation: germinal vesicle (GV), metaphase I (MI) and metaphase II (MII). Mature oocytes underwent ICSI while immature oocytes were further cultured until maturity before ICSI was performed. The rates of fertilization and good quality embryos at day 3 were evaluated.

***Results*:** A progressive and significant increase in the rates of oocyte recovery and MII oocyte recovery were observed from group A follicles compared to the other groups ($p < 0.001$). The fertilization rate of mature and in vitro matured oocytes, as well as the rate of good quality embryos showed a tendency to increase from group A to group C follicles, but not significantly. The corresponding fertilization rates were 78 and 55.3% ($p < 0.001$) for mature and in vitro matured oocytes, respectively.

***Conclusion*:** Collection of oocytes from small follicles, especially with a mean diameter less than 10 mm, and in vitro maturation of immature oocytes before fertilization may allow the total number of good quality and transferable embryos to be increased.

KEY WORDS: Follicular sizes; in vitro maturation; metaphase II oocyte; oocyte recovery.

INTRODUCTION

In an in vitro fertilization program, controlled ovarian hyperstimulation is frequently used for obtaining more embryos to be transferred and frozen. Unfortunately, not all follicles develop in synchrony and there are always different sizes and different stages of oocyte development present.

Follicular size and serum estradiol concentration are commonly used as indicators of oocyte maturity during controlled ovarian hyperstimulation. Human chorionic gonadotrophin (HCG) has been administered when leading follicle(s) reached the size of 16–18 mm (1) or when the majority of large follicles reached 18–20 mm (2). Oocytes from follicles with mean diameters greater than 12 mm have been reported to have significantly higher fertilization and cleavage rates (1). A positive correlation has also been found between follicular size and the presence of metaphase II oocytes (2–4). Dubey *et al.* (5) suggested that measurement of follicular size before retrieval might be the best indicator of the fertilization potential of oocytes in an IVF cycle. However, in an

[1] Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[2] To whom correspondence should be addressed at Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Rama IV road, Bangkok 10330, Thailand; e-mail: kanoksorn@hotmail.com.

1058-0468/03/0400-0143/0 © 2003 Plenum Publishing Corporation

intracytoplasmic sperm injection (ICSI) program, the data are still scanty regarding the follicular size and the fertilization rate. Furthermore, at the present time, the process of in vitro maturation has a role to mature underdeveloped oocytes to a stage appropriate for ICSI.

This prospective study was undertaken to determine the correlation between the follicular size and oocyte recovery, metaphase II oocyte recovery, as well as the fertilization rate from mature and immature oocytes and embryo quality in an ICSI program.

MATERIALS AND METHODS

Data were obtained from patients undergoing ICSI in the reproductive medicine unit, Chulalongkorn Memorial hospital, during an 8-month period from January to August 2000. A total of 72 ovarian stimulation cycles were studied.

Patients received ovarian stimulation according to the short GnRHa (gonadotrophin releasing hormone agonist) (buserelin, Suprefact injectable; Hoechst AG, Frankfurt, Germany) or long GnRHa (buserelin, Suprefact nasal; Hoechst AG, Frankfurt, Germany) protocols depending on their age, basal FSH level, and previous history of ovarian stimulation. Follicular stimulating hormone (Metrodin HP; Serono, Aubonne, Switzerland) or recombinant follicular stimulating hormone (Gonal-F; Serono, Aubonne, Switzerland; or Puregon; Organon, Oss, Holland) was administered from day 3 of the cycle. Human chorionic gonadotriphin (HCG 10,000 IU, Profasi; Serono, Aubonne, Switzerland; or Pregnyl; Organon, Oss, Holland) was given when at least two follicles had mean diameters of 18 mm.

Thirty-six hours after the administration of HCG, oocyte recovery was performed transvaginally with a 16-G double lumen Cook needle (Cook; Cook Group Company, Queenland, Australia) with an aspiration pressure of 110 mmHg. Before aspiration, the size of each follicle was measured by transvaginal ultrasound (Aloka; model SSD-500, Aloka Co, LTD, Japan) as a mean of two measurements in 1 two-dimensional plane. The ultrasound was performed by single investigator (A. Triwitayakorn). The follicles were classified into three groups according to their diameters: group A included those with diameters less than 10 mm, group B comprised those in the 10–14 mm range, while group C contained follicles larger than 14 mm. Individual follicles were punctured under direct ultrasound guidance and aspirated to a 10-mL tube. If an oocyte was not recovered, the follicle would be flushed for maximum of four times with 2 mL of T6 with 25 mM HEPES-buffered media. During all manipulations, these three follicular groups were treated separately.

For the ICSI procedure cumulus and corona radiata cells were removed 2–3 h after oocyte retrieval with hyaluronidase 80 IU/mL (Type VIII; Sigma Chemical Co, St Louis, MO) and mechanical pipetting. The oocytes were classified into three groups according to their nuclear maturation: germinal vesicle (GV), metaphase I (MI), and metaphase II (MII). ICSI was performed on all MII oocytes followed standard techniques (6). Oocytes were cultured individually in IVF media (IVF-20; IVF Science Scandinavia, Gothenburg, Sweden) for 18 h after which they were examined for fertilization. Normal fertilization was determined by the presence of two pronuclei (2PN) and two polar bodies. During the first 2 days embryos were cultured in IVF media (G1.2; IVF Science Scandinavia, Gothenburg, Sweden) and for embryonic days 3–4, they were cultured in a further IVF media (G2.2; IVF Science Scandinavia, Gothenburg, Sweden). The quality of the embryos was evaluated 72 h after ICSI by morphologic appearance (fragmentation, granularity, number and size of blastomeres) and graded on scale of 1–4 according to Yovich *et al.* (7). The embryos with grade ≥ 3 were considered to be of good quality. A maximum of three embryos was transferred after 96 h of ICSI. The rest of the embryos were frozen at the 2PN stage.

Immature oocytes (GV and MI) were further cultured separately in Human tubal fluid media (HTFM; Irvine Scientific, Santa Ana, CA, USA) with 10% heat inactivated maternal serum (MS). Following culture, the maturity of the oocytes was determined at 6, 24, 28, and 48 h after oocyte recovery. The oocytes, which were mature at the time of checking, were subjected to ICSI and underwent the procedure described above.

Statistical Analysis

The results are reported as the mean $\pm$ SD. The statistical analyses used were the Pearson χ^2-test and χ^2 for linear trend for categorical data. A p value of <0.05 was considered significant. Statistical analysis was performed with the Statistical Package for the Social Sciences (version 10.0, SPSS Inc., Chicago, IL).

Results

Nine hundred and ninety-one follicles obtained from 72 ICSI cycles were studied. Table I shows the

Table I. Patients Characteristics and Stimulation Outcomes

Characteristics and outcomes	Results
Age (years)[a]	35.95 ± 4.56
No. of ICSI cycles	72
Short GnRHa protocol	38
Long GnRHa protocol	34
No. of follicles per cycle[a]	13.61 ± 7.49
No. of oocytes retrieved per cycle[a]	11.24 ± 6.39

Note. ICSI = intracytoplasmic sperm injection; GnRHa = gonadotrophin releasing hormone agonist.
[a] Mean ± SD.

patients' characteristics and stimulation outcomes. Tables II and III show the correlation between follicular groups and outcomes. A higher percentage of GV oocytes were obtained from group A follicles (20.5%) and a higher percentage of MII oocytes were collected from group C follicles (93.3%). The oocyte recovery and MII oocytes rates were significantly correlated with the follicular diameters ($p < 0.001$).

Concerning the fertilization rate by ICSI, the difference was not significant between the follicular groups and the fertilization rate from mature oocytes (MII) or in vitro mature oocytes (GV and MI). However, the comparison of the fertilization rate between all mature and all in vitro mature oocytes showed a significant difference ($p < 0.001$), as shown in Table IV.

A progressive and significant increase in the rate of oocyte recovery and also the percentage of MII oocytes was observed from group A to group C follicles ($p < 0.001$). The 127 embryos (24%) that were destined to be transferred were assessed for quality at 72 h after ICSI, showing no significant difference between the follicular groups and the rate of embryos scored as good ($p = 0.4$).

DISCUSSION

In the present study, we found a significantly higher recovery rate for oocytes from large compared to small follicles (Table II). This is in agreement with previous publications (1,4,8). The possible reasons of this finding may be the difference in processes between small and large follicles that allow the oocyte-cumulus cell mass to become free-floating in the antral fluid just before follicle rupture. In the human ovary, hyaluronic acid, synthesized by the cumulus cells and stimulated by midcycle FSH peak through its receptor, disperses the cumulus cell prior to ovulation (9,10). The expression of the FSH-receptor (FSH-R) varies according to the follicular size and FSH-R is abandant in preovulatory follicles (11,12). Another possible reason may be due to the process of oocyte recovery. In this study, we used a 16-guage double lumen Cook needle with an aspiration pressure of 110 mmHg to obtain the oocytes from all follicles, as recommended by Yovich *et al.* (7). This method was also supported by Russell (13) who used 17-gauge, 30-cm long Cook aspiration needle and 80–100 mmHg pressure to retrieve from small follicles. On the other hand, Trounson *et al.* (14) and Cha *et al.* (15) used a specially designed aspiration needle and a vacuum aspiration pressure of 7.5 kPa (56.25 mmHg), which was half of that commonly used for stimulated patients, to retrieve the immature oocytes from unstimulated patients with polycystic ovary syndrome (PCOS).

The positive correlation between follicular size and the presence of MII oocytes also proved to be significant (Table III), the same as observed in previous publications (2–4). This finding may reflect a higher proportion of immature oocytes in the small compared to the large follicle group and can also explain a reduction in the fertilization and cleavage rates in the small size follicle group after conventional IVF (1,2,5,16,17). In the ICSI cycles, we found that the fertilization rate of MII oocytes, obtained from the three follicle groups, had tendency to increase from the small to the large follicle groups, but this was not significant. Our results are in agreement with those of Ectors *et al.* (2) and Bergh *et al.* (17). Despite the smaller follicular diameter in each follicle group as compared to other publications, our fertilization rate was still higher than that of Ectors *et al.* (2) (78%

Table II. Correlation Between Follicular Groups and Oocyte Recovery

	Group A (<10 mm)	Group B (10–14 mm)	Group C (>14 mm)
No. of follicles	127	314	470
No. of oocytes recovered	73	250	402
Oocyte recovery rate (%)[a]	57.3	79.6	85.5

Note. A maximum of four flushes was performed if no oocyte was recovered.
[a] Statistically significant difference ($p < 0.001$; χ^2-test and χ^2 for linear trend).

Table III. Correlation Between Follicular Groups and Outcomes

	Group A (<10 mm)	Group B (10–14 mm)	Group C (>14 mm)
MII oocyte (%) (*n*)[a]	68.5 (50)	81.2 (203)	93.3 (375)
2PN from MII (%) (*n*)[b]	70.0 (35)	75.4 (153)	80.5 (302)
2PN from GV and MI (%) (*n*)[b]	47.8 (8)	53.5 (23)	68.8 (11)
Embryos scored as good (%) (*n*)[b]	16.7 (1)	33.3 (12)	41.2 (35)

Note. GV = germinal vesicle oocyte; MI = metaphase I oocyte; MII = metaphase II oocyte; *n* = number; 2PN = two pronuclei; scored as good = score ≥ 3.
[a] Statistically significant difference ($p < 0.001$; χ^2-test and χ^2 for linear trend).
[b] No statistically significant difference.

vs. 58.4%) and similar to Bergh *et al*. (17) (78% vs. 72%). We used only MII oocytes in the ICSI procedure, so this is probably explained by a higher fertilization rate in ICSI than in conventional IVF (17). We also propose that although oocytes originating from smaller follicles, especially diameter less than 10 mm, have a lesser potential of completing nuclear maturation, they may still have sufficient maternal factors to achieve successful fertilization.

In this study, we have demonstrated that the fertilization rate of in vitro mature oocytes, obtained from three follicular groups, increased from small to large follicular groups (although not significantly,Table III). We also found that fertilization rate after ICSI was significantly higher in mature oocytes than in in vitro mature oocytes (Table IV, 78% vs. 55.3%) in contrast to Nogueira *et al*. (18) who demonstrated a similar fertilization rate obtained after ICSI between in vitro and in vivo mature oocytes (70.3%) from stimulated patients with male and tubal factor infertility. However, the fertilization rate of in vitro mature oocytes after ICSI in our study is similar to that obtained in stimulated patients without PCOS (56.4%) (19). The exact reason for this trend of decrease in fertilization rate of in vitro mature oocytes after ICSI is not known.

Nagy *et al.* (20) demonstrated that an extremely low fertilization rate was usually obtained after standard insemination of matured GV oocytes, obtained from ovulation induction. This low fertilization rate is probably due to the altered characteristics of the zona pellucida, a result of the longer in vitro culture before insemination. He also suggested that ICSI is the best option in this circumstance. However, Trounson *et al*. (21) suggested that ICSI can only really be justified for in vitro mature oocytes in couples where the male partner has low quality semen or where the female partner has PCOS; but not in normally cycling non-PCOS patients.

Table IV. Fertilization Rate Between Mature and In Vitro Mature Oocytes

	Mature oocytes	In vitro mature oocytes
No. of oocytes	628	76
Normal fertilization[a]	490	42
Fertilization rate (%)	78	55.3

[a] Statistically significant difference ($p < 0.001$; χ^2 test).

It should be noted that this study was limited by the small number of oocytes graded at 72 h after ICSI. The number of good quality embryos from the small follicle group tended to be lower than that from another two groups as supported by Wittmaack *et al.* (1), Miller *et al*. (16), and Ectors *et al*. (2), but this was not significant (Table III). We also showed that oocytes from smaller follicles yield acceptable fertilization rates and good quality embryos and it thus seems worthwhile also to aspirate these follicles (17) despite of the high proportion of immature oocytes were obtained. Immature oocytes may undergo nuclear maturation and successful fertilization, yet not have completed cytoplasmic maturation, as indicated by the ability to support embryo development and successful implantation (22–24). Nogueira *et al*. (18) speculated that in these oocytes, the lack or defect of some cellular events involving cytoskeletal organization might lead to a mitotic spindle deficiency, resulting in embryos with nuclear disorganization or with chromosomal abnormalities. This could explain a low success rate after replacement of these embryos (19,21,23,25). However, higher pregnancy rate has recently been reported in unstimulated patients with PCOS (15) as well as in unstimulated regularly menstruating women (26).

In conclusion, our results indicate that collection of oocytes from small follicles with a mean diameter less than 10 mm and in vitro maturation of immature oocytes may allow the total number of good quality and transferable embryos to be increased. However, the developmental potential as well as pregnancy and implantation rate after fertilization of embryos from immature oocytes still remain unknown and warrant further study.

ACKNOWLEDGMENTS

We greatly appreciated the support given by all the staff members of the Reproductive Medicine Unit.

REFERENCES

1. Wittmaack FM, Tureck RW, Kreger DO, Mastroianni L, Jr, Blasco L, Lessey BA: Effect of follicular size on oocyte retrieval, fertilization, cleavage and embryo quality in in vitro fertilization cycles: A 6-years data collection. Fertil Steril 1994;62:1205–1210
2. Ectors FJ, Vanderzwalmen P, Hoeck JV, Nijs M, Verhaegen G, Delvigne A, Schoysman R, Leroy F: Relationship of human follicular diameter with oocyte fertilization and development after in-vitro fertilization or intracytoplasmic sperm injection. Hum Reprod 1997;12:2002–2005
3. Tsuji K, Sowa M, Nakano R: Relationship between human oocyte maturation and different follicular sizes. Biol Reprod 1985;32:413–417
4. Scott RT, Hofmann GE, Muasher SJ, Acosta AA, Kreiner DK, Rosenwaks Z: Correlation of follicular diameter with oocyte recovery and maturity at the time of transvaginal follicular aspiration. J In Vitro Fertil Embryo Transfer 1989;6:73–75
5. Dubey AK, Wang HA, Duffy P, Penzias AS: The correlation between follicular measurements, oocyte morphology, and fertilization rates in an in vitro fertilization program. Fertil Steril 1995;64:787–790
6. Van Steirteghem AC, Liu J, Joris H, Nagy ZP, Cecile J, Tournaye H, Derde MP, VanAssche E, Devroey P: Higher success rate by intracytoplasmic sperm injection than subzonal insemination. A report of a second series of 300 consecutive treatment cycles. Hum Reprod 1993;8:1055–1060
7. Yovich J, Grudzinskas G: The Management of Infertility: A Manual of Gamete Handling Procedures. Oxford, Heinemann Medical, 1990
8. Blackledge DG, Thomas WP, Turner SR, Richardson PA, Matson PL, Yovich JL: Transvaginal ultrasonically-guided oocyte pick-up. Med J Aust 1986;145:300
9. Vanderhyden BC, Telfer EE, Eppig JJ: Mouse oocytes promote proliferation of granulosa cells from preantral and antral follicles in vitro. Biol Reprod 1992;46:1196–1204
10. Suchanek E, Simunic V, Juretic D, Grizelj V: Follicular fluid contents of hyaluronic acid, follicle-stimulating hormone and steroids relative to the success of in vitro fertilization of human oocytes. Fertil Steril 1994;62:347–352
11. Oktay K, Briggs D, Gosden RG: Ontogeny of follicle stimulating hormone receptor gene expression in isolated human ovarian follicle. J Clin Endocrinol Metab 1997;82:3748–3751
12. Minegishi T, Tano M, Igarashi M, Rokukawa S, Abe Y, Ibuki Y, Miyamoto K: Expression of follicle-stimulating hormone receptor in human ovary. Eur J Clin Invest 1997;27:469–474
13. Russel JB: Immature oocyte retrieval combined with in vitro oocyte maturation. Hum Reprod 1998;13(Suppl):63–70
14. Trounson A, Wood C, Kausche A: In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994;62:353–362
15. Cha KY, Han SY, Chung HM, Choi DH, Lim JM, Lee WS, Ko JJ, Yoon TK: Pregnancies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. Fertil Steril 2000;73:978–983
16. Miller KF, Goldberg JM, Falcone T: Follicular size and implantation of embryos from in vitro fertilization. Obstet Gynecol 1996;88:583–586
17. Bergh C, Broden H, Lundin K, Hamberger L: Comparision of fertilization, cleavage and pregnancy rates of oocytes from large and small follicles. Hum Reprod 1998;13:1912–1915
18. Nogueira D, Staessen C, Van de Velde H, Van Steirteghem A: Nuclear status and cytogenetics of embryos derived from in vitro-matured oocytes. Fertil Steril 2000;74:295–298
19. Ludwig M, Finas DF, Al-Hasani S, Diedrich K, Ortmann O: Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients. Hum Reprod 1999;14:354–358
20. Nagy ZP, Loccufier A, Cecile J, Devroey P, Liu J, Van Steirteghem A: Pregnancy and birth after intracytoplasmic sperm injection of in vitro matured germinal vesicle stage oocyte: Case report. Fertil Steril 1996;65:1047–1050
21. Trounson A, Anderiesz C, Jones GM, Kausche A, Lolatgis N, Wood C: Oocyte maturation. Hum Reprod 1998;13(Suppl):52–62
22. Morgan PM, Warikoo PK, Bavister BD: In vitro maturation of ovarian oocytes from unstimulated rhesus monkeys: Assessment of cytoplasmic maturity by embryonic development after in vitro fertilization. Biol Reprod 1991;45:89–93
23. Barnes FL, Crombie A, Gardner DK, Kausche A, Lacham-Kaplan O, Suikkari AM, Tiglias J, Wood C, Trounson AO: Blastocyst development and birth after in vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum Reprod 1995;10:101–105
24. Trounson AO, Bongso A, Szell A: Maturation of human and bovine primary oocytes in vitro for fertilization and embryo production. Singapore J Obstet Gynecol 1996;27:78–84
25. Fulka J, First NL, Moor RM: Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. Mol Hum Reprod 1998;4:41–49
26. Mikkelsen AL, Smith S, Lindenberg S: Impact of oestradiol and inhibin A concentrations on pregnancy rate in in-vitro oocyte maturation. Hum Reprod 2000;15:1685–1690