Developmental capacity of in vitro–matured human oocytes retrieved from polycystic ovary syndrome ovaries containing no follicles larger than 6 mm

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Objective: To test the developmental competence of oocytes in a nonhCG-triggered in vitro maturation (IVM) system when oocyte-cumulus complexes (OCC) are retrieved from antral follicles with a diameter of <6 mm.
Design: Prospective cohort study.
Setting: Tertiary university-based referral center.
Patient(s): From January 2010 to September 2011, 121 patients with polycystic ovaries/polycystic ovary syndrome underwent 239 IVM cycles in total. In 58 of these cycles (44 patients), all antral follicles had a diameter of <6 mm on the day of oocyte retrieval.
Intervention(s): NonhCG-triggered IVM of oocytes, fresh or vitrified/warmed embryo transfer (ET).
Main Outcome Measure(s): Oocyte diameter, maturation rate, fertilization rate, embryo development and morphology, implantation rate, clinical pregnancy rate, ongoing pregnancy rate.
Result(s): Oocyte retrieval yielded 16.7 OCC/cycle, and 50.8% of oocytes completed IVM. The mean oocyte diameter increased from 108.8 ± 4.3 μm to 111.9 ± 4.1 μm after IVM. Mean fertilization rate was 63.7%, and 45.4% of 2-pronuclei oocytes developed into a morphologically good-quality embryo on day 3 after intracytoplasmic sperm injection. Fresh ET resulted in two ongoing pregnancies (2/37; 5.4%). Deferred vitrified-warmed ET led to an ongoing pregnancy rate of 34.6% (9/24). Three healthy babies were born and eight pregnancies were still ongoing.
Conclusion(s): Oocytes retrieved from follicles with a diameter of <6 mm grow during a 40-hour IVM culture can acquire full competence in vitro, as illustrated by their development into healthy offspring. Endometrial quality appears to be a crucial determinant of pregnancy after nonhCG-triggered IVM. (Fertil Steril® 2012;98:503–7. ©2012 by American Society for Reproductive Medicine.)
Key Words: In vitro maturation, antral follicles, oocyte diameter, nonhCG-triggered

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Patients with polycystic ovaries (PCO) or polycystic ovary syndrome (PCOS) (1, 2) have an increased risk of ovarian hyperstimulation syndrome (OHSS) after conventional gonadotropin stimulation and assisted reproductive technologies (ART) (3). Clinical strategies to prevent OHSS include replacement of hCG as the final oocyte maturation trigger by a GnRH agonist and elective freezing of all oocytes or embryos. These measures reduce the risk of OHSS significantly but do not eliminate it (4). In vitro maturation (IVM) is currently the only ART with the potential to completely avoid the occurrence of OHSS in patients with PCO/PCOS.

Reports of clinical outcomes of IVM of oocytes from follicles with a diameter of <6 mm are scarce (5, 6) and have suggested poor embryologic and clinical outcomes. It has therefore

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been proposed, at least in hCG-primed IVM cycles, that immature oocytes should be obtained from follicles with a diameter of 6–12 mm (7); oocytes retrieved from follicles with a diameter of <6 mm would be able to mature, but they would lack the capacity for fertilization and proper embryo development (5, 8).

The acquisition of oocyte developmental competence and the relationship with the follicular diameter in domestic mammalian species has been described in rabbits and sheep. In these species, it has been demonstrated that the presence of all follicular cell types, including granulosa and theca cells, is a prerequisite for the completion of oocyte maturation (9, 10). Furthermore, proper mRNA synthesis and protein storage in the oocyte are also essential for oocyte maturation and subsequent acquisition of embryo developmental competence (11, 12).

It appears that oocyte maturation capacity is higher when the oocyte is derived from larger antral follicles compared with small antral follicles (13, 14). In pigs, the capacity to resume meiosis is achieved in oocytes retrieved from follicles of 0.8 mm diameter, and completion of meiosis is achieved only in follicles ≥2 mm (13). Similar observations have been made in other domestic animals, such as goats and new world primates (14, 15). Apart from follicular diameter, oocyte diameter has also been correlated with the capacity to complete meiosis, acquire oocyte developmental capacity, and support embryo development in large mammals (16–19), monkeys, and humans (20–22).

In the series presented here, we report the developmental capacity of oocytes from patients with PCO/PCOS. All oocyte-cumulus complexes (OCC) were retrieved from ovaries in which no follicles larger than 6 mm were observed on the day of oocyte retrieval. The aim of this study was to investigate whether oocytes derived from these small follicles would be able to grow, mature, be fertilized, and produce viable embryos after maturation in a nonhCG-primed IVM system.

MATERIALS AND METHODS

Patient Population

This study was approved by the local ethical committee, and each of the patients gave written informed consent. IVM treatment was performed from January 2010 to September 2011. During that period, patient data, cycle characteristics and outcomes were prospectively collected in 121 consecutive patients with PCO or PCOS (according to the revised Rotterdam criteria [27, 28]), who underwent 239 IVM cycles in total at our center. In 58 of these cycles, undertaken by 44 consecutive patients, there were no follicles observed with a diameter >6 mm by vaginal ultrasound at the time of oocyte retrieval (Supplemental Fig. 1, available online at www.fertstert.org). 61.4% (27/44) of patients were amenorrhoeic, 29.5% (13/44) were oligomenorrhoeic, and 9.1% (4/44) were patients with ultrasound-only PCO and regular cycles.

Immature Oocyte Retrieval

All patients received 150 IU/d highly purified hMG (Menopur; Ferring Pharmaceuticals) or highly purified human FSH (Fostimon; IBSA) daily for 3 days, starting on day 3 after menstruation. When the endometrial thickness reached >5 mm, transvaginal oocyte retrieval was scheduled for the following day. No hCG trigger was administered. OCC were retrieved with a 17-gauge single-lumen needle (K-OPS-1230-VUB; Cook Medical). During the ultrasound-guided procedure, two-dimensional diameter measurements were performed in all follicles. The aspiration pressure was 70 mm Hg. Follicular aspirates were filtered (Falcon 1060; 70 μm mesh size) and OCC were collected from the culture dish. Collected OCC were matured for 40 hours in the Medicult IVM System (Origio) supplemented with 75 mIU/mL FSH, 100 mIU/mL hCG, and 10% human albumin solution (Vitrolife). After IVM, mature oocytes were inseminated by intracytoplasmic sperm injection (ICSI) as described previously (23). Oocytes and embryos were cultured individually in 25-μL medium droplets covered with mineral oil. Embryos were cultured until day 3 in sequential Sage cleavage media (Cooper Surgical).

Only embryos with good morphology were selected for transfer and/or cryopreservation based on morphology in the morning of day 3 after ICSI (24). Embryo transfer was performed under ultrasound guidance using a soft catheter (K-Soft 5100; Cook). In case of a fresh embryo transfer, the choice between a single (SET) or double (DET) embryo transfer depended on the patient's age and the rank of the trial, in accordance with Belgian legislation (single embryo transfer below the age of 36 years in the first cycle, dual embryo transfer thereafter).

Fresh Embryo Transfer or Elective Embryo Freezing

If the endometrial thickness was <6 mm on day 3 after ICSI, embryo transfer was canceled and patients were offered elective vitrification of all embryos of good quality. In that case, vitrified-warmed embryo transfer in an ulterior artificial endometrial priming cycle was performed. Only embryos with good morphology were selected for fresh transfer and/or cryopreservation based on the morphology in the morning of day 3 after ICSI (24). Embryo vitrification was performed using closed CBS-VIT High Security (HS) straws (Cryo Bio system) in combination with DMSO-EG-S as the cryoprotectant (Irvine Scientific Freeze Kit), as previously described (25).

Outcome Parameters

A normal rise in serum hCG on two consecutive occasions from 11 days after embryo transfer onward indicated a pregnancy. The implantation rate represented the ratio between the number of gestational sacs and the total number of embryos transfered. A clinical pregnancy was defined as a pregnancy with an intrauterine gestational sac seen at transvaginal ultrasound scan ≥5 weeks after embryo transfer (26).

Oocyte Diameter Measurement

Oocyte diameter was assessed as previously described by Cavilla et al. (21). Briefly, the oocyte diameter was calculated as

the mean of the two-length diameter of the oocyte passing through the oocyte's centroid. Images were taken under an inverted microscope (Nikon) with Hoffman contrast modulation optic by using computerized image analysis software (Octax EyeWare).

Statistical Analysis

For categoric variables (clinical outcomes), Fisher exact test was used. Because the noncategoric variables (oocyte diameter) did not show a normal distribution, Wilcoxon test and Kruskal-Wallis test were performed. Differences were considered to be statistically different when $P<.05$. All the statistical analyses were done using the Graphpad Prism program, version 4 for Windows (Graphpad Software).

RESULTS

The body mass index, age, gonadotropin priming dosage and hormonal and steroid profiles are presented in Table 1. All oocytes collected were in germinal vesicle (GV) stage and the cumulus cells were compact. As presented in Table 2, 1,274 OCC (an average of 16.7 ± 10.2 OCC/cycle) were retrieved in total. After written informed consent, 307 OCC were donated for scientific research, and 967 OCC were allocated to the patients. Further results and analysis relate to the latter OCC group only, not to the OCC donated for research. The average IVM rate was 50.8% (491/967), with an average yield of 8.5 ± 5.6 metaphase II (MII) embryos/cycle. The mean fertilization rate after ICSI was 63.7%, conferring an average of 5.4 ± 4.2 zygotes/cycle. On day 3 after ICSI, 45.4% (142/313) of fertilized oocytes (an average of 2.4 ± 2.6 embryos/cycle) had good morphologic embryo quality (>6 cells and classified as grade 1 or 2 [24]) and were eligible for embryo transfer or freezing.

Embryo Transfer Outcomes

In 9 IVM cycles there was no embryo transfer, owing to suboptimal embryo development. In 12 cycles, all available good-quality embryos were vitrified on day 3 after ICSI, because of suboptimal endometrial thickness (<6 mm). A fresh transfer of one or two cleavage-stage embryos was performed in 37 IVM cycles, with a mean number of 1.35 ± 0.51 transfered embryos/cycle. Additionally, 24 vitrified/warmed embryo transfers (1.32 ± 0.49) were performed in an artificial endometrium priming cycle (Supplemental Fig. 2, available online at www.fertstert.org).

Overall, fresh embryo transfer yielded a positive pregnancy test in 13.5% (5/37); the implantation rate was 10.2% (5/49) and the clinical pregnancy 5.4% (2/37) per embryo transfer. Significantly better clinical outcomes were obtained after vitrified-warmed embryo transfer in an artificial cycle: This approach resulted in a positive pregnancy test of 50.0% (12/24) per embryo transfer; the implantation rate was 35.1% (13/37) and the clinical pregnancy rate 37.5% (9/24). Three patients had delivered a healthy girl and eight singleton pregnancies were still ongoing at the time of writing. Table 3 summarizes the comparative clinical outcomes of fresh and vitrified-warmed IVM embryo transfer.

Relation Between Oocyte Diameter and Maturation Status

Images were taken from all oocytes at the start of IVM culture and after removal of cumulus cells from the oocyte at 40 hours. In total, 140 oocytes were evaluated before and 131 were evaluated after denudation; 9 oocytes had degenerated. The mean oocyte diameters before and after IVM culture were

TABLE 1

Baseline patient characteristics and in vitro maturation cycle parameters.

No. of patients	44
No. of cycles	58
BMI (kg/m^2)	28.0 ± 6.9
Age (y)	28.5 ± 3.4
Gonadotropin priming	
Total injection dose (IU)	465 ± 237
Days of stimulation	4.2 ± 2.5
Day 3 AMH (μg/L)	16 ± 11.2
Day 3 E_2 (ng/L)	49.4 ± 16.7
Day 3 FSH (IU/L)	4.9 ± 1.8
Day 3 LH (IU/L)	8.5 ± 5.8
E_2 on day of OR (ng/L)	439 ± 337.8
FSH on day of OR (IU/L)	5.6 ± 1.3
LH on day of OR (IU/L)	6.5 ± 3.5
Endometrium thickness on day of OR (mm)	7.0 ± 2.0

Note: Values are mean ± SD. AMH = antimüllerian hormone; BMI = body mass index; OR = oocyte retrieval.

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.

TABLE 2

Embryo development outcome.

	Overall	Per cycle
Total no. of follicles punctured	2226	39.1 ± 16.1
Total no. of OCC retrieved	1,274 (57.2%)	22 ± 15.1
Total no. of OCC[a]	967	16.7 ± 10.2
No. of MII oocytes[a]	491 (50.8%)	8.5 ± 5.6
Total no. of oocytes resuming meiosis[a]	698 (72.2%)	12.0 ± 7.3
No. of fertilized 2PN oocytes[a]	313 (63.7%)	5.4 ± 4.2
No. of cleaved embryos[a]	288 (92.0%)	5.0 ± 4.0
No. of good-quality embryos[a]	142 (45.4%)	2.4 ± 2.6

Note: OCC = oocyte-cumulus complexes; PN = pronuclei.
[a] 307 OCC allocated for research were excluded from analysis.

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.

TABLE 3

Clinical outcomes of fresh and vitrified-warmed IVM embryo transfer.

	Fresh ET	Vitrified-warmed ET	*P* value
Clinical pregnancy rate	2/37 (5.4%)	9/24 (37.5%)	.004
Positive hCG	5/37 (13.5%)	12/24 (50.0%)	.003
Implantation rate	5/49 (10.2%)	13/37 (35.1%)	.007

Note: ET = embryo transfer.

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.

TABLE 4

Measured oocyte diameter before and after in vitro maturation (IVM) in nonhCG-triggered IVM cycles.

	Mean ± SD (range)
Oocyte diameter before IVM culture (μm)	108.8 ± 4.3[a] (93–119)
Oocyte diameter after IVM culture (μm)	111.9 ± 4.1[a] (95–124)
MII oocyte diameter after IVM (μm)	112.8 ± 3.7[b] (103–124)
MI oocyte diameter after IVM (μm)	112.2 ± 3.8 (106–121)
GV oocyte diameter after IVM (μm)	109.9 ± 4.7[b] (95–117)

Note: GV = geminal vesicle; MI = metaphase I; MII = metaphase II.
[a] Wilcoxon test showing a statistically significant different oocyte diameter before and after IVM culture ($P<.001$).
[b] Kruskal-Wallis test showing a statistically significant difference between oocyte diameters ($P<.05$).

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.

108.8 ± 4.3 μm and 111.9 ± 4.1 μm, respectively ($P<.001$). Of the 131 oocytes measured after IVM culture, 71 (50.7%) were at MII stage, with an average oocyte diameter of 112.8 ± 3.7 μm, 25 oocytes were MI (average oocyte diameter of 112.2 ± 3.8 μm), and 35 oocytes were at GV stage (average oocyte diameter 110.0 ± 4.7 μm; Table 4). Supplemental Figure 2 shows the histograms of the oocyte diameter distribution at the time of oocyte retrieval and after IVM culture, and the distribution of the oocyte diameter according to the maturation status after IVM.

DISCUSSION

This study demonstrates, in a large series of consecutive IVM cycles in PCO/PCOS patients, that a nonhCG-primed IVM system is capable of producing developmentally competent oocytes leading to healthy offspring, even when these oocytes are retrieved from ovaries where the largest follicular diameter does not exceed 6 mm.

Earlier IVM trials recommended oocyte retrieval when follicles reach a diameter of 6 mm (5, 8, 27) to 12 mm (27). Nevertheless, patients with PCO/PCOS constitute a heterogeneous population with divergent endocrine profiles and there is a considerable proportion of patients in whom antral follicles will not grow beyond a diameter of 6 mm after stimulation. In the present series of minimally gonadotropin-primed patients, the entire cohort of antral follicles remained <6 mm in 24.3% (58/239) of cycles at the time of oocyte retrieval. Oocyte retrieval was scheduled when the endometrial thickness reached ≥5 mm, regardless of the size of the antral follicles and without hCG trigger. The clinical outcome after embryo transfer (in deferred transfer cycles) was similar to clinical outcomes published by other groups in hCG-primed IVM systems (7, 28–31). Although good embryonic development outcomes were obtained in these published series, albeit with oocytes retrieved from follicles with a larger diameter (32, 33), our findings prove that human oocytes retrieved from antral follicles ≤6 mm can have developmental capacity. In our series, follicles were exposed to a minimal dose of hCG (highly purified hMG contains 10 IU hCG per 75 IU FSH) during a minimal stimulation protocol. During 40 hours of IVM, OCC were exposed to 75 mIU/mL FSH and 100 mIU/mL hCG that had been added the basal maturation medium.

The intrinsic capacity of the mammalian oocyte to undergo nuclear maturation and to acquire developmental competence has been correlated with oocyte diameter (34). In human, oocyte diameter increases from ~30 μm in primary follicles to ~115 μm in graafian follicles over an 8-week period (35). The diameter of human in vivo–matured oocytes ranges from ~110 μm to ~120 μm (36). In patients with PCOS, in vivo–matured oocytes retrieved from follicles >15 mm after conventional gonadotropin stimulation and hCG priming have a smaller oocyte diameter compared with normo-ovulatory patients (37), which seems to suggest that oocyte diameter may be influenced by differences in the intraovarian milieu. Cavilla et al. (21) described an oocyte diameter increase of 3 μm when human oocytes retrieved from follicles of <10 mm in patients with PCO were cultured in vitro for 48 hours, which corresponds to an 8% increase of the initial volume. In oocytes retrieved from antral follicles of <6 mm, we found an oocyte diameter increase of 3.1 μm after 40 hours of in vitro culture, which corresponds to an 8.1% increment of the initial volume (i.e., an increase of 59,300 μm^3). These observations are similar to those of Cavilla et al., although incubation was shortened by 8 hours. Although in vitro–matured oocytes in our study had a significantly larger diameter than arrested oocytes (Table 4; Supplemental Fig. 2), we were not able to correlate oocyte diameters before and after IVM culture with the developmental potential of individual oocytes, because OCC were cultured in groups. Others found that the diameter of oocytes retrieved from follicles of 10–14 mm was similar to the diameter of oocytes from follicles of <10 mm, and they could not demonstrate a correlation with developmental competence (38).

In conclusion, in patients with PCO/PCOS, oocytes retrieved from antral follicles with a diameter of <6 mm after minimal gonadotropin stimulation but no hCG trigger are able to acquire competent nuclear and cytoplasmic maturation in vitro, support embryo development, and develop into healthy offspring. Further research is needed to optimize the clinical and laboratory approach of IVM treatment, tailored to the specific follicular constellation within the ovaries of patients with PCO/PCOS. Morphologic assessment of oocytes, including oocyte diameter, might serve as a valuable tool in the laboratory, but it needs further evaluation. Molecular studies in OCC retrieved from small antral follicles should enable detection of pathways conducive to meiotic maturation in the oocytes. On a similar note, there is great potential for culture media supplementation of the in vitro culture system; addition with oocyte growth factors might enhance cytoplasmatic maturation (39, 40). Finally, a differential culture approach might allow ART practitioners in the future to exploit the full spectrum of the numerous antral follicles in PCO/PCOS and should improve clinical outcomes of IVM treatment.

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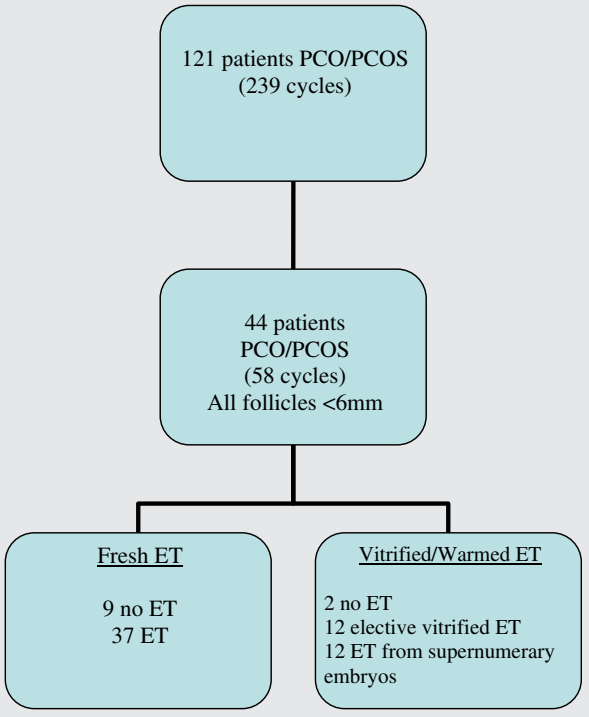
REFERENCES

1. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
2. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004;19:41–7.
3. MacDougall MJ, Tan SL, Balen A, Jacobs HS. A controlled study comparing patients with and without polycystic ovaries undergoing in-vitro fertilization. Hum Reprod 1993;8:233–7.
4. Huang JY, Chian RC, Tan SL. Ovarian hyperstimulation syndrome prevention strategies: in vitro maturation. Semin Reprod Med 2010;28:519–31.
5. 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;62:353–62.
6. Barnes FL, Crombie A, Gardner DK, Kausche A, Lacham-Kaplan O, Suikkari AM, et al. Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum Reprod 1995;10:3243–7.
7. Son WY, Chung JT, Herrero B, Dean N, Demirtas E, Holzer H, et al. Selection of the optimal day for oocyte retrieval based on the diameter of the dominant follicle in hCG-primed in vitro maturation cycles. Hum Reprod 2008; 23:2680–5.
8. Trounson A, Anderiesz C, Jones G. Maturation of human oocytes in vitro and their developmental competence. Reproduction 2001;121:51–75.
9. Thibault C, Gerard M, Menezo Y. Preovulatory and ovulatory mechanisms in oocyte maturation. J Reprod Fertil 1975;45:605–10.
10. Trounson AO, Willadsen SM, Moor RM. Reproductive function in prepubertal lambs: ovulation, embryo development and ovarian steroidogenesis. J Reprod Fertil 1977;49:69–75.
11. Zheng P, Patel B, McMenamin M, Moran E, Paprocki AM, Kihara M, et al. Effects of follicle size and oocyte maturation conditions on maternal messenger RNA regulation and gene expression in rhesus monkey oocytes and embryos. Biol Reprod 2005;72:890–7.
12. Caixeta ES, Ripamonte P, Franco MM, Junior JB, Dode MA. Effect of follicle size on mRNA expression in cumulus cells and oocytes of Bos indicus: an approach to identify marker genes for developmental competence. Reprod Fertil Dev 2009;21:655–64.
13. Motlik J, Crozet N, Fulka J. Meiotic competence in vitro of pig oocytes isolated from early antral follicles. J Reprod Fertil 1984;72:323–8.
14. Gilchrist RB, Nayudu PL, Hodges JK. Maturation, fertilization, and development of marmoset monkey oocytes in vitro. Biol Reprod 1997;56:238–46.
15. Crozet N, Ahmed-Ali M, Dubos MP. Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture in vitro. J Reprod Fertil 1995;103:293–8.
16. Jimenez-Macedo AR, Anguita B, Izquierdo D, Mogas T, Paramio MT. Embryo development of prepubertal goat oocytes fertilised by intracytoplasmic sperm injection (ICSI) according to oocyte diameter. Theriogenology 2006; 66:1065–72.
17. Otoi T, Fujii M, Tanaka M, Ooka A, Suzuki T. Oocyte diameter in relation to meiotic competence and sperm penetration. Theriogenology 2000;54:535–42.
18. Anguita B, Jimenez-Macedo AR, Izquierdo D, Mogas T, Paramio MT. Effect of oocyte diameter on meiotic competence, embryo development, p34 (cdc2) expression and MPF activity in prepubertal goat oocytes. Theriogenology 2007;67:526–36.
19. Fair T, Hyttel P, Greve T. Bovine oocyte diameter in relation to maturational competence and transcriptional activity. Mol Reprod Dev 1995;42:437–42.
20. Durinzi KL, Saniga EM, Lanzendorf SE. The relationship between size and maturation in vitro in the unstimulated human oocyte. Fertil Steril 1995; 63:404–6.
21. Cavilla JL, Kennedy CR, Byskov AG, Hartshorne GM. Human immature oocytes grow during culture for IVM. Hum Reprod 2008;23:37–45.
22. Peluffo MC, Barrett SL, Stouffer RL, Hennebold JD, Zelinski MB. Cumulus-oocyte complexes from small antral follicles during the early follicular phase of menstrual cycles in rhesus monkeys yield oocytes that reinitiate meiosis and fertilize in vitro. Biol Reprod 2010;83:525–32.
23. Joris H, Nagy Z, van de Velde H, de Vos A, van Steirteghem A. Intracytoplasmic sperm injection: laboratory set-up and injection procedure. Hum Reprod 1998;(13 Suppl 1):76–86.
24. van Royen E, Mangelschots K, de Neubourg D, Valkenburg M, van de Meerssche M, Ryckaert G, et al. Characterization of a top quality embryo, a step toward single-embryo transfer. Hum Reprod 1999;14:2345–9.
25. van Landuyt L, Verpoest W, Verheyen G, de Vos A, van de Velde H, Liebaers I, et al. Closed blastocyst vitrification of biopsied embryos: evaluation of 100 consecutive warming cycles. Hum Reprod 2011;26:316–22.
26. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520–4.
27. Smitz JE, Thompson JG, Gilchrist RB. The promise of in vitro maturation in assisted reproduction and fertility preservation. Semin Reprod Med 2011; 29:24–37.
28. Tan SL, Child TJ. In-vitro maturation of oocytes from unstimulated polycystic ovaries. Reprod Biomed Online 2002;(4 Suppl 1):18–23.
29. Son WY, Chung JT, Demirtas E, Holzer H, Sylvestre C, Buckett W, et al. Comparison of in-vitro maturation cycles with and without in-vivo matured oocytes retrieved. Reprod Biomed Online 2008;17:59–67.
30. Chian RC. In-vitro maturation of immature oocytes for infertile women with PCOS. Reprod Biomed Online 2004;8:547–52.
31. Mikkelsen AL, Lindenberg S. Benefit of FSH priming of women with PCOS to the in vitro maturation procedure and the outcome: a randomized prospective study. Reproduction 2001;122:587–92.
32. Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online 2009;19:343–51.
33. Son WY, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. Hum Reprod Update 2010;16:675–89.
34. Bao S, Obata Y, Carroll J, Domeki I, Kono T. Epigenetic modifications necessary for normal development are established during oocyte growth in mice. Biol Reprod 2000;62:616–21.
35. Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Hum Reprod 1986;1:81–7.
36. Veeck LL. An atlas of human gametes and conceptuses: an illustrated reference for assisted reproductive technology. New York: Parthenon; 1999.
37. Marquard KL, Stephens SM, Jungheim ES, Ratts VS, Odem RR, Lanzendorf S, et al. Polycystic ovary syndrome and maternal obesity affect oocyte size in in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril 2011;95:2146–9.
38. Son WY, Chung JT, Dahan M, Reinblatt S, Tan SL, Holzer H. Comparison of fertilization and embryonic development in sibling in vivo matured oocytes retrieved from different sizes follicles from in vitro maturation cycles. J Assist Reprod Genet 2011;28:539–44.
39. Raty M, Ketoja E, Pitkanen T, Ahola V, Kananen K, Peippo J. In vitro maturation supplements affect developmental competence of bovine cumulus-oocyte complexes and embryo quality after vitrification. Cryobiology 2011;63:245–55.
40. Gilchrist RB. Recent insights into oocyte-follicle cell interactions provide opportunities for the development of new approaches to in vitro maturation. Reprod Fertil Dev 2011;23:23–31.

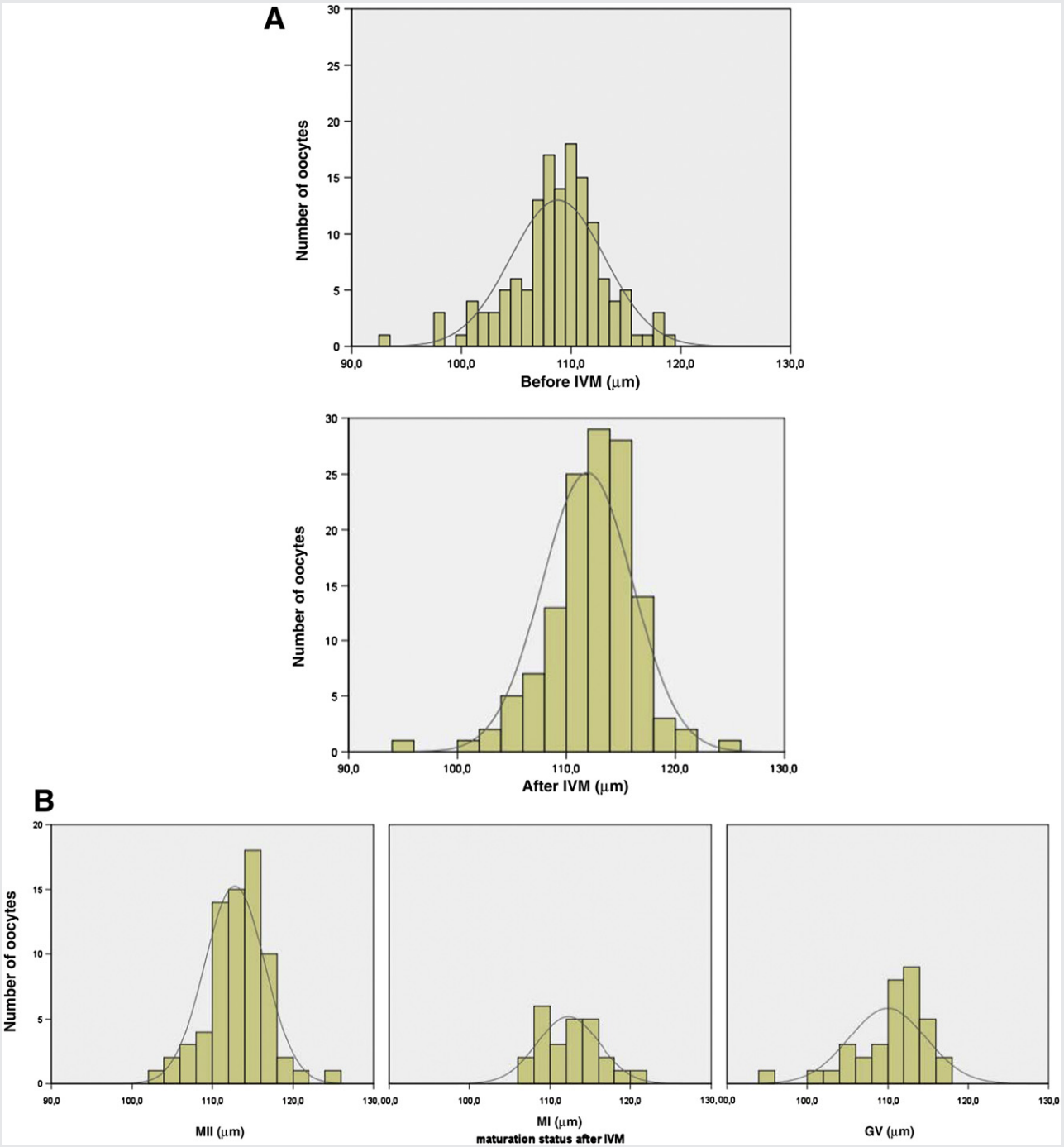
SUPPLEMENTAL FIGURE 1



Distribution of the cycles according to the embryo transfer. ET = embryo transfer; PCO = polycystic ovaries; PCOS = polycystic ovary syndrome.

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.

SUPPLEMENTAL FIGURE 2



(A) Histogram distribution of oocyte diameter before and after in vitro maturation (IVM) culture. (B) Histogram distribution of oocyte diameter according to the maturation status after IVM culture.

Guzman. IVM of oocytes from follicles <6 mm. Fertil Steril 2012.