Elevated circulating micro-ribonucleic acid (miRNA)-200b and miRNA-429 levels in anovulatory women

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Objective: To study the role of micro-RNA (miRNA)-200b and miRNA-429 in human ovulation and to measure their expression levels in ovulatory and anovulatory patients.
Design: Micro-RNA-200b and miRNA-429 expression analysis in human serum and granulosa cells at different phases of the ovulation cycle in normal cycling women and women undergoing assisted reproductive technology cycles.
Setting: University-affiliated hospital and academic research laboratory.
Patient(s): Forty women (7 normally ovulating, 15 normally ovulating with pure male infertility factor, and 18 with polycystic ovary syndrome) were included in this study.
Intervention(s): None.
Main Outcome Measure(s): The expression profile of circulating miRNAs and granulosa cells was assessed by means of real-time quantitative reverse transcription–polymerase chain reaction analysis.
Result(s): We identified miRNA-200b and miRNA-429 in the sera of all women tested. These miRNA expression levels were elevated during the early follicular phase of the cycle compared with serum levels during the early luteal phase. Anovulatory women, diagnosed with polycystic ovary syndrome, expressed significantly higher levels of miRNA-200b and miRNA-429 compared with spontaneously ovulating women. Ovulation induction with exogenous gonadotropins during an IVF cycle reduced these levels to the levels measured in normal ovulating women.
Conclusion(s): Our findings suggest an involvement of miRNA-200b and miRNA-429 in the pituitary regulation of human ovulation. Although it is unclear whether this altered miRNA expression profile is a cause or a result of anovulation, the levels of these molecules in the serum of anovulatory women may serve as serum biomarkers for the ovulation process. (Fertil Steril® 2017;107:269–75. ©2016 by American Society for Reproductive Medicine.)
Key Words: miRNA-200b, miRNA-429, ovulation, PCOS, pituitary, serum levels

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Ovulation of a mature and viable oocyte and the formation of a functional corpus luteum are essential for conception and the establishment of pregnancy. Numerous studies have shown that the LH surge initiates transcriptional up- and down-regulation of genes, including cytokines, transcription factors, and matrix-remodeling proteins within periovulatory granulosa cells (1). However, posttranscriptional gene regulation with respect to ovarian function, outside of oocyte development, has been largely overlooked.

Micro-RNAs (miRNAs) are endogenous small noncoding RNA molecules of approximately 23 nucleotides in length, which are known to form a novel class of regulatory determinants

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of cellular signaling pathways, with a large set of target messenger RNAs (mRNAs) proposed for every single miRNA (2). It has been postulated that modulation of a single miRNA might be an effective way to target entire pathways for clinical benefit (3, 4).

The role of miRNA in follicular signaling and ovulation has been described in various systems, but the specific miRNAs involved and their mechanism of action are yet to be revealed (5–7). Carletti and Christenson (8) demonstrated that 212 known miRNAs are expressed in mouse mural granulosa cells, and of these, 13 were regulated by the LH surge. Additionally, disruption of miRNA processing seems to result in altered ovarian morphology and gene expression (9). A granulosa cell–specific Dicer knockout showed an accelerated early follicular recruitment, increased follicular degeneration, and alterations in genes involved in follicular development, such as *Amh1*, *Cyp17a1*, and *Cyp19a1*. These studies demonstrated the potential role for miRNAs in follicular development and function (9).

A recent murine study demonstrated that miRNA-200b and miRNA-429, members of the miRNA 200 family, strongly affect ovulation in female mice (10). Double knockout mice lacking miRNA-200b and miRNA -429 displayed a defective hypothalamic–pituitary–ovarian axis and failed to ovulate (11). The involvement of the miRNA 200 family in humans has been tied to tumorogenesis, particularly in the epithelial to mesenchymal transition (12, 13). This process is an important component of corpus luteum formation, especially during the intense angiogenesis occurring after the LH signal of ovulation is received (14). Interestingly, when miRNA-200b and miRNA-429 double knockout mice were super-ovulated by injecting pregnant mare serum gonadotropin and hCG, the miRNA-200b and miRNA-429 double knockout females ovulated, similar to control females. This finding suggests that impaired hormonal regulation prevented these mice from ovulating naturally and that ovulation can be activated by exogenous hormones. Among the putative target mRNAs listed, the 3′-untranslated regions of both mouse and human *ZEB1* and *ZEB2* have the highest number of loci that are complementary to the seed sequence of miR-200b and miR-429 (15). Specific knockout of these miRNAs in the mice pituitary increased *ZEB1* mRNA expression (11). In the mouse, ZEB1 protein is known to be a nuclear repressor of the LH gene, leading to inhibition of LH transcription and abolishment of the LH surge, which is vital for ovulation (11, 16). The miR-200b cluster is present in humans, and the upstream regions of human *LHB* gene also contain ZEB1-binding sites (11). These similarities imply that the roles of these miRNAs in maintaining normal ovulation in the mouse may also be applicable to human reproductive physiology.

To investigate the role of these two miRNAs in human ovulation, we analyzed their expression pattern during ovarian stimulation for IVF in anovulatory and ovulatory women, as well as in naturally ovulating women. Because miRNAs could not be measured directly in the human pituitary, we measured their serum levels during the different phases of the ovulatory cycle according to the known stability and resistance to degradation of miRNAs in body fluids (17, 18). We also measured the expression levels of miR-200b and miR-429 in the target organ of the reproductive axis, in freshly isolated granulosa lutein cells (GLCs).

MATERIALS AND METHODS

Subjects

The Hadassah Hebrew University Medical Center Institutional Review Board approved this study (HMO-0110-09), and all subjects gave written informed consent to participate in the study. All women were under 35 years of age. The first group was composed of normally cycling women, with a regular menstrual cycle of 27–30 days, without recent use of hormonal treatment, history of pelvic inflammatory disease, or any other general health problem. Group 2 included normally cycling women undergoing IVF treatment solely because of male factor infertility (MFI). The anovulatory women (group 3) were diagnosed with polycystic ovary syndrome (PCOS) according to the Rotterdam revised criteria (19). These women underwent IVF treatment after failure to conceive with at least four cycles of controlled ovarian stimulation and insemination.

Ovarian Stimulation Protocol

Women undergoing IVF were treated by the short GnRH antagonist protocol. In brief, controlled ovarian stimulation was initiated on day 2 or 3 of a spontaneous cycle. An initial dose of 150–225 IU recombinant FSH (Gonal-F [Merck-Serono] or Puregon [MSD]) or highly purified hMG (Menopur [Ferring Pharmaceuticals]) was administered. From day 6 onward, the gonadotropin dose was estimated according to serum E_2 levels and a transvaginal ultrasound scan. When a leading follicle reached 13–14 mm, a GnRH antagonist (Cetrotide [Merck-Serono] or Orgalutran [MSD]) was administered at 0.25 mg/d. Final oocyte maturation was triggered with the use of 250 μg recombinant hCG (Ovitrelle [Merck-Serono]) as soon as the mean diameters of two follicles were $\geq$18 mm. Oocyte retrieval was scheduled 36 hours after hCG injection. All accessible follicles were harvested, and oocytes were collected from follicular aspirates and subjected to fertilization. The residual follicular fluid aspirates containing GLCs were collected for further investigation.

Specimens

Blood samples were collected at two time points during the ovarian cycle, and serum was aliquoted and immediately stored at −80°C until used. The first sample was collected on day 3 of the cycle, before any gonadotropin treatment was administered, representing the early follicular phase. The second sample was collected at the early luteal phase, around day 14 in the first group, or during ovum pick up in the IVF patient groups. Ovulation was determined in the first group by measurement of serum levels of P and LH. Granulosa lutein cells originating from follicular fluid aspirates were

collected for further investigation from all IVF recruited patients.

Human GLC Purification

Follicular fluids were collected and filtered through 40 μm cell strainer (BD Biosciences) in order to retain the granulosa cell clusters and remove smaller cells. Subsequently repeated cell washing with phosphate-buffered saline (PBS) and sedimentation with centrifugation are used to remove any residual red blood cells that are also treated cells with hemolytic buffer ACK solution (0.15 M NH4Cl, 1.0 mM KHCO3, and 0.1 nM EDTA) for 15 minutes at 37°C, as previously described (20). The cells were then washed three times with 10 mL of PBS (300 × *g*, 5 minutes), and the pellet was resuspended with 10 mL Dulbecco's Modified Eagle Medium/F12 medium (Biological Industries). Subsequently, GLCs were separated by 15 mL Lymphoprep (Axis-Shield) according to the manufacturer's protocol. Collected cells were transferred into a new tube and washed three times with PBS. Purified cells were immediately processed, and RNA was isolated.

Isolation of GLC RNA

Ribonucleic acid extraction from freshly isolated GLCs was performed using the mirVana isolation kit (Applied Biosystems, Life Sciences) protocol for total RNA isolation (including miRNAs) according to the manufacturer's instructions. Briefly, the granulosa cells pellet was resuspended in five volumes of lysis/binding buffer and 1/10 volume of miRNA homogenate additive and subsequently incubated on ice for 10 minutes. A low concentration of ethanol was added to the samples, which were subsequently mixed and bound to a filter cartridge by centrifugation. In the next step, a higher concentration of ethanol was added to the eluent, allowing the shorter RNAs to be immobilized to filter cartridges during centrifugation and subsequently eluted. Ribonucleic acid elution was performed in 50 μL elution buffer. Ribonucleic acids were stored at −80°C until used.

Isolation of Serum RNA

Ribonucleic acid from serum was extracted using the miRNeasy serum/plasma kit (Qiagen) according to the manufacturer's instructions. Briefly, 450 μL of serum were thawed on ice and centrifuged at 16,000 × *g* and at 4°C for 10 minutes to remove cellular debris. Thereafter 2 mL of QIAzol lysis reagent were added to 400 μL of supernatant. After incubation for 5 minutes, 25 fmol of exogenous synthetic cel-miR-39 (*Caenorhabditis elegans* cel-miR-39) (Qiagen) were spiked in the samples (before the addition of chloroform). Total RNA including small RNA was extracted using column-based purification according to the miRNeasy serum/plasma kit manufacturer's instructions and eluted in 25 μL of nuclease-free water. Ribonucleic acids were stored at −80°C until used. In humans the cel-miR-39 sequence does not exist, and thus its addition allows controlling sample-to-sample variation of the RNA isolation efficiency (21–24). Previous studies demonstrated that synthetic spike-in miRNA performs better for normalizing sample input than the most stably expressed endogenous miRNAs because its abundance is less affected by sample variations (25).

Reverse Transcription

In all samples, complementary DNA (cDNA) was synthesized using the TaqMan miRNA reverse transcription (RT) kit and miRNA-specific stem-loop primers (Applied Biosystems, Life Sciences) in 15 μL of total volume reaction. In the case of serum RNA, each reaction consisted of 3 μL of eluted RNAs and 12 μL of master mix (6.16 μL of nuclease-free H_2O, 3 μL of TaqMan miRNA RT-specific primer, 1.5 μL of RT buffer, 0.19 μL of 20 U/μL RNase inhibitor, 0.15 μL of 100 mmol/L deoxynucleotide Triphosphates, and 1 μL of 50 U/μL MultiScribe Reverse Transcriptase [Applied Biosystems, Life Sciences]). In the case of GLC RNA, 60 ng of total RNA per GLC sample (in a volume of 3 μL) were reverse transcribed into cDNA using the Multiscribe RT System (Applied Biosystems, Life Sciences). The RT reaction mixture was incubated at 16°C for 30 minutes, at 42°C for 30 minutes, and at 85°C for 5 minutes and then was held at 4°C. A no-RT negative control was included in each experiment to ensure that the polymerase chain reaction (PCR) products were not due to contamination by genomic DNA. The negative control produced no detectable signal in any of the experiments.

Quantitative RT-PCR

The miR-429 and miR-200b levels were quantified using TaqMan miRNA assays (Applied Biosystems, Life Sciences), according to the manufacturer's protocols. Quantitative RT-PCR was performed in a final volume of 20 μL containing 3 μL of cDNA template (serum samples), 6 μL of nuclease-free water, 1 μL of 20× primer/probe mix from the TaqMan miRNA assay, and 10 μL of 2× TaqMan Universal Master Mix II, no UNG (Applied Biosystems, Life Sciences). In the case of GLC, 1.3 μL of cDNA template was used for each GLC sample. All the reactions were run in duplicate on the StepOnePlus system (Applied Biosystems) under the following cycling conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Cycle threshold (Ct) values were calculated using StepOne Software version 2.3 (Applied Biosystems). Template-free controls for both RT and PCR were included in each experiment to ensure target-specific amplification. In the case of granulosa cells, the expression level of snRNA U6 was used as the internal expression normalization reference for the target miRNAs.

Expression levels of serum miRNAs were normalized to those of cel-miR-39 and determined by the $2^{-\Delta Ct}$ method in which ΔCt was calculated as follows: ΔCt = Ct (miRNA of interest) − Ct (cel-miR-39) (21, 22, 24–27).

Statistical Analysis

Statistical analyses were performed with IBM SPSS version 19. Background parameters of PCOS and MFI subjects were compared with the Mann-Whitney *U* test. Serum miRNA results were compared among all three groups, between PCOS and MFI IVF patients, PCOS and ovulatory women and normally cycling women either or not undergoing IVF with the

TABLE 1

Clinical characteristics of PCOS and MFI patients.

Characteristic	PCOS (n = 18)	MFI (n = 15)	*P* value
Age (y)	26.9 ± 4.3	26.8 ± 4.7	NS
BMI (kg/m^2)	29.3 ± 7.1	23.6 ± 3.3	.03
Day-3 FSH (IU/L)	5.3 ± 1.4	6.2 ± 1.9	NS
Day-3 LH (IU/L)	8.25 ± 3.47	7.13 ± 5.41	NS
Peak E_2 (pmol/mL)	7,273 ± 4,661	8,620 ± 3,447	NS
Oocytes retrieved (n)	11 ± 7	13 ± 5	NS

Note: Values are reported as mean ± SD. NS = nonsignificant.

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Kruskall-Wallis test. The Wilcoxon test was applied to compare control subjects' day-3 vs. day-14 miRNA outcomes. Analyses were two-sided, and exact significance applied as appropriate. *P* values of $<.05$ were considered statistically significant.

RESULTS

Clinical Characteristics

Forty women (7 normally ovulating, 15 normally ovulating with pure MFI, and 18 with PCOS) were recruited to this study. The clinical characteristics of the anovulatory and MFI participants in the study are summarized in Table 1. Age and FSH level on early follicular phase were comparable between the groups, yet body mass index (BMI) was significantly higher in the PCOS women than in the MFI group (29.3 ± 4.3 kg/m^2 vs. 23.6 ± 3.3 kg/m^2, respectively). Average basal LH levels in PCOS patients was slightly elevated compared with the MFI group, but did not reach significant difference (8.25 ± 3.47 IU/L vs. 7.13 ± 5.4 IU/L, respectively).

Cycle characteristics, including E_2 levels on the day of hCG injection and the number of oocytes retrieved, were similar in the two IVF groups, indicating similarly controlled ovarian hyperstimulation protocol.

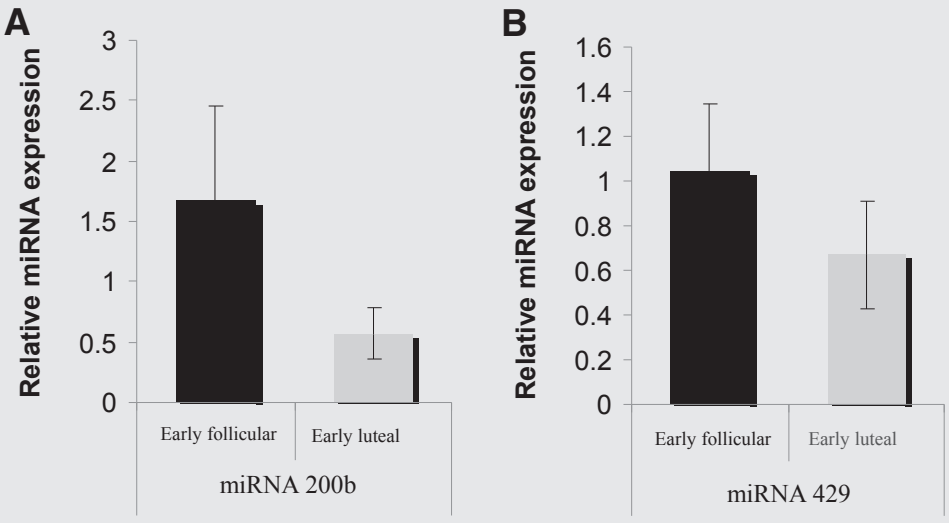
miRNA-200b and miRNA-429 Expression during the Normal Ovarian Cycle

The expression levels of miRNA-200b and miRNA-429 in the serum of the nontreated normal ovulatory women during the ovarian cycle were established through RNA analysis of serum from normally ovulating women. Micro-RNA-200b and miRNA-429 expression levels in the serum demonstrated a different pattern in the transition from early follicular phase (cycle day 3) to early luteal phase. Whereas the expression of both miRNAs was relatively low (Ct >30), miRNA-200b relative expression levels (RQ) were found to be approximately 60% higher (1.7 ± 0.8) than miRNA-429 during the early follicular phase (1.05 ± 0.33) (Fig. 1A and B). Upon ovulation there was a decrease in the expression level of both miRNAs: miRNA-200b RQ during early luteal phase dropped from 1.67 (±0.79) at the early follicular phase to 0.57 (±0.21). At the same time, miRNA-429 relative expression levels dropped from 1.05 (±0.3) at the early follicular phase to 0.67 (±0.3) in the early luteal phase.

miRNA-200b and miRNA-429 Expression in Anovulation

To explore a possible involvement of miRNA-200b and miRNA-429 in human ovulation dysfunction, the miRNA-200b and miRNA-429 expression profile of anovulatory patients was compared with that of ovulatory women undergoing IVF (MFI group). Serum analysis at the early follicular

FIGURE 1



Relative expression of circulating miRNA-200b and miRNA-429 in normal ovulating women. Expression levels of miRNA-200b (**A**) and miRNA-429 (**B**) in serum at day 3, early follicular phase (n = 7) and day 14, early luteal phase (n = 7) of the ovarian cycle were analyzed by quantitative RT-PCR and normalized to cel-miR-39. Error bars indicate ±SEM.

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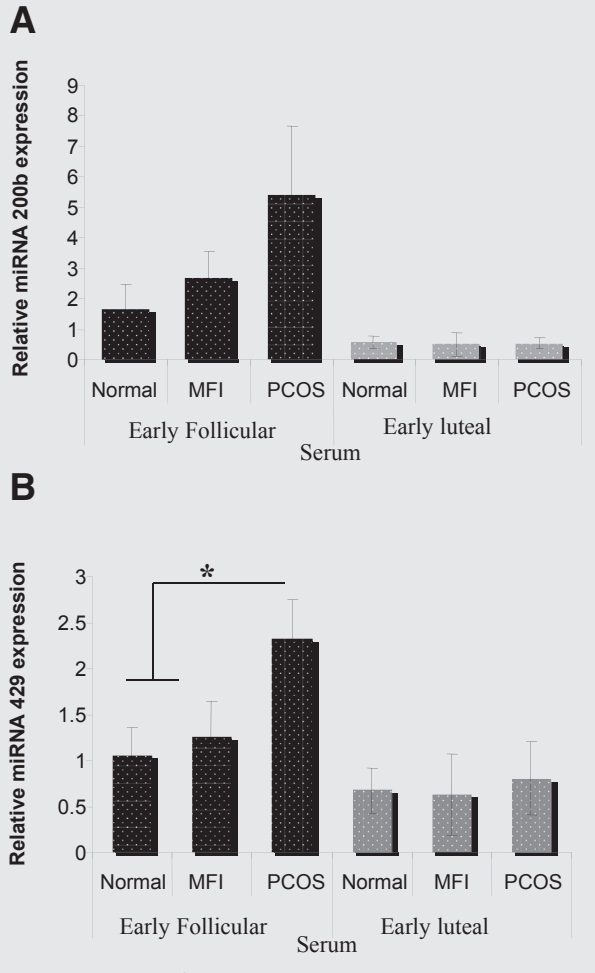
phase revealed elevated expression of both miRNA-200b (Fig. 2A) (P=.06) and miRNA-429 (P=.002) (Fig. 2B) in PCOS patients before treatment. Specifically, serum miRNA-429 was relatively lower than miRNA-200b at the early follicular phase of the ovulatory phase but was twice as high in PCOS (average 2.3 ± 0.4) than in the regularly ovulatory IVF and non-IVF women (1.2 ± 0.4 [P=.002] and 1.0 ± 0.4 [P=.002]) (Fig. 2B).

The miRNA-200b expression demonstrated the same trend but did not reach statistical significance. The miRNA-200b expression was more than twice as high in PCOS patients (average 5.4 ± 2.3) than in any of the normally ovulating IVF and non-IVF groups (1.6 ± 0.8 [P=.06] and RQ 2.3 ± 0.9 [P=.005]) (Fig. 2A).

Thirty-six hours after ovulation induction, the expression levels of both miRNAs were considerably decreased in all groups analyzed (Fig. 2A and B). The same pattern of low expression levels was observed for miRNA-200b and miRNA-429 (Fig. 2A and B). The suppression of both miRNA-200b and miRNA-429 expression at the early luteal phase was significantly more pronounced in the anovulatory patients; the relative level of miRNA-200b dropped from 5.4 (±2.3) at early follicular phase to 0.54 (±0.16), and that of miRNA-429 dropped from 2.3 (±0.4) to 0.8 (±0.4) at early luteal phase.

FIGURE 2



Relative expression of miRNA-200b and miRNA-429 in anovulatory patients with PCOS, MFI, and in normal subjects. Expression levels of miRNA-200b (**A**) and miRNA-429 (**B**) in serum at early follicular phase (n = 6) and 36 hours after LH surge (n = 5) were analyzed by quantitative RT-PCR and normalized to cel-miR-39. Error bars indicate ±SEM. *P<.05.

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miRNA-200b and miRNA-429 Expression in GLCs

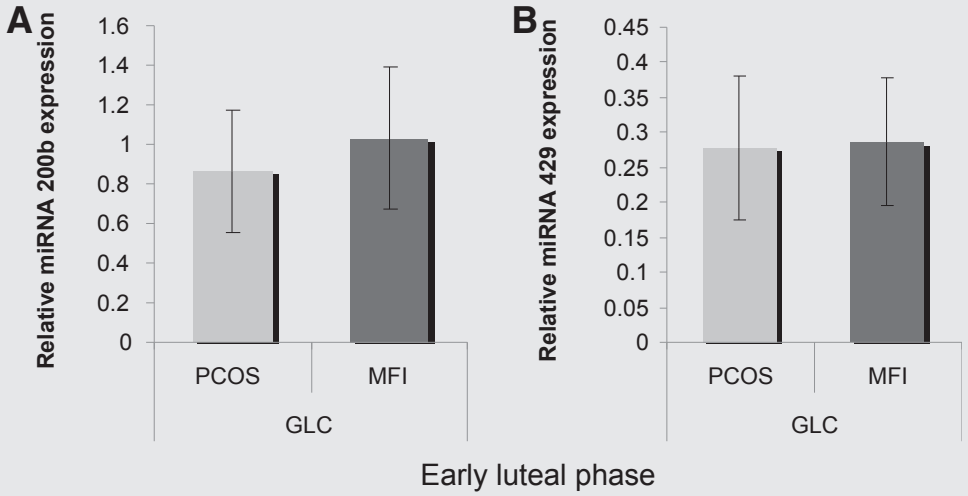
Although the expression profile of both miRNAs in the serum may be a reflection of their expression in the pituitary, which cannot be measured directly in humans, we sought to assess their expression in the target organ, the ovary. Because granulosa cells in the ovarian follicle respond to gonadotropin signaling and are involved in the processes of folliculogenesis and oocyte maturation, the expression profile of both miRNA-200b and miRNA-429 was also analyzed in GLCs from both IVF-treated groups (anovulatory and ovulatory). Very low expression levels were detected for the two miRNAs in granulosa cells, with miRNA-200b expression relatively higher (1.03 ± 0.36) than miRNA-429 (0.28 ± 0.1). No significant expression difference of any of these miRNAs was identified in the GLCs of the two groups analyzed (Fig. 3A and B).

DISCUSSION

Our study showed the expression pattern of miRNA-200b and miRNA-429 in human serum during naturally occurring and induced ovulation cycles. To our knowledge (literature search on August 30, 2016 using keywords "miRNA-200b," "miRNA-429," "serum," "ovulation"), this is the first record of the expression of miRNA-200b and miRNA-429 in human serum through the ovulation cycle. Our results show that at two time points during normal ovulatory cycles, miRNA-200b and miRNA-429 expression is decreased from the early follicular phase (time point 1) toward the early luteal phase (time point 2). This may imply a role for these miRNAs in human ovulation. These findings correlate with a recent study in mice (11) showing the expression of these miRNAs in the mouse pituitary. In mouse disrupted gene knockout of miRNA-200b and miRNA-429, the females were sterile and regained their fertility only after superovulation with exogenous gonadotropins. These findings elaborate the hypothesis that these miRNAs act as regulators of hormonal pituitary function. Thus, we postulated that PCOS as a typical condition of central dysregulation may show a disrupted miRNA expression profile, which leads to anovulation.

In accordance with this speculation, we revealed a distinct difference in the expression pattern of these two miRNAs in the serum of anovulatory (PCOS) women at early follicular phase. The higher levels of miRNA-200b and miRNA-429 in anovulatory women dropped after gonadotropin stimulation and after ovulation induction to levels comparable to those in ovulatory women, showing a similar restoration of ovarian function as that observed in mice (11). No difference in these miRNA levels were noted in the human GLCs of ovulatory and anovulatory women, hence

FIGURE 3



Relative expression of miRNA-200b and miRNA-429 in GLCs from anovulatory women (PCOS) (n = 11) and from ovulatory women (MFI) (n = 8) undergoing IVF. Expression levels of miRNA-200b (**A**) and miRNA-429 (**B**) in GLCs, 36 hours after LH surge were analyzed by quantitative RT-PCR and normalized to U6. Error bars indicate ±SEM.

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supporting the hypothesis of central rather than ovarian miRNAs regulation in humans.

In recent years, several studies have indicated that miRNAs play critical roles in almost all ovarian biological processes, including ovulation, folliculogenesis, follicular atresia, and luteal development (28–31) Previous reports demonstrated that specific deletion of Dicer, which led to absence of miRNA synthesis in gonadotropin-producing cells, resulted in altered gonadotropin homeostasis, leading to reproductive dysfunction (32). Knockout mice lacking Dicer in their gonadotropin-producing cells were rescued with supplementation of exogenous hormones. This work supports the assumption that fertility defects that evolved from reduced miRNA activity are secondary to suppressed gonadotropin production and action (32). Thus, the identification of specific miRNAs that selectively target a regulatory mRNAs, such as of the ZEB family, may provide in the future a novel in vivo therapeutic tool to manipulate pituitary gonadotropin secretion.

Our data also show a difference in the BMI between ovulatory and anovulatory patients. It is unclear whether the BMI itself affects miRNA-200b and miRNA-429 levels. A possible mechanism that associates miRNA-200b and miRNA-429 overexpression, anovulation, obesity, and insulin resistance was proposed in a recent study in mice (33), showing that miR-200b and miR-429 expression is leptin-dependent. The authors showed that hypothalamic silencing of miR-200 increased the expression level of the leptin receptor and insulin receptor substrate 2, caused a reduction in body weight gain, and restored liver insulin responsiveness in the female mice (33) Thus, blocking these two specific miRNAs may, at least partially, overcome the leptin- and insulin-resistant phenotype. This finding in mice echoes in humans, where high levels of miRNA-200b and miRNA-429 are now linked to anovulation, obesity, and insulin resistance typical of PCOS. Future studies should compare miRNA-200b and miRNA-429 levels in lean and obese women with PCOS not currently undergoing gonadotropin stimulation.

Our study has some important limitations. We measured the miRNA levels in serum of anovulatory women at only two time points; before starting ART treatment and after induced ovulation with exogenous gonadotropins. Therefore we cannot extrapolate the full miRNA profiles throughout the cycle. Treatment was initiated in anovulatory women according to their hormonal profile, with no relation to the exact date of the menstruation or duration of anovulation. Although miRNA expression profiles may vary with the duration of anovulation, one may assume high miRNA-200b and miRNA-429 levels in anovulatory states. We suggest here that noncyclic hormonal profile is associated with the elevated levels of miRNA-200b and miRNA-429 that decrease upon ovulation. Although the results of this study suggest a temporal association between changes in miRNA-200b and miRNA-429 levels and ovulation, the data presented are not sufficient to support a causal mechanism between dropping miRNA-200b and miRNA-429 levels and ovulation. We also cannot prove a causative role between elevated miRNA-200b and anovulation.

Direct measurement of miRNA production by the human pituitary is impossible in vivo; therefore, we used the unique feature of miRNA stability in the serum and measured serum levels of the miRNAs as an alternative that may reflect pituitary expression. Circulating extracellular miRNAs are considered important players and promising biomarkers for a variety of physiologic and disease processes (17,18,34,35). The elevated serum levels of miRNA-200b and miRNA-429, even without the certainty of their tissue origin, may be

significant and their temporal change with the ovulation cycle remarkable.

In conclusion, our study identified a pair of miRNAs, 200b and 429, in human serum with a temporal downward change during the normal ovulatory cycle. Whereas in anovulatory women these miRNAs were overexpressed and their high levels dropped only after administration of exogenous gonadotropins. This may reflect a pituitary dysregulation of ovulation in PCOS patients. We suggest that these miRNAs are involved in ovarian function via a mechanism of pituitary control and may serve as serum biomarkers for the ovulation process.

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