Cumulus cell-oocyte complexes retrieved from antral follicles in IVM cycles: relationship between COCs morphology, gonadotropin priming and clinical outcome

Mariabeatrice Dal Canto · Fausta Brambillasca ·
Mario Mignini Renzini · Giovanni Coticchio ·
Maria Merola · Monia Lain · Elena De Ponti ·
Rubens Fadini

Received: 16 February 2012 / Accepted: 28 March 2012 / Published online: 18 April 2012
© Springer Science+Business Media, LLC 2012

Abstract

Purpose To assess retrospectively the developmental potential of different types of cumulus cell-oocyte complexes (COCs) derived from IVM cycles.

Methods IVM cycles were performed in natural cycles or after HCG, FSH, or FSH/HCG priming. COCs recovered were morphologically characterized in different types: compact (CC) or expanded (EC) cumulus mass but including an immature oocyte, and expanded cumulus mass enclosing a mature oocyte (EC-MII). Embryo developmental competence was investigated analysing exclusively cycles in which all transferred embryos derived from the same COC category.

Results Fertilization rates did not differ significantly. Significant differences in pregnancy rates (14.5%, 10.0% and 27.6% in the CC, EC, and EC-MII categories, respectively) were observed. Likewise, significant differences in implantation rates (8.9%, 6.3% and 19.1% in the CC, EC, and EC-MII categories, respectively) were found. Overall, priming with FSH/HCG had a beneficial effect on pregnancy and implantation rates, while no priming or HCG alone generated oocytes with poor competence.

Capsule In IVM cycles, morphological evaluation at the time of collection can predict the developmental ability of different COCs. FSH/HCG priming has a positive effect on oocyte competence.

M. Dal Canto · F. Brambillasca · M. Mignini Renzini ·
G. Coticchio (✉) · M. Merola · M. Lain · R. Fadini
Biogenesi Reproductive Medicine Centre, Istituti Clinici Zucchi,
V Zucchi, 24,
Monza, Italy
e-mail: coticchio.biogenesi@grupposandonato.it

E. De Ponti
Department of Medical Physics, San Gerardo Hospital,
Monza, Italy

Conclusions In IVM cycles, morphological evaluation at the time of collection can predict the developmental ability of different COCs. FSH/HCG priming has a positive effect on oocyte competence.

Keywords Oocytes · Cumulus cell-oocyte complexes ·
In vitro maturation · Pregnancy · Implantation

Introduction

Immature fully-grown mammalian oocytes matured in vitro can fertilize, develop into embryos and generate live births [1]. Through in vitro maturation (IVM), disadvantages of ovarian stimulation, such as gonadotropin costs and risk of ovarian hyperstimulation syndrome (OHSS), can be avoided. Both women with polycystic ovaries [2] and normo-ovulatory [3] can benefit from IVM.

To improve oocyte quality of IVM cycles, oocyte recovery may be preceded by very mild priming with follicle stimulating hormone (FSH), possibly accompanied by human chorionic gonadotropin (HCG) administration [3–8]. The use of HCG has drawn particular interest for its ability to initiate meiotic resumption and other events associated to oocyte maturation, such as cumulus cell expansion, before the oocyte is retrieved and placed in culture. Because of growth stage heterogeneity among antral follicles, HCG priming can lead to the recovery cell-oocyte complexes (COCs) of diverse morphological and functional categories. In most cases, COCs are retrieved in a compact form (CC), with multiple layers of tightly connected cumulus cells enclosing a germinal vesicle (GV) stage oocyte. Alternatively, the cumulus mass may show a varying degree of expansion

(EC), enclosing an oocyte at the GV stage or that has undergone GV breakdown (GVBD), but not emission of the polar body I (PBI). Less frequently, COCs may display cumulus expansion and an oocyte with a visible PBI (EC-MII) [4]. The different meiotic stages of these oocytes may be indicative of diverse developmental fates. In effect, it has been episodically reported that oocytes with a PBI surrounded by an expanded cumulus mass give rise to embryos with a higher ability to develop to blastocyst in comparison to oocytes of the other two COCs categories [3, 9–12]. However, the precise relationship between COC constitution and potential to establish a viable pregnancy has not been thoroughly investigated, leaving still open the question of the assessment of oocyte quality in IVM cycles. In the present study, we characterized the morphology of COCs recovered in IVM cycles and appraised their competence to develop in viable embryos and implant in dependence of gonadotropin priming regimen.

Methods

Data of this study, approved by the local IRB, were obtained from 1096 IVM treatments performed from January 2005 to December 2009. Informed consent was obtained from treated couples. Patients had an indication for standard IVF or ICSI because of infertility due to male or tubal factor, stage I or II endometriosis, polycystic ovary syndrome (PCOS) with chronic anovulation, or PCO defined as more than 10 follicles in one plane at ultrasound scan. Female inclusion criteria were: 24–38 years; FSH < 11 mIU/ml on cycle day 3; 13–31 kg/m² body mass index; maximum one previously failed IVF or IVM attempt. Women with other endocrine abnormalities such as hyperprolactinaemia or thyroid dysfunction, were excluded. Oocytes were recovered in natural non-primed cycles (A) or after HCG (B), FSH (C) or FSH/HCG (D) priming, according to a randomization aimed at establishing possible differences in alternative IVM schemes [3]. Women characteristics did not differ significantly among the different priming regimens.

After basal ultrasound scan, women were monitored for follicular growth until a leading follicle of 10–14 mm in diameter and an endometrial thickness of >4 mm were observed. Under those conditions, oocyte retrieval was scheduled to occur within 24 h in Group A and C or after 36–38 h in group B and D. Oocyte retrieval was performed under transvaginal ultrasound guidance using a single lumen aspiration needle (code 4551-E2 Ø 17, gauge 35 cm; Gynetics, Lommel, Belgium) connected to a vacuum pump (pressure 80–100 mmHg; Craft Pump, Rocket Medical, Washington, UK). During oocyte collection, all women received sedation with Propofol (Astra-Zeneca, Basiglio, Italy). Follicular aspirates containing COCs were collected

in a single 50-ml tissue culture flask containing 15 ml of pre-warmed Flushing Medium with heparin (code no. 10760125; Medicult, Jyllinge, Denmark). Follicular aspirates were filtered through a 70 µm cell strainer (code no. 352350; Becton-Dickinson, Buccinasco, Italy) and washed twice with Flushing Medium. COCs were detected under a stereomicroscope and thoroughly washed.

COCs were examined and classified according to cumulus oophorus morphology and stage of oocyte maturation as described below.

- Compact Cumulus (CC) COCs included a GV-stage oocyte surrounded by multiple layers of cubical and tightly compacted cumulus cells. The cell vestment was classified as continuous or partially interrupted.
- Expanded Cumulus (EC) COCs were those constituted by an immature oocyte (GV- or GVBD-stage) surrounded by a dark corona radiata and an expanded and loose cumulus mass.
- Expanded Cumulus metaphase II (EC-MII) COCs consisted of a mature oocyte showing an extruded PBI surrounded by an expanded corona radiata and an expanded and loose cumulus mass. Nude or atretic GV-stage oocytes were discarded.

After recovery, COCs were transferred to a single well petri dish containing 0.5 ml of IVM medium (Vial 2 of IVM system medium, code no. 82214010; Jyllinge, Medicult) supplemented with recombinant FSH 0.075 IU/ml (Merck Serono, Rome, Italy), HCG 0.10 IU/ml (Merck Serono, Rome, Italy), and inactivated 10% maternal serum. Immature oocytes surrounded by compact cumulus cells were cultured at 37°C in a 6% CO₂ humidified atmosphere for 30 h. Oocytes associated to an expanded cumulus mass were cultured for 3 h and again observed under a stereomicroscope using the spreading technique to assess their meiotic stage [10]. GV-stage oocytes were returned to maturation medium leaving intact the cumulus cell vestment. In the absence of a visible GV, COCs were treated with 20 IU/ml hyaluronidase solution (Sage, Pasadena, USA) to remove cumulus cells. Only oocytes showing the PBI were used for insemination on the same day. GV-stage or GVBD oocytes recovered after cumulus cell removal were discarded. After 30 h of culture, all remaining COCs were treated with 20 IU/ml hyaluronidase solution to remove cumulus cells. In compliance with the law regulating assisted reproduction in Italy during the period of study, maximum three mature oocytes were inseminated. MII-stage oocytes suitable for insemination were selected. Oocytes were inseminated by ICSI. Fertilization was assessed 16–18 h after microinjection and confirmed by the presence of two pronuclei and two polar bodies.

Pre-zygotes were cultured in a four-well Petri dish in 0.5 ml of IVF medium or ISM1 (code no. 10500060 and 10315060; Medicult, Jyllinge, Denmark). Embryos were

cultured until day 2 or 3. Embryo quality was evaluated by observing the percentage of anucleate cytoplasmic fragments and number and symmetry of blastomeres. As prescribed by the above mentioned law, all resulting embryos were transferred without selection. Each embryo transfer included in the study was performed using only unselected embryos derived from the same oocyte category.

Embryo transfers were carried out 48–72 h after fertilization by using a Gynetics soft catheter (Semtrac 5-2000 SET-Gynetics, Lommel, Belgium). All women received oral oestradiol hemihydrate supplementation, 6 mg/day (17 β E2; Novo-Nordisk, Rome, Italy) starting on the day of oocyte retrieval. Luteal support was provided by intravaginal progesterone supplementation (Progesterone; Rottapharm, Monza, Italy) 600 mg/day starting 1 day later.

Pregnancy was tested 12–13 days after transfer by quantitative definition of serum β -HCG.

In case of pregnancy, oestrogen and progesterone supplementation was continued until the 12th week of gestation. Clinical pregnancy was defined by the presence of a gestational sac, with or without fetal heartbeat, at transvaginal ultrasound examination 2 weeks after β -HCG testing.

Analysis of fertilization, clinical pregnancy and implantation rates versus COC morphology and priming treatment was performed by Fisher's exact test or Chi Squared test depending on absolute frequencies in the table (Stata Corporation, 1999, Texas, U.S.A.). A level of $P < 0.05$ was adopted for significance.

Results

In 1096 consecutive IVM cycles, 6113 COCs were recovered of which 3753 were classified as CC, 1303 as EC, 780 as EC-MII, and 277 as degenerate. CC and EC oocytes were matured in vitro (Table 1), achieving an overall maturation rate of 52.1 % (2636/5056). EC-MII oocytes, already mature at pick-up, were not included in the calculation of the

maturation rate. Only 2316 metaphase II oocytes were inseminated in compliance with the Italian law which, during the period of study, prohibited the insemination of more than 3 oocytes per cycle. The overall fertilization rate (Table 1) was 73.8 % (1710/2316). In 827 cycles, transferred embryos derived from a single COC category. In particular, replacements performed with embryos derived from CC, EC and EC-MII oocytes were 491, 90, and 246, respectively.

Data were initially analyzed irrespective of the type of priming regimen. The difference in maturation rate between the CC (47.0 %; 1766/3753) and EC (66.8 %; 870/1303) groups (Table 1) was statistically significant ($p < 0.0001$). No differences in fertilization rate were observed among different types of oocytes (Table 1). The overall clinical pregnancy rate per transfer was 17.9 % (148/827). Clinical pregnancy rates per transfer were 14.5 %, 10.0 % and 27.6 % in the CC, EC, and EC-MII categories, respectively (Table 1). Some differences were statistically significant (EC-MII vs. CC, $p < 0.0001$; EC-MII vs. EC $p < 0.0001$). A total number of 168 gestational sacs was reported. This corresponded to an overall implantation rate of 11.8 % (168/1424). The implantation rates were 8.9 %, 6.3 % and 19.1 % in the CC, EC, and EC-MII categories, respectively (Table 1). The differences were significant comparing EC-MII vs. CC ($P < 0.0001$) and EC-MII vs. EC groups ($P < 0.0001$).

The analysis was subsequently extended to ascertain possible correlations between COC classification, gonadotropin regimen and clinical outcome.

No significant differences in fertilization rates of EC and EC-MII oocytes were observed in different priming regimens (Fig. 1). A significantly lower fertilization was found in CC oocytes in regimen D (FSH/HCG) versus regimens A (no priming) and B (HCG) ($p = 0.001$ and $p = 0.03$ respectively).

In HCG cycles (Fig. 2), the pregnancy rate per embryo transfer (CPRt) obtained from EC-MII oocytes was higher than the one achieved with EC oocytes (16.7 % and 0 %,

Table 1 Maturation and fertilization rates of different COC categories. The difference in maturation rate between the CC and EC groups was statistically significant ($p < 0.0001$). No differences in fertilization rate

	Maturation rate (no.)	Fertilization rate (no.)	Clinical pregnancy rate (no.)	Implantation rate (no.)
CC	47.0 (1766/3753)	75.0 (984/1312)	14.5 (71/491)	8.9 (75/842)
EC	66.7 (870/1303)	74.6 (227/304)	10.0 (9/90)	6.3 (9/143)
EC-MII	n.a.	71.3 (499/700)	27.6 (68/246)	19.1 (84/439)

The table also describes the clinical outcome of embryo replacements in which transferred embryos derived from a single category of oocytes. Statistically significant differences were found in pregnancy (EC MII vs. CC $p < 0.0001$; EC MII vs. EC $p < 0.0001$) and implantation rates (EC MII vs. CC $p < 0.0001$; EC MII vs. EC $p < 0.0001$). Data were analyzed by the Fisher test

were observed among different types of oocytes. Data were analyzed by the chi square test

Fig. 1 Fertilization rates of different categories of oocytes (CC, EC and EC-MII) in IVF cycles performed after different priming regimens. A significantly lower rate was found in CC oocytes of FSH/HCG regimen versus no priming (+, $p=0.001$) and HCG (§, $p=0.03$)

$p=0.07$) and comparable to the CC group (18.4%), while the pregnancy rate in the EC group was significantly lower than the CC group (0% and 18.4%, $p=0.01$).

Among different priming schemes, the differences in CPRt involving CC oocytes were not significant (13.2%, 18.4%, 8.4%, and 10% respectively for regimens A, B, C, and D), although the difference between the C and D groups approached significance ($p=0.060$). The CPRt obtained using EC oocytes derived from the HCG regimen (group B) was significantly lower in comparison to the one of EC oocytes recovered in FSH/HCG cycles (group D) (0.0% and 15.3% respectively, $p=0.018$). No differences were recorded in clinical pregnancy rates of EC-MII oocytes derived from different priming strategies.

In treatments A and C, where only CC oocytes were collected, no differences were noted in clinical pregnancy rates.

In the HCG group (Fig. 3), the implantation rate derived from EC-MII oocytes was significantly higher than the one achieved with EC oocytes (13.3% and 0%, $p=0.04$) and comparable to the CC group (10.4%). The implantation rate in the EC group was significantly lower than the CC group (0% and 10.4%, $p=0.02$).

Implantation rates from regimens A, B, C and D involving CC oocytes were 8.2%, 10.4%, 11.6%, and 5.9% respectively.

The difference in implantation rate of CC oocytes between the FSH (group C) and the FSH/HCG (group D) was significant (11.6% and 5.9% respectively, $p=0.04$). Likewise, the implantation rates of EC oocytes were significantly different between the HCG and FSH/HCG groups (0% and 15.3%, respectively; $p=0.007$).

No differences were recorded in implantation rates of EC-MII oocytes derived from different priming strategies.

Fig. 2 Rates of pregnancy per transfer (CPRt) in cycles in which transferred embryos developed from a single category of oocytes. No differences were found among CC oocytes of different groups although a trend was observed in the comparison between regimen C and regimen D ($p=0.060$). In group B (regimen HCG) the differences were significant by comparing EC vs. CC (+, $p=0.012$). In regimen D the differences were significant by comparing EC vs. EC MII (§, $p<0.0001$). The CPRt of the EC group in regimen B was also significantly lower in comparison to regimen D (°, $p=0.018$)

Fig. 3 Rates of implantation in cycles in which transferred embryos developed from a single category of oocytes. Statistically significant differences were found by comparing oocyte categories in regimen B: EC vs. CC $p=0.010$ (+); EC vs. EC-MII $p=0.039$ (^); regimen D: EC-MII vs. CC $p<0.0001$ (#); EC-MII vs. EC $p=0.037$ (\$). Implantation rate within the EC group was lower in regimen B in comparison to regimen D $p=0.007$ (\$). The difference in implantation rate of CC oocytes between group C and group D) was significant (£, 11.6% and 5.9% respectively, $p=0.04$)

In FSH/HCG cycles, significantly higher implantation rates were obtained from EC-MII oocytes in comparison to the EC (19.3% and 6.3%, $p=0.04$) and CC oocytes (19.3% and 5.9%, $p<0.0001$). No differences were observed between EC and CC oocytes.

Finally, in view of the emerging importance of EC-MII oocytes, we compared the clinical outcome of FSH/HCG cycles, in which at least one transferred embryo derived from EC-MII oocytes, with the one obtained in cycles with no HCG priming (no priming or FSH priming). Pregnancy rates were 29.5% (64/217) and 15.5% (55/363), respectively ($p<0.0001$).

Discussion

Morphologically distinct cumulus-oocyte-complexes (COCs) are retrieved during an IVM procedure as an effect of different priming regimens. While these characteristics may predict biological (maturation and fertilization rates) and clinical (pregnancy and implantation rates) potential, a clear definition of the relationship between COC organization and priming strategy has yet to be achieved. In absence of gonadotropin administration, only compact cumulus masses including a GV-stage oocyte are obtained [2, 13]. The use of HCG, when follicles reach a size of 10–12 mm, causes variable degrees of cumulus expansion and meiotic progression. In this case, about 70% of oocytes may be found at the GV stage within a compact cumulus mass, 10–20% of COCs display an expanded cumulus configuration associated to meiotic resumption (GVBD), and 6–20% are accompanied by complete progression to MII [4].

Previous studies suggested that oocytes of COCs displaying an expanded cumulus give rise to embryos with higher developmental ability, supporting the notion of a beneficial effect of HCG stimulation [11]. However, a detailed relational

analysis is lacking. Here we provide a thorough analysis indicating that metaphase II oocytes developed from different types of COCs exhibit diverse developmental competencies attributable in part to the type of gonadotropin priming.

COCs having already undergone cumulus expansion and oocyte meiotic maturation (EC-MII) at the time of recovery develop into embryos with higher developmental competence. Despite EC-MII COCs represent a minority (6–20%) of all retrieved COCs, they have a major positive impact in determining the clinical outcome of an IVM treatment.

The fact that, even after HCG administration, the majority of COCs appear compact (CC) is not surprising [3]. In fact, most antral follicles available for recovery in an IVM cycle are small (4–10 mm) and, as such, not responsive to luteinizing hormone (LH).

In the present study, we observed a significant difference in the overall maturation rate of CC and EC oocytes, irrespective of priming regimen. CC COCs show a limited ability to undergo GVBD and progress to MII (47.0%) after 30 h of culture, a period of time sufficient for meiotic maturation [14].

EC oocytes show an elevated maturation rate (66.8%). In this oocyte population, expansion of the cumulus shows a trend towards higher meiotic competence. Immature oocytes within expanded cumulus (EC) reinitiated maturation in vivo in response to HCG administration. As a consequence, it seems likely that cumulus cells elicit activation of the signaling pathway that sustains both the resumption and completion of meiosis in vitro. Importantly, while fertilization rates of in-vitro matured (CC and EC) oocytes were comparable to oocytes that matured in vivo (EC-MII), any distinctions in developmental ability between in vivo and in vitro matured oocytes cannot be appreciated at the fertilization stage. In contrast, we observed that implantation rates obtained from oocytes matured in vivo (EC-MII, 19%) were

higher than those obtained from *in vitro* matured oocytes (CC, 8.9% and EC, 6.3%).

These findings emphasize the clinical implications of COC selection for embryologists working with IVM protocols. Moreover, the findings help to resolve different results obtained from different clinics where diverse gonadotrophin regimens are applied.

Comparison of pregnancy and implantation rates among different COC categories and priming regimens indicates that EC-MII oocytes generated in HCG-primed cycles (group B) produce the same results irrespective of whether FSH was also supplemented. However, when FSH was added (group D), the percentage of EC-MII available was significantly higher [3]. Such considerations warrant further studies. From a clinical standpoint, to maximize the clinical efficiency of IVM, our large data sets confirm that HCG (with or without FSH) is crucial to obtain at least a fraction of mature and developmentally competent oocytes at the time of retrieval. Moreover, implantation rates for EC-MII oocytes (19.1%) approach those normally achieved in stimulated cycles in our IVF program, under otherwise comparable conditions (data not shown). The superior developmental ability of EC-MII oocytes in comparison to those properly defined as *in-vitro* matured (CC) leads inevitably to a semantic question. In fact, it may be argued that cycles involving HCG priming (with or without FSH) are not “pure” IVM treatments, but rather an approach that combines in the same cycle mild stimulation (which accounts for EC-MII oocytes and requires only IVF) and IVM (definition justified by the recovery and systematic use of immature oocytes). Therefore, a novel more appropriate classification of HCG-primed IVM cycles would be welcome. On another hand, a relatively subtle difference may be discerned between HCG-primed IVM cycles and mild stimulation. In fact, while in the former mature oocytes are retrieved from mid-antral follicles of 10–13 mm in diameter, in the latter MII oocytes derived from larger follicles, usually ≥ 18 mm in diameter.

While the competence of embryos developed from the EC class was low, despite the occurrence of GVBD *in vivo* and completion of maturation *in vitro*, success rates were improved with a previous exposure to FSH. Our data indicate an enhancement in oocyte quality of oocytes due to FSH since this condition yielded a higher implantation rate (10.7% in group D versus 0% in group B).

Possible explanations for the low competence of EC oocytes might be attributed to defective signalling and/or *in vitro* maturation conditions. In particular, because in many EC oocytes meiotic resumption occurred before recovery, as indicated by the absence of the GV at retrieval, it is plausible that a culture period of 30 h would be in excess of the time required for meiotic transition from metaphase I to metaphase II. If meiotic maturation is achieved within a

time considerably less than 30 h, it may happen that these oocytes remain in culture for too many hours before insemination, thus undergoing a process of *in vitro* aging, with detrimental consequences on oocyte quality.

CC-COCs represent a valuable source that can contribute to the overall clinical outcome, irrespective of priming regimens. Further improvements of IVM success rate will depend critically on the ability to mature these oocytes *in vitro*, considering also that they represent the largest proportion of retrieved material. The reasons of a general limited developmental potential of CC oocytes in comparison of EC-MII are not completely known, but likely involve or underscore our lack of optimal media maturation conditions.

More studies are needed to understand better the meiotic resumption process and to improve the maturation *in vitro*. Our results suggest a direction for future work in this area. We observed a lower, even if not significant, implantation potential of CC oocytes after FSH/HCG supplementation comparing the other regimens (group D versus groups A, B and C). This can be explained considering that using FSH/HCG strategy the whole pool of immature follicles is induced to mature. A part of follicles can easily produce EC and EC-MII oocytes, while some small antral follicles, which could contain oocytes with particularly pronounced condition of immaturity, can only produce CC-COCs with very poor potential.

In conclusion, in this study we described the significance of morphological characteristics of COCs recovered in IVM cycles, providing also evidence that developmental destiny of these classes of COCs is different. Distinct COCs produce embryos with different competences that can affect the success rate. Based on our studies, we recommend that the gonadotropin regimens that produce the higher percentage of EC-MII oocytes should be applied and a substantial effort should be made to improve the maturation of EC and CC oocytes.

Further improvement of IVM treatment will depend on the ability to increase the maturation potential of CC and EC oocytes in order to optimize production of competent embryos based upon higher implantation rate. In this light, factoring in new information on oocyte metabolism and architectural requirements of oocyte-cumulus cell association in development of culture media, will hopefully achieve procurement of high quality oocytes in which nuclear-cytoplasmic synchronization has been achieved.

References

- Schroeder AC, Eppig JJ. The developmental capacity of mouse oocytes that matured spontaneously *in vitro* is normal. *Dev Biol.* 1984;102:493–7.

2. 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.
3. 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.
4. Chian RC, Buckett WM, Too LL, Tan SL. Pregnancies resulting from *in vitro* matured oocytes retrieved from patients with polycystic ovary syndrome after priming with human chorionic gonadotropin. *Fertil Steril*. 1999;72:639–42.
5. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod*. 2000;15:165–70.
6. Chian RC. In-vitro maturation of immature oocytes for infertile women with PCOS. *Reprod Biomed Online*. 2004;8:547–52.
7. 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.
8. Lin YH, Hwang JL, Huang LW, Mu SC, Seow KM, Chung J, et al. Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes. *Hum Reprod*. 2003;18:1632–6.
9. Son WY, Lee SY, Chang MJ, Yoon SH, Chian RC, Lim JH. Pregnancy resulting from transfer of repeat vitrified blastocysts produced by in-vitro matured oocytes in patient with polycystic ovary syndrome. *Reprod Biomed Online*. 2005;10:398–401.
10. Son WY, Yoon SH, Lee SW, Ko Y, Yoon HG, Lim JH. Blastocyst development and pregnancies after IVF of mature oocytes retrieved from unstimulated patients with PCOS after in-vivo HCG priming. *Hum Reprod*. 2002;17:134–6.
11. 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.
12. Yang SH, Son WY, Yoon SH, Ko Y, Lim JH. Correlation between *in vitro* maturation and expression of LH receptor in cumulus cells of the oocytes collected from PCOS patients in HCG-primed IVM cycles. *Hum Reprod*. 2005;20:2097–103.
13. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. *Hum Reprod*. 1999;14:1847–51.
14. Combelles CM, Cekleniak NA, Racowsky C, Albertini DF. Assessment of nuclear and cytoplasmic maturation in in-vitro matured human oocytes. *Hum Reprod*. 2002;17:1006–16.