Antral follicle counts (AFC) predict ovarian response and pregnancy outcomes in oocyte donation cycles

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Abstract

Purpose Antral follicle count (AFC) is used as a marker of ovarian response. We assessed its value in predicting pregnancy outcomes in ovum donation cycles by retrospective review.

Methods Oocyte donors ($n=94$) underwent ovarian hyperstimulation using rFSH and GnRH-antagonists. Recipients were synchronized using GnRH-agonist down-regulation followed by fixed dose of estrogen and progesterone following hCG. Outcomes measured included correlation of AFC to pregnancy outcomes and cycle characteristics in those with and without clinical and ongoing-delivered cycles.

Results AFC significantly correlated with clinical [Exp β 1.12; 95% CI: 1.02–1.23, $p<0.05$] and ongoing-delivered pregnancy [Exp β 1.10; 95% CI: 1.01–1.20, $p<0.05$]. Significantly greater AFC, total and M-2 oocytes, and

cycles resulting in cryopreserved embryos were seen in clinical and ongoing-delivered cycles.

Conclusions AFC predicts cycle stimulation responses and clinical outcomes and may serve as a guide for dosing protocols and in choosing to proceed with the most optimal cycle.

Keywords Antral follicle count · In-vitro fertilization · Pregnancy outcomes · Oocyte donation

Capsule AFC Predicts Cycles Stimulation Response in Clinical and Ongoing Pregnancy Outcomes.

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Introduction

The success of in vitro fertilization and embryo transfer (IVF-ET) oocyte donation cycles is primarily dependent upon the recruitment and development of multiple follicles to ovulation induction hyperstimulation leading to the development of multiple, high quality embryos for selection for embryo transfer. Much like in autologous cycles, non-invasive markers of ovarian reserve including age [1], early follicular phase FSH [2–4], estradiol [5–6], inhibin B [7, 8], and anti-Mullerian hormone [9–12] have been used in an attempt to select the optimal ovum donor who will yield the highest number and best quality oocytes. However, none of these are particularly reliable, especially because young fertile women typically have normal baseline hormonal markers [13] and their accuracy of predicting pregnancy outcomes is at best limited [14].

The antral follicle count (AFC) (2 to 10 mm in both ovaries) in the early follicular phase correlates with ovarian reserve [15] and low AFC is a sign of ovarian aging. Some have proposed that AFC may be a better single predictor of ovarian reserve than age or any endocrine markers and appears to be an earlier objective finding when compared to a rise in serum FSH levels [16, 17]. A number of studies in

IVF cycles including two meta-analyses [17–26] have found AFC to be a useful predictor of poor ovarian response to gonadotropin stimulation. AFC may help with counseling and selection of appropriate treatment protocols and dosing regimens as AFC correlates with the number of recruited follicles and the number of retrieved oocytes.

Nonetheless, even with the abundance of studies evaluating cycle stimulation characteristics, there are only a few studies that suggest AFC is useful in predicting autologous IVF cycles pregnancy outcomes. According to some reports, lower pregnancy, clinical pregnancy, and live birth rates are noted in women with AFC ≤ 10 [19–21, 26]. In contrast, Barreto Melo reported AFC could not predict oocyte quality, embryo development, and clinical pregnancy rates in oocyte donation cycles [28]. They attributed the findings to the different patient populations where subjects are typically older and infertile in autologous cycles in contrast to oocyte donors who are younger and presumably fertile. The present study was undertaken to further add to the literature regarding AFC and its predictive value in oocyte donation cycles primarily with respect to clinical and ongoing-delivered pregnancy rates and secondarily to cycle stimulation characteristics using GnRH-antagonists and blastocyst embryo transfers.

Materials & methods

This study was reviewed and approved by the Western Institutional Review Board.

Design and AFC

From January 2005 to December 2008, a retrospective review of oocyte donation cycles ($n=119$) with documented total AFC was undertaken. All transvaginal ultrasounds (TVS) were performed by SL following 1 to 2 months of oral contraceptives (Mircette®, 0.02 mg ethinyl estradiol and 0.15 mg desogestrel, Duramed Pharmaceuticals, Inc, Pomona, NY), for cycle synchronization starting on cycle day-2 or 3 of a withdrawal bleed using a GE 3600-7 mHz vaginal transducer. All follicles measuring 2–10 mm on both ovaries were counted and recorded with the total number of AFC per patient used for calculation [28].

Subjects

First time donor cycles with at least one fully expanded blastocyst and used gonadotropin releasing hormone antagonist (GnRH-ant) were included in the study for evaluation. Those recipient cycles with uterine myoma(s) >3 cm, severe male factors (<1 million/cc or TESE), and cleavage stage

embryo transfers were excluded from analysis resulting in 94 fresh oocyte donation cycles for review.

All oocyte donors ($n=94$) (age: 26.0 ± 3.7 years (yrs), mean $\pm$ standard deviation (SD), range 20–34 yrs) had normal menstrual cycles of 25 to 32 days duration, no clinical evidence of polycystic ovarian syndrome, normal body mass index, cycle day-3 serum follicle stimulating hormone (FSH) level, and a normal pelvic ultrasound.

Donor protocol

Ovarian stimulation was started on cycle day 3 following an OCP withdrawal bleed using daily injections of 150 to 300 IU of recombinant follicle stimulating hormone (rFSH) [Follistim TM; Organon Inc., West Orange, NJ, USA]. The use of OCPs is not known to affect AFC. All cycles were monitored by serum estradiol levels starting on cycle day 3 and TVS and serum estradiol levels on cycle day 4. Dosage was adjusted according to response. GnRH-antagonist (Ganirelix, 0.25 mg, subcutaneously daily [Organon Inc., West Orange, NJ, USA] was begun when lead follicles were 13–14 mm. When ≥ 3 follicles reached 18–20 mm, urinary hCG 10,000 IU was given and was followed by transvaginal oocyte aspiration 36 h later [29, 30]. Cycles were cancelled for poor response, defined as ≤ 4 dominant follicles on the day of human chorionic gonadotropin (hCG) or hyperresponse with serum estradiol $>6,500$ pg/mL [28].

Recipient protocol

All recipients underwent a fluid contrast sonography (sonohysterography) and mock embryo transfer within 6 months of an embryo transfer cycle. All cycles were synchronized to the donors' using GnRH-agonist down-regulation followed by fixed dose of 2 mg estradiol two times daily (BID) (Estrace). Endometrial thickness and echogenic pattern were obtained 10 to 11 days after initiation of Estrace. Progesterone (P4) 50 mg intramuscularly BID ($n=30$; 33%) or 200 mg suppository three times daily (TID) ($n=62$; 67%) supplementation was initiated following the day of hCG for the oocyte donor.

Intracytoplasmic sperm injection and embryo transfer

All oocytes were fertilized by ICSI. All normally fertilized embryos were cultured sequentially in Vitrolife's G1.3 and G2.3 media, supplemented with 5% protein. A gas phase of 7% CO₂, 5% O₂ and 88% N₂ was used in a humidified incubator. Embryos were cultured individually in drops under

oil (Vitrolife's Ovoid [Vitrolife Inc., Englewood, CO, USA]). At least 4 good quality cleavage stage embryos were required to continue culture to the blastocyst stage (≥ 6 cells and grade 2 (slight to moderately uneven cells and slight to moderate fragmentation)). Embryos were assessed on day 3 and day 5. Blastocyst quality was evaluated on day 3 and day 5 according to inner cell mass and outer cell mass morphology and the presence of expansion or hatching. Two to three ($n=2$, these patients were consented regarding risks of higher orders multiples and the issues related to selective reduction) embryos were transferred transcervically 5 days post retrieval under ultrasound guidance. Pregnancy was confirmed by serum β -hCG 7 to 10 days following embryo transfer. Clinical and ongoing pregnancies were defined as the presence of an intrauterine gestational sac at 6 weeks and a fetal heart rate(s) at 12 weeks of gestation, respectively. A multiple pregnancy was regarded as one pregnancy.

Measured outcomes

The following primary outcome measures were assessed: 1) the diagnostic accuracy of AFC to predict clinical pregnancy and ongoing-delivered pregnancy in donor egg cycles and 2) differences in cycle stimulation characteristics in those with and without clinical and ongoing-delivered pregnancy. Secondary outcomes were the predictive value of AFC to stimulation characteristics including days of stimulation (DOS), # of total IUs used, serum E2 on of hCG administration, total # oocytes, and total # M-2 oocytes.

Assays

Serum samples were assayed for FSH (DPC, Los Angeles, CA) and estradiol (DPC, Los Angeles, CA) on CD-3 using CIA Immulite $\rightarrow$ kits. Intra and interassay CV for FSH were 5.4% and 8.1% and 6.3% and 6.4% for estradiol, respectively.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package, version 11.0 (SPSS Inc., Chicago, IL). Data were expressed as mean $\pm$ SD. Unpaired student's *t* test was used to test the significance of differences in means between groups and chi-square-test was used to assess the significance of categorical comparisons. Pearson's correlation was used for pair wise associations. In a general linear model, univariate regression analysis was used to determine the effect of demographic parameters (donor age and donor

BMI, and recipient age) and ovarian reserve variables (cycle day-3 FSH and AFC) on cycle stimulation characteristics. Backward selection of parameters was applied, using $p<0.05$ and $p<0.10$ for entry or deletion, respectively. Logistic regression was used to determine these variables as determinants of clinical pregnancy and ongoing-delivered cycles. Receiver operator characteristics were used to calculate the area under the ROC curve (AUC) to assess the predictive accuracy of the logistic models, yielding values from 0.5 (no predictive power) to 1.0 (perfect prediction). Significance was defined as a p -value <0.05 .

Results

Ninety-four initiated donor cycles resulted in 85 retrievals (90.4%) 7 cancelled for poor response, 2 cancelled for hyperresponse, and 84 embryo transfers (89.4%). Fifty-six of the cycles resulted in a pregnancy (59.6% per initiated cycle and 66.6% per ET); 50 in a clinical pregnancy (53.2% per initiated cycle and 59.5% per ET); and 43 in an ongoing-delivered pregnancy (45.7% per initiated cycle and 51.2% per ET). Twenty-nine of the clinical pregnancies (58%) resulted in a multiple gestations (27- two sacs and 2-three sacs).

The mean age of oocyte donors and recipients were 25.9 ± 3.9 years, range 20–34 yrs and 40.5 ± 5.3 years, range 30–55 yrs, respectively. The donor mean day 3 serum FSH was 5.6 ± 2.4 mIU/ml. The mean BMI was $22.4\text{ kg/m}^2\pm 2.3$, (range 19–28 kg/m^2) and $25.9\text{ kg/m}^2\pm 4.6$, (range 19–33 kg/m^2) for the donors and recipients, respectively. For the oocyte donor, the mean DOS was 11.2 ± 1.6 days and the mean total dosage of gonadotropin used was 2730 ± 988 IU. The mean number of embryos transferred was 2.1 ± 0.5 .

Logistic regression model was used to assess the effects of AFC, donor and recipient age, donor BMI and #ET as model variables for both clinical and on-going-delivered pregnancy. AFC significantly correlated with clinical pregnancy [Exp β 1.12; 95% CI: 1.02–1.23, $p<0.05$] and ongoing-delivered pregnancy [Exp β 1.10; 95% CI: 1.01–1.20, $p<0.05$].

Differences in clinical variables with respect to those with and without clinical and ongoing-delivered pregnancy are depicted in Tables 1 and 2. Only the following variables were significantly higher in cycles leading to clinical pregnancy when compared the unsuccessful ones: AFC (16.4 ± 6.5 vs. 13.2 ± 4.7 $p=0.003$), retrieved total oocytes (22.7 ± 11.4 vs. 16.6 ± 7.8 , $p=0.007$), # M-2 oocytes (20.0 ± 11.4 vs. 13.6 ± 6.6 , $p=0.006$). Similarly, the same parameters including the % of cycles resulting in cryopreserved embryos were higher in those cycles that lead to an ongoing-delivered pregnancy compared to those without and ongoing-delivered pregnancy: AFC (16.3 ± 6.9 vs. 13.2 ± 4.7 , $p=0.01$), retrieved total

Table 1 Comparison of variables in those with and without clinical pregnancy

	Cycles with clinical pregnancy (<i>n</i> =50)	Cycles without clinical pregnancy (<i>n</i> =44)	p-value
Age of donor (yrs.)	25.6±4.0	26.4±3.9	0.36
Cycle day-3 FSH (mIU/mL)	5.2±2.5	5.7±2.4	0.37
Donor BMI (kg/m ²)	22.5±2.2	22.2±2.4	0.44
Age of recipient (yrs.)	39.9±5.7	41.0±5.9	0.38
Recipient BMI (kg/m ²)	26.8±4.6	24.4±4.6	0.27
AFC	16.4±6.5	13.2±4.7	0.003
Starting dose (IUs)	251±55	255±50	0.74
Total # gonadotropins (IUs)	2599±1047	2884±901	0.17
DOS	11.2±1.7	11.1±1.6	0.86
E2 day of hCG, pg/mL	2518±1431	2089±1431	0.17
# Follicles >12 mm	21.5±10.3	18.4±11.0	0.15
# oocytes retrieved	22.7±11.4	16.6±7.82	0.007
#M2 oocytes	20.0±11.4	13.6±6.6	0.006
Fertilization Rate (%)	70.0±17.6	68.7±17.1	0.22
# Embryos Transferred	2.1±0.5	2.1±0.5	0.24
% Cryopreservation	72	43	0.19
# Cryopreserved Embryos	5.1±3.8	3.4±2.1	0.09

oocytes (23.1±11.8 vs. 17.1±7.5, $p=0.007$), # M-2 oocytes (20.3±12.0 vs. 14.4±6.9, $p=0.01$), and % of cycles resulting in cryopreserved embryos (72% vs. 49%, $p=0.04$).

Further analysis of AFC in cancelled cycles (9.4±2.6) for a poor response failed to show a difference from those cycles that were without a clinical (12.9±4.6, $p>0.05$) or ongoing-delivered pregnancy (12.9±4.6, $p>0.05$), but AFC was significantly less than in those cycles with a clinical

pregnancy (16.4±6.5, $p<0.05$) and ongoing-delivered pregnancy (16.3±6.9, $p<0.05$).

With respect to secondary outcome measures, AFC negatively correlated with total dosage of gonadotropins used ($r=-0.44$, $P<0.001$) and positively correlated with serum E2 on day of hCG ($r=0.36$, $p<0.001$), number of follicles >12 mm on the day of hCG (0.443, $p<0.001$), number of oocytes retrieved ($r=0.431$, $p<0.001$), and # M-

Table 2 Comparison of variables in those with and without ongoing-delivered cycles

	Cycles with ongoing-delivered pregnancy (<i>n</i> =43)	Cycles without ongoing-delivered pregnancy (<i>n</i> =51)	p-value
Age of Donor (yrs.)	25.3±4.1	26.5±3.8	0.13
Cycle day-3 FSH (mIU/mL)	5.2±2.5	5.7±2.4	0.37
Donor BMI (kg/m ²)	22.5±2.0	22.2±2.5	0.65
Age of Recipient (yrs.)	39.5±5.9	41.2±5.6	0.17
Recipient BMI (kg/m ²)	27.3±4.8	24.3±4.4	0.18
AFC	16.3±6.9	13.2±4.7	0.01
Starting dose (IUs)	252±55	253±51	0.87
Total # gonadotropins (IUs)	2656±1082	2794±904	0.51
DOS	11.3±1.6	11.0±1.7	0.33
E2 day of hCG, pg/mL	2474±1458	2184±1532	0.35
# Follicles >12 mm	21.6±10.5	18.8±10.5	0.21
# oocytes retrieved	23.1±11.8	17.1±7.5	0.007
#M2 oocytes	20.3±12.0	14.4±6.9	0.01
Fertilization Rate (%)	68.3±16.5	67.6±18.6	0.85
# Embryos Transferred	2.1±0.5	2.1±0.5	0.98
% Cryopreservation	72	49	0.04
# Cryopreserved Embryos	4.9±3.7	4.1±3.0	0.41

2 oocytes ($r=0.37$, $p<0.01$), (Figs. 1 and 2, Table 3). AFC did not correlate with donor age, basal FSH, days of stimulation and fertilization rates ($p>0.05$). Data is summarized in Table 3.

Using an AFC cut-off of ≤ 10 had a sensitivity of 75% and a specificity of 66.7% to identify cycle cancellation. An AFC cut-off of ≤ 9 had a sensitivity of 71% and a specificity of 83.7% to identify poor response. An AFC cut-off of ≤ 13 had sensitivity of 71.4% and specificity of 59.1% to identify clinical pregnancy. Finally, an AFC cut-off of ≤ 13 ; had a sensitivity of 75.5% and specificity of 52.9% to identify an ongoing-delivered pregnancy, respectively. The predictive values are shown in Table 4 and a respective plot is shown in Fig. 2. Using an AFC >13 vs <13 , clinical pregnancy rates were 66% vs. 37%, $p<0.01$) and using >13 vs <13 , ongoing-delivered pregnancy rates were 58% vs. 35%, $p<0.05$, respectively.

Discussion

To our knowledge, this is the second report in the literature to describe the predictive value of AFC in oocyte donation cycles for both clinical and ongoing delivered pregnancies. This study suggests that while AFC is useful in predicting clinical and ongoing-delivered pregnancies in oocyte donation cycles, it is not predictive of cycles that result in cancellation.

In agreement with the results of the prospective trial in oocyte donation cycles [28], AFC was a good predictor of ovarian response much like in autologous IVF cycles in our study. In contrast to our study, Barreto Melo et al. could not use AFC to predict oocyte/embryo quality or IVF outcomes

Fig. 2 ROC plot for AFC and ongoing-delivered pregnancy

if the cycle resulted in an embryo transfer. According to them, an AFC of <12 had the optimal predictive value for poor response. While oocyte donors with AFC <10 had significantly higher cancellation rates. If cycles resulted in an embryo transfer, there were no differences in pregnancy outcomes among the groups they studied. The authors suggested that oocyte donors with low AFC do not necessarily have poor oocyte quality in the face of normal basal FSH; rather, they simply may have a lower capacity to respond to ovarian hyperstimulation [28].

Fig. 1 Plot of correlation analysis between the number of M2 oocytes retrieved and AFC. The regression curve delineates the significant correlation between AFC and M2 oocytes retrieved ($r=0.37$, $p<0.001$)

Table 3 Correlation analysis of AFC to demographic and cycle stimulation characteristics-outcomes

Parameter	r-value	p-value
Age	0.039	0.71
BMI kg/m ²	0.067	0.79
FSH mIU/mL	0.033	0.46
Estradiol pg/mL	0.051	0.83
# total gonadotropins	-0.44	<0.001
Days of Stimulation	0.157	0.135
Cycle d-3 E2	0.442	0.004
Cycle d-5 E2	0.44	<0.001
Peak E2	0.364	<0.001
# Oocytes retrieved	0.431	<0.001
# M-II oocytes	0.371	0.001
% Fertilization	0.062	0.591
Cancellation	0.123	0.17
Pregnant	0.396	<0.001
Clinical Pregnant	0.354	0.001

Table 4 Results of ROC analysis for different parameters depending on AFC

Parameter	Optimum AFC cutoff	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Cancelled Cycles	≤ 10	0.664	0.462–0.866	75	66.7	22	95
Cancelled cycles for poor response	≤ 9	0.811	0.688–0.933	71	83.7	35.7	94.5
Cycles resulting in ET	≤ 10	0.652	0.468–0.837	74.7	60	22	95
CP/initiated cycle	≤ 13	0.683	0.572–0.793	71.4	59.1	65	66
Ongoing-Delivered cycle/initiated cycle	≤ 13	0.638	0.521–0.755	75.5	52.9	67.5	54.7

Recent meta-analyses to determine the predictive capacity of AFC measurements for pregnancy outcome [25, 26] in autologous IVF cycles deemed that AFC is poor at best and has limited clinical value for pregnancy prediction. More recently, Maseelall et al. reported in autologous IVF cycles that AFC as a primary outcome measure was a significant predictor of live birth rates and miscarriage rates [27]. Based on their finding, these investigators counsel patients who have an AFC ≤ 10 compared to women with an AFC > 11 , that their chance of live birth is lower, miscarriage rates and cycle cancellation are higher, that they will require more gonadotropins, and are likely to have less retrieved oocytes. However, they do not use AFC to exclude patients from IVF treatment.

While our results in oocyte donation cycles demonstrate a difference in cycles with and without clinical and ongoing-delivered pregnancies, we need to be cautious in these conclusions and should not necessarily exclude donors with AFC ≤ 13 , particularly when close to half of cycles (47%) with AFC ≤ 13 resulted in cryopreserved embryos as well. Given that not all recipients had undergone a frozen transfer cycle, cumulative pregnancies including both fresh and frozen cycles could impact our findings.

A flaw in our study design was the individual dosing of rFSH initially used during follicular recruitment (150 to 300 IU rFSH). Specific to ongoing-delivered cycles, the starting doses were similarly distributed in those with and without ongoing-delivered pregnancies: 150 rFSH-12% ($n=5$) vs 6% ($n=3$); 225 rFSH-35% ($n=15$) vs 41% ($n=21$); and 300 rFSH-53% ($n=23$) vs 53% ($n=27$) respectively. In addition, the use of OCPs was not consistent in duration length which could have impacted on AFC, though no data in the literature suggests an OCP effect on AFC.

Unlike in our study, the starting dose for all cycles in the study of Barreto-Melo was fixed at 225 IU/day, however, both rFSH and urinary gonadotropins were used and dosing was adjusted between 2 to 5 days of stimulation. In addition, GnRH-agonists were used for pituitary suppression and embryo transfers were performed either at the cleavage stage or blastocyst stage, which may have impacted on their clinical outcomes. Though studies suggest that GnRH-agonists do not

result in a significant change in AFC and AFC can reliably be measured before or after pituitary down-regulation [31]. Our study controlled for these variables by only including cycles using rFSH; dosing adjustments after 3 days of ovarian stimulation; GnRH-antagonists treated cycles; and blastocyst only embryo transfers.

This study corroborates the many other trials that support AFC as a useful tool for predicting ovarian response to ovulation induction, though many of these trials focus on autologous ovulation induction cycles [15–26]. This is especially important in oocyte donation cycles, where younger women often have normal ovarian reserve markers including basal serum FSH. Predicting ovarian response before ovarian hyperstimulation is useful in selecting optimal oocyte donors particularly in programs that may share the oocytes retrieved between 2 or 3 couples and may be helpful in tailoring the dosage of gonadotropins where concerns exist for both adequate responses and ovarian hyperstimulation.

We feel that excluding donors with AFC ≤ 14 as a cutoff in the face of normal basal FSH is a bit extreme and using an AFC of this threshold would preclude the use of presumably many fertile donors. Instead, we use this data to counsel our recipient couples parlaying the use of all markers of ovarian reserve including age, basal FSH, and AFC to guide us in our stimulation protocol. Ongoing pregnancy may be less with an AFC ≤ 14 , but if the recipient is willing to proceed with the cycle, we would cancel treatment if the oocyte donor fails to have a serum estradiol > 45 pg/ml after 4 days of ovarian stimulation (no pregnancy below this threshold). Conversely, if the donor's serum estradiol is > 600 pg/ml after 4 days of stimulation, then we caution about proceeding given the oocyte donor's risk of hyperstimulation. Lastly, we counsel against oocyte sharing in the face of AFC ≤ 13 .

In conclusion, AFC should be used as a measure of ovarian reserve testing in oocyte donation cycles and appears to be useful in guiding treatment and dosing protocols for oocyte donors. An absolute AFC, which should be program specific, can be used to predict clinical outcomes and counsel recipient couples regarding cancellation rates and clinical outcomes in selecting optimal oocyte donors and in considering proceed-

ing forward with oocyte retrieval. Further study is needed to verify these finding and assess if different dosing regimens based on AFC would impact outcomes.

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