High levels of mineralocorticoids in preovulatory follicular fluid could contribute to oocyte development

Rita Sneeringer, M.D.,[a,b,c] *Alan S. Penzias, M.D.,*[a,b,c] *Brent Barrett, Ph.D.,*[a] *and Anny Usheva, Ph.D.*[b]

[a] Beth Israel Deaconess Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Boston; [b] Department of Medicine, Division of Endocrinology, Harvard Medical School, Boston; and [c] Boston IVF, Waltham, Massachusetts

Objective: To identify aldosterone and precursors in ovarian follicles and to relate levels of mineralocorticoids to previously described renin-angiotensin system follicular fluid content.
Design: Experimental.
Setting: Academic laboratory and affiliated large private practice.
Patient(s): Women undergoing oocyte retrieval for in vitro fertilization (IVF).
Main Outcome Measure(s): The concentrations of mineralocorticoids were measured in plasma and follicular fluid. Granulosa cell mRNA expression and oocyte receptor content were evaluated.
Result(s): High concentrations of preovulatory aldosterone and corticosterone were measured in follicular fluid (419.5 ± 122.2 and 218,383 ± 124,143 pg/mL, respectively). Increased mineralocorticoid levels are found in follicular fluid compared with in plasma and in large follicles compared with in small. Plasma aldosterone levels increase before ovulation. Granulosa cell gene expression that promotes aldosterone production and accumulation of corticosterone is increased in younger patients. Aldosterone receptors are localized to the surface of human oocytes.
Conclusion(s): High levels of aldosterone and its precursor, corticosterone, were found in ovarian follicles. This combined with the presence of aldosterone receptors on oocytes suggests a possible role for aldosterone in oocyte development. (Fertil Steril® 2011;95:182–7. ©2011 by American Society for Reproductive Medicine.)

Key Words: Follicular fluid, aldosterone, corticosterone, mineralocorticoids, ovary, ovarian, human, renin-angiotensin system

The follicle within the human ovary is designed to store, nurture, and release an oocyte capable of fertilization with the resultant creation of a human life. While some molecular pathways are clearly described, others remain to be deciphered. One example is the ovarian renin-angiotensin system (OvRAS). Extensive research has described high follicular fluid content (1–5) and peak levels in the periovulatory time period (6–8) of several OvRAS components. Evidence for local action includes the presence of angiotensin II receptors in bovine ovarian follicles (9) and the in vitro production of renin and prorenin from human theca cells cultures (10, 11). OvRAS components may regulate angiogenic factors, which are integral to the development of normal ovulatory follicles. Follicular fluid levels of renin and prorenin correlate with development of fertility competent oocytes (12), likely via production of angiotensin II, an angiogenic factor and stimulator of vascular endothelial growth factor. In patients with ovarian hyperstimulation syndrome, an example of exaggerated angiogenesis, significantly elevated renin and angiotensin II levels are reported in both plasma and ascites fluid (13). At the opposite extreme, polycystic ovary patients have lower levels of follicular prorenin (14, 15) and generally have poorer oocyte quality (16). Thus, there are several strong correlations described in the literature suggesting an important role for OvRAS in folliculogenesis and oocyte development.

However, all of the active components of the OvRAS may not have been explored. Outside of the ovary, the renin-angiotensin pathway is intimately linked to the production of aldosterone. Primate studies have suggested the presence of aldosterone precursors and aldosterone pathway enzyme activity in the ovary (17). To our knowledge, the presence of ovarian mineralocorticoids has not been previously evaluated in humans. We hypothesized that aldosterone could also be an important active component of the OvRAS. In this study, we report the presence of aldosterone and its obligate precursor, corticosterone, within the preovulatory human ovarian follicle. We propose that aldosterone may play an important role in folliculogenesis and contribute to optimizing the environment for normal oocyte development.

MATERIALS AND METHODS

Institutional Review Board Approval/Site

This study was approved by the Beth Israel Deaconess Medical Center Institutional Review Board (IRB). All patients were treated at Boston IVF, a large infertility clinic affiliated with Beth Israel Deaconess Medical Center. Informed consent was obtained from all subjects at the time of oocyte retrieval during an IVF cycle that provided granulosa cells (GCs), follicular fluid aspirates, and plasma. The IRB waived consent for the study of deidentified discarded oocytes.

Subjects

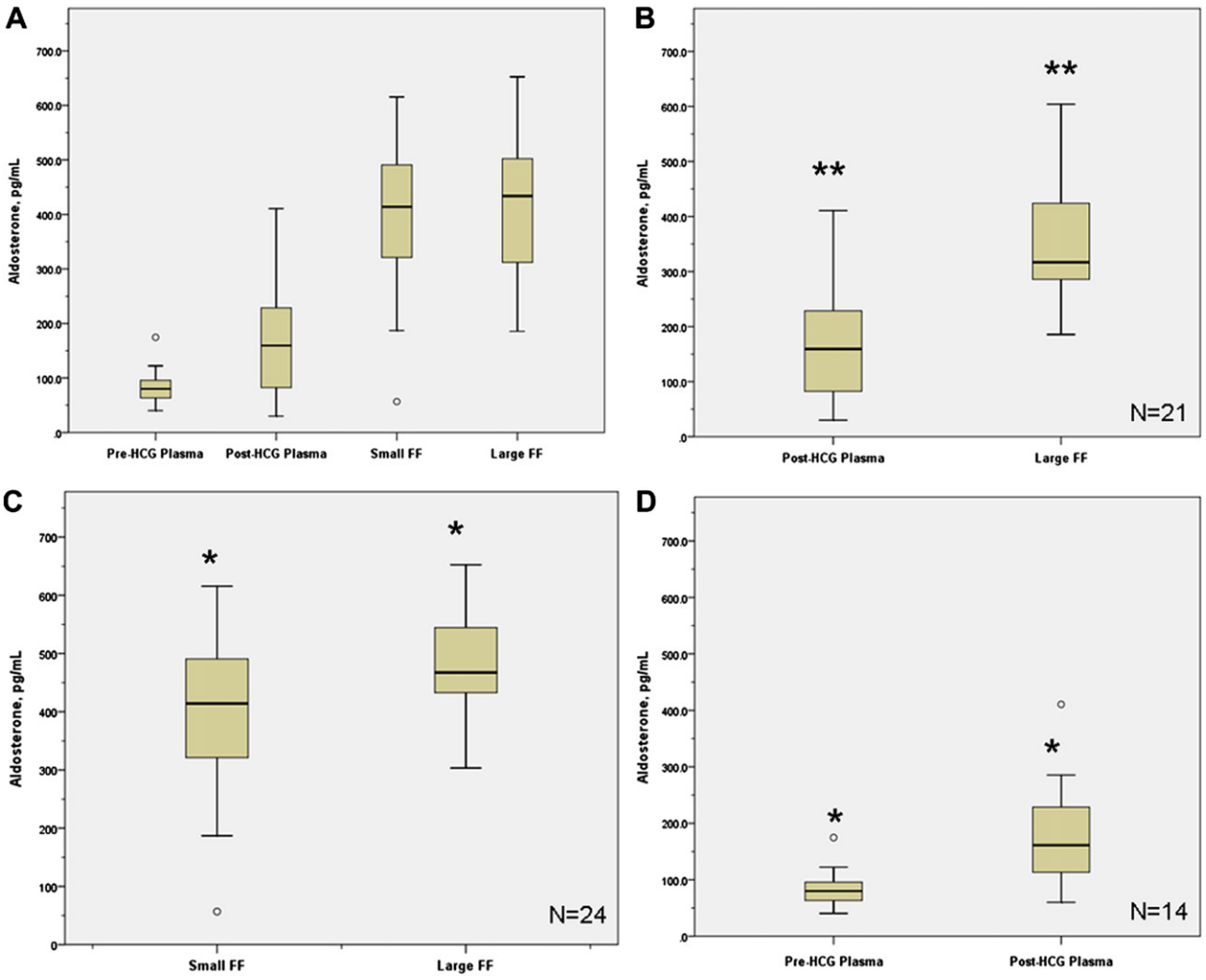
Patients undergoing oocyte retrieval for the purpose of IVF were eligible to participate (n = 44). Patients were categorized as reproductively young if

Received September 21, 2009; revised May 18, 2010; accepted June 15, 2010; published online August 2, 2010.
R.S. has nothing to disclose. A.S.P. has nothing to disclose. B.B. has nothing to disclose. A.U. has nothing to disclose.
This study was supported by Beth Israel Deaconess Medical Center (Internal).
Presented at the American Society for Reproductive Medicine 2007 meeting, which was held in Washington, D.C., and SGI 2007, which was held in Reno, Nevada.
Reprint requests: Anny Usheva, Ph.D., Harvard Medical School, Beth Israel deaconess Medical Center, 3 Blackfan Circle, CLS-701, Boston, Massachusetts 02115 (FAX: 617-735-2627; E-mail: ausheva@bidmc.harvard.edu).

Copyright ©2011 American Society for Reproductive Medicine, Published by Elsevier Inc.
0015-0282/$36.00
doi:10.1016/j.fertnstert.2010.06.028

FIGURE 1

Aldosterone (Aldo) content in follicular fluid (FF) and plasma samples is measured by EIA and presented in standard box plot format. All samples are presented in Figure 1A, and paired comparisons are presented in Figures 1B–1D. Paired *t*-tests were used for all comparisons. Significant differences are labeled with asterisks: $^{**}P<.001$ and $^{*}P=.001$.



Sneeringer. Aldosterone in human ovarian follicular fluid. Fertil Steril 2011.

under 35 years of age and reproductively old at age 40 years or more. All patients underwent controlled ovarian hyperstimulation as described elsewhere (18). Follicle aspirates from two to three large follicles (>16 mm) and two to three small follicles (<10 mm) were collected, processed, and stored separate from one another. All other follicle aspirates were pooled within study subjects. Plasma samples from discarded blood collection before administration of hCG (pre-hCG) and on the day of oocyte retrieval (post-hCG) were obtained when possible.

Follicular Fluid, Plasma, and GC Processing

Follicular fluid and plasma samples were processed as described elsewhere (18). GCs were isolated from the cellular pellet remaining after follicular fluid centrifugation. This pellet was resuspended in 0.1% trypsin solution, incubated for 10–15 minutes, and centrifuged for 20 minutes on a 45% PureCeption gradient. The GC rich layer was suspended in phosphate-buffered saline and archived at −80°C after the adding Qiagen RLT buffer with beta-mercaptoethanol. The mRNA purification was completed as per the Qiagen RNeasy Mini protocol (Valencia, CA).

Aldosterone and Corticosterone Quantification

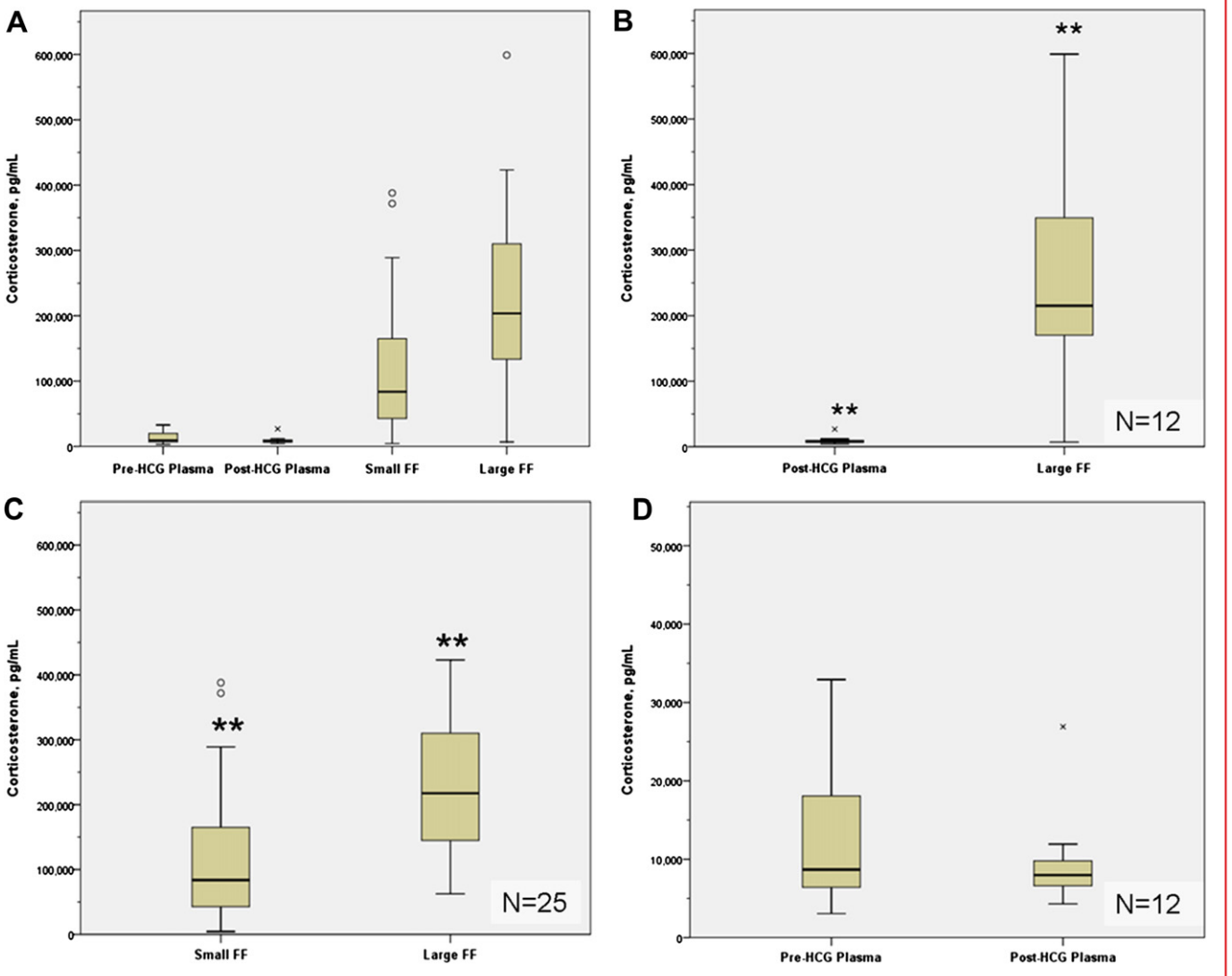
Commercially available enzyme linked immunoassays (EIA) were used to quantitate aldosterone (Alpco Diagnostics, Salem, NH) and corticosterone (Cayman Chemical Company, Ann Arbor, MI) concentrations in follicular fluid and plasma. Controls and standards were analyzed in accordance with manufacturer recommendations and met the criteria for quality assurance. All samples were analyzed in duplicate. For the corticosterone EIA, samples were diluted (samples 1:2500, high performance liquid chromatography [HPLC] fractions 1:10) to achieve concentrations in the center of the standard curve.

Determination of Free and Bound Aldosterone Content

Follicular fluid samples from a subset of patients with a sufficient availability of pooled fluid were fractionated by HPLC (Pharmacia, Sweden) on a Superose 6 size exclusion column. Particle molecular weight of the molecules and their complexes was determined by light scattering using the supplier's software (Wyatt Technology, Santa Barbara, CA). Estimation of free and bound aldosterone content was based on the estimated molecular mass of particles within specific fractions combined with the content of aldosterone as

FIGURE 2

Corticosterone (CS) content in follicular fluid (FF) and plasma samples is measured by EIA and presented in standard box plot format. All samples are presented in Figure 2A, and paired comparisons are presented in Figures 2B–2D. Paired *t*-tests were used for all comparisons. Significant differences are labeled with asterisks: $^{**}P < .001$.



Sneeringer. Aldosterone in human ovarian follicular fluid. Fertil Steril 2011.

determined by EIA (see above). Unbound aldosterone eluted in the last fractions, corresponding to a molecular mass 360 Da. Patients who ultimately became pregnant were compared with those who did not achieve a pregnancy.

Glanulosa Cells mRNA Microchip Analysis

Total mRNA from pooled GCs of three young and three old patients were compared on two identical microarrays (Agilent Human Genome CGH Microarray 44K). The microarray methods were described elsewhere (19). GeneSpring software (Agilent) was used to generate lists of selected genes that differed at least twofold between the study groups. Ingenuity Systems Pathway Analysis Software (Redwood City, CA) was used to interpret the microchip data. Specifically, the software identified gene pathways that were significantly different between our two groups, which included pathways that affect mineralocorticoid content.

Oocyte Immunohistochemistry

Nonfertilized oocytes with intact zona pellucida were fixed with 4% paraformaldehyde and washed with 0.1% Tween/0.05% Saponin solution. Surrounding GCs were removed during these washing steps. The oocytes were blocked with a 5% horse serum/0.1% Tween/0.05% Saponin solution. Primary antibodies were applied overnight at 4°C: Abcam (Cambridge, MA); angiotensin II receptor 1 (AIIR1, mouse monoclonal clone H3122, 1:20 dilution); and ABR aldosterone receptor (AldoR, mouse monoclonal clone H10e4c9f, 1:100 dilution). Control oocytes were incubated in filtered washing solution. After another series of washes, all oocytes were incubated at room temperature with fluorescently labeled secondary antibody (Alexa 430 nm, 1:200 dilution) and Hoechst stain (1:5000 dilution) for 2 hours. Fluorescence was then assessed by fluorescence microscopy (Zeis LM, Germany).

Statistical Analysis

SPSS version 15.0 (Chicago, IL) software was used for statistical analysis. The following parametric tests were used after confirming a normal distribution of data: the paired *t*-test for comparison of aldosterone or corticosterone content among patients who had multiple samples measured and the Pearson correlation coefficients for comparison of associations of aldosterone

TABLE 1

mRNA gene expression of regulators of the aldosterone pathway.

Abbreviated gene name	Gene name	Fold change	Gene function
Aldosterone production			
AGTR1	Angiotensin II receptor 1	−3.058	Necessary for the production of aldosterone via angiotensin II
ALOX12	Arachidonate 12-lipooxygenase	−6.897	Protein increases aldosterone production by activating CYP11B2 (aldosterone synthase)
AMZ2	Archaelysin family metallopeptidase 2	+3.210	Increases hydrolysis of angiotensin III
MMELI	Membrane metallo-endopeptidase-like 1	−7.576	Increased hydrolysis of angiotensin I
WNT4	Wingless-type MMTV integration site	−3.390	KO mouse decreases aldosterone production (via CYP11B2 and STAR)
Corticosterone quantity			
APOE	Apolipoprotein E	+3.616	Apo E KO increases corticosterone
CYP19A1	Aromatase	+2.560	Protein increases corticosterone
HSD11B1	Hydroxysteroid, dehydrogenase 11 Beta 1	+4.178	KO mouse increases corticosterone
MECP2	Methyl CpG binding, protein 2	+1.100	Mutant MECP2 increases corticosterone
NPY2P	Neuropeptide Y receptor	+4.560	NPY KO increases corticosterone
	Nuclear receptor 3C1	+4.570	Mutant or KO NR3C1 increases corticosterone
SCG5	Secretogranin V	+5.344	KO mouse increases corticosterone

Note: Fold changes are shown with young patients as the reference group: "+" for increased mRNA expression and "−" for decreased mRNA expression in old patients. KO = knockout.

Sneeringer. Aldosterone in human ovarian follicular fluid. Fertil Steril 2011.

concentration with demographic and outcomes variables. Significance was at a level of $\alpha = .05$.

RESULTS

High Concentration of Aldosterone in Preovulatory Follicles

Forty-four patients had follicular fluid from large follicles analyzed for aldosterone. Among these 44 patients, additional samples were obtained and analyzed for aldosterone content: small follicles (24 patients), pre-hCG plasma (14 patients), and post-hCG plasma (21 patients). Paired comparisons (paired *t*-test for all paired comparisons) of samples revealed significantly higher mean concentrations of aldosterone in large follicles compared with in both pre-hCG (339.5 ± 115.6 vs. 85.5 ± 33.9 pg/mL, $P<.001$, n = 14 pairs) and post-hCG plasma (343.5 ± 107.8 vs. 159.4 ± 96.7 pg/mL, $P<.001$, Figure 1B, n = 21 pairs). A small, but statistically significant elevation of aldosterone content was seen in large follicles compared with in small follicles (485.4 ± 86.4 vs. 397.7 ± 137.4 pg/mL, $P=.001$, Figure 1C, n = 24 pairs). Finally, plasma aldosterone concentration was found to increase after administration of hCG (85.5 ± 33.9 vs. 179.2 ± 92.5 pg/mL, $P=.001$, Figure 1D, n = 14 pairs). The aforementioned comparisons are shown in standard box plot format in Figure 1 to illustrate the distribution of data and to highlight clear differences in aldosterone content between samples. Of note, the aldosterone level in follicular fluid was markedly higher than the level in the matched plasma samples. The aldosterone content in the follicular fluid was also found to be markedly higher than the plasma reference range (mean, 105 pg/mL; range, 25–315 pg/mL). The content in the plasma samples was found to be generally within the range.

Aldosterone level weakly correlated with some demographic and outcome variables. Higher levels of aldosterone were associated (Pearson's correlation coefficient for all associations) with younger age ($R = -0.185$), greater body mass index ($R = 0.286$), higher number of oocytes retrieved ($R = +0.179$), and higher peak E_2 levels ($R = +0.111$). Plasma and follicular fluid aldosterone levels were strongly correlated ($R = +0.881$).

High Concentration of Corticosterone in Preovulatory Follicles

Corticosterone content in the follicular fluid paralleled the aldosterone findings above. Forty patients had follicular fluid samples from large follicles analyzed for presence of the aldosterone precursor corticosterone. Among these 40 patients, additional samples were assayed for corticosterone content in small follicles (25 patients), pre-hCG plasma (14 patients), and post-hCG plasma (12 patients). The mean concentration of corticosterone was significantly increased in the large follicles compared with in matched pre-hCG plasma (245,693 ± 161,774 vs. 12,170 ± 8,790 pg/mL, $P<.001$, n = 14 pairs) and post-hCG plasma samples (245,693 ± 161,774 vs. 9,404 ± 5,921 pg/mL, $P<.001$, Figure 2B, n = 12 pairs). The large follicles had significantly more corticosterone than the small follicles (220,364 ± 92,812 vs. 139,571 ± 114,318 pg/mL, $P<.001$, Figure 2C, n = 25 pairs). There was no difference in corticosterone content between pre-hCG and post-hCG plasma samples (12,170 ± 8,790 vs. 9,404 ± 5,921 pg/mL, $P=.318$, Figure 2D, n = 12 pairs). As with aldosterone, corticosterone concentration in the follicular fluid was higher than the reference range (900–3,900 pg/mL). The median corticosterone concentration in follicular fluid was approximately 50 times the upper limit of the reference range, and median plasma concentration was approximately double the upper limit.

Free and Bound Aldosterone Increased in Pregnant Patients

The free and bound content of aldosterone among three patients who achieved a pregnancy was increased compared with between two

patients who did not achieve a pregnancy for the majority of eluted fractions. Specifically, the profile for the pregnant group had a higher proportion of aldosterone bound to large complexes (fractions with estimated molecular mass of 213–451 kDa) and higher free aldosterone (fraction with estimated molecular mass of 360 Da) compared with nonpregnant patients. The mean age, number of follicles, and cycle number were similar for the two groups, but peak E_2 was higher in the pregnant group (pregnant: 35 years, 7.7 follicles, 2.3 cycles, 2,266.7 pg/mL; not pregnant: 33 years, 7.5 follicles, 2.0 cycles, 1,051 pg/mL).

Granulosa Cells mRNA Analysis

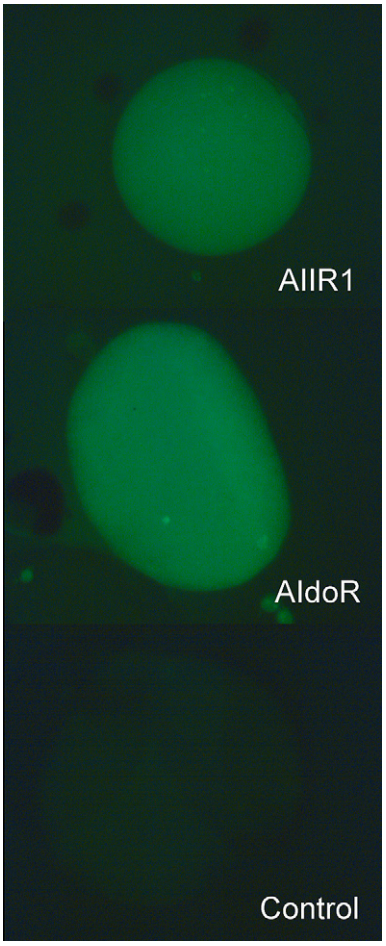
The total RNA from GCs of three young and three older patients was compared by a differential gene expression profiling by microgene chip analysis. Comparing the level of mRNA content in young versus old patients favored production of aldosterone and the accumulation of corticosterone in young patients. The magnitude of changes and the specific gene functions (20–34) for differentially expressed mRNAs are summarized in Table 1. Of note, the mRNA expression in the corticosterone quantity pathway is primarily associated with genes that inhibit accumulation. The higher mRNA content of these genes in old patients is therefore associated with decreased corticosterone accumulation. These data support the hypothesis that specific coordination in the level of expression of genes that enhance aldosterone production and corticosterone accumulation occurs in the young patients who have the best fertility prognosis.

Oocyte Immunohistochemistry: Aldosterone Receptor Presence on Human Oocytes

Immunohistochemistry for the aldosterone receptor (AldoR) revealed a strong signal in comparison with controls (Figure 3). Specific weaker staining was also seen with the AIIR1 antibody. Both AldoR and AIIR1 staining were seen diffusely over the oocyte. Based on concomitant Hoechst staining, the receptors did not appear to be localized to the nucleus but more likely are on the oocyte membrane or within the cytoplasm. The exact site of the receptors was unclear.

FIGURE 3

Oocyte immunohistochemistry. Aldosterone receptor (AldoR) revealed a strong signal in comparison with controls. Specific weaker staining was seen with the angiotensin II antibody (AIIR1).



Sneeringer. Aldosterone in human ovarian follicular fluid. Fertil Steril 2011.

DISCUSSION

This is the first report of the presence of mineralocorticoids in the human ovarian follicle. Specifically, high levels of aldosterone and its precursor corticosterone are found in the follicular fluid of women undergoing IVF. Content is significantly increased in follicular fluid compared with in plasma, and peak levels are noted before ovulation. Additionally, we report preliminary evidence of local aldosterone synthesis and aldosterone receptors on the oocyte. Our observations are novel findings in ovarian biology and add to prior knowledge of an OvRAS.

Previously published studies revealed the presence of an OvRAS that is most active during the preovulatory time period (1–8), mimicking the elevations in mineralocorticoids described here. Outside of the ovary, aldosterone is an active component of the renin-angiotensin system system and is tightly regulated by angiotensin II (35). The same regulation scheme may be active in the ovary. Further, OvRAS with aldosterone may play a role in folliculogenesis or oocyte maturation. The increased aldosterone in preovulatory follicular fluid, in larger follicles most likely to contain mature oocytes, and among younger patients with more oocytes are some observations that are consistent with this claim. Alternatively, aldosterone level may be influenced by another undetermined factor. For example, estrogen is weakly correlated with aldosterone in our study.

The site of aldosterone synthesis is unclear. In our study, we demonstrated both higher levels of aldosterone in follicular fluid compared with in plasma and a differential expression of genes associated with aldosterone production and corticosterone accumulation in GCs of younger patients. Although these observations are consistent with ovarian production of aldosterone, there are some inconsistencies. We did not demonstrate a difference in CYP11B2 (aldosterone synthase) mRNA expression, a critical enzyme in the synthesis pathway for aldosterone. Future studies designed to specifically address the question of the site of aldosterone synthesis will provide much needed information. Data supporting ovarian synthesis would challenge the classic dogma that mineralocorticoids are produced exclusively in the adrenal gland (35) and provide some explanation for clinical examples of rare aldosterone-secreting tumors in the ovary (36–38).

In summary, this is the first study to demonstrate high levels of mineralocorticoids in the preovulatory ovarian follicular fluid

of humans. Aldosterone may be the active component of the OvRAS, conferring an improved fertility prognosis with the attainment of sufficient follicular levels. Further study on the site of aldosterone production and its role within the ovary is needed.

Acknowledgments: The authors thank the following for contributions to this research: Yevgeniya Monisov and Dr. Yoo Sang Wook for their assistance in the laboratory, the Boston IVF staff for their assistance in obtaining clinical samples, and Dimcho Bachvarov of Laval University in Quebec for his expertise in performing the gene chip analysis.

REFERENCES

1. Fernandez LA, Tarlatzis BC, Rzasa PJ, Negro-Vilar AF, DeCherney AH, Natolin F, et al. Renin-like activity in ovarian follicular fluid. Fertil Steril 1985;44:219–23.
2. Glorioso N, Atlas SA, Laragh JH, Jewelewicz R, Sealey JE. Prorenin in high concentrations in human ovarian follicular fluid. Science 1986;233(4771):1422–4.
3. Culler MD, Tarlatzis BC, Lightman A, DeCherney AH, Negro-Vilar A, Noftolin F, et al. Angiotensin II-like immunoreactivity in human ovarian follicular fluid. J Clin Endocrinol Metab 1986;62(3):613–5.
4. Ezra Y, Simon A, Yaron A, Laufer N, Navot D. Angiotensin-I-converting enzyme and its correlation with human follicular fluid steroids. Eur J Obstet Gynecol Reprod Biol 1992;44:71–5.
5. Nielsen AH, Schauser KH, Svenstrup B, Poulsen K. Angiotensin converting enzyme in bovine ovarian follicular fluid and its relationship with oestradiol and progesterone. Reprod Domest Anim 2002;37: 81–5.
6. Lightman A, Tarlatzis BC, Rzasa PJ, Lennard D, DeCherney AH, Naftolin F, et al. The ovarian renin-angiotensin system: renin-like activity and angiotensin II/III immunoreactivity in gonadotropin-stimulated and unstimulated human follicular fluid. Am J Obstet Gynecol 1987;156:808–16.
7. Sealey JE, Cholst I, Glorioso N, Weintraub ID, James G, Laragh JH, et al. Sequential changes in plasma luteinizing hormone and plasma prorenin during the menstrual cycle. J Clin Endocrinol Metab 1987;65:1–5.
8. Loret de Mola JR, Goldfarb JM, Hecht BR, Babbo CJ, Friedlander MA. Gonadotropins induce higher active renin levels in the follicular fluid of normal and hyperstimulated cycles. Gynecol Endocrinol 1999;13:155–60.
9. Nielsen A, Hagemann A, Svenstrup B, Nielsen J, Poulsen K. Angiotensin II receptor density in bovine ovarian follicles relates to tissue renin and follicular size. Clin Exp Pharmocol Physiol 1994;21:463–9.
10. Paulson RJ, Do YS, Hsueh WA, Eggena P, Lobo RA. Ovarian renin production in vitro and in vivo: characterization and clinical correlation. Fertil Steril 1989;51:634–8.
11. Do YS, Sherrod A, Lobo RA, Chen SW, Kjos S, Hsueh WA, et al. Human ovarian theca cells are a source of renin. Proc Natl Acad Sci 1988;85: 1957–61.
12. Bokal EV, Vrtovec HM, Virant Klun I, Verdenik I. Prolonged HCG action affects angiogenic substances and improves follicular maturation, oocyte quality and fertilization competence in patients with polycystic ovarian syndrome. Hum Reprod 2005;20:1562–8.
13. Fan Y, Chen G. Association between renin activity, angiotensin II and ovarian hyperstimulation syndrome. Zhonghua Fu Chan Ke Za Zhi 2001;36: 647–50.
14. Vrtacnik-Bokal E, Meden-Vrtovec H, Verdenik I. Uterine arterial blood flow and the substances of ovarian renin-angiotensin system in women with polycystic ovaries undergoing in vitro fertilization. Eur J Obstet Gynecol Reprod Biol 2006;124:77–81.
15. Vrtacnik-Bokal E, Meden-Vrtovec H, Osredkar J, Verdenik I. Follicular fluid renin concentration in patients with polycystic ovaries treated with gonadotrophins in an in vitro fertilisation programme. Clin Chem Lab Med 2003;41:663–7.
16. Homburg R, Berkowitz D, Levy T, Feldberg D, Ashkenazi J, Ben-Rafael Z. In vitro fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. Fertil Steril 1993;60:858–63.
17. Fru KN, VandeVoort CA, Chaffin CL. Mineralocorticoid synthesis during the periovulatory interval in macaques. Biol Reprod 2006;75:568–74.
18. Von Wald T, Monisova Y, Hacker M, Yoo S, Penzias A, Reindollar R, et al. Age-related variations in follicular apolipoproteins may influence human oocyte maturation and fertility potential. Fertil Steril 2009.
19. Zimon A, Erat A, Von Wald T, Bachvarov D, Reindollar R, Usheva A, et al. Genes invoked in the ovarian transition to menopause. Nucl Acid Res 2006;34:3279–87.
20. Bird IM, Hanley NA, Word RA, Mathis JM, McCarthy JL, Mason JI, et al. Human NCI-H295 adrenocortical carcinoma cells: a model for angiotensin II responsive aldosterone secretion. Endocrinology 1993;133:1555–61.
21. Gu J, Wen Y, Mison A, Nadler JL. 12-lipoxygenase pathway increases aldosterone production, 3',5'-cyclic adenosine monophosphate response element-binding protein phosphorylation, and p38 mitogen-activated protein kinase activation in H295R human adrenocortical cells. Endocrinology 2003;144:534–43.
22. Díaz-Perales A, Quesada V, Peinado JR, Ugalde AP, Suárez MF, Gomis-Rüth FX, et al. Identification and characterization of human archaemetzincin-1 and -2, two novel members of a family of metalloproteases widely distributed in archaea. J Biol Chem 2005;280:30367–75.
23. Ikeda K, Emoto N, Raharjo SB, Saiki K, Yokoyama M, Matsuo M, et al. Molecular identification and characterization of novel membrane-bound metalloprotease, the soluble secreted form of which hydrolyzes a variety of vasoactive peptides. J Biol Chem 1999;274:32469–77.
24. Heikkila M, Peltoketo H, Leppaluoto J, Ilves M, Vuolteenaho O, Vainio S. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. Endocrinology 2002;143:4358–65.
25. Thorngate FE, Strockbine PA, Erickson SK, Williams DL. Altered adrenal gland cholesterol metabolism in the apoE-deficient mouse. J Lipid Res 2002;43:1920–6.
26. Li X, Nokkala E, Yan W, Santti R, Makela S, Poutanen M, et al. Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase. Endocrinology 2001;142:2435–42.
27. Harris HJ, Kotelevtsev Y, Mullins JJ, Seckl JR, Holmes MC. Intracellular regeneration of glucocorticoids by 11beta-hydroxysteroid dehydrogenase (11beta-HSD)-1 plays a key role in regulation of the hypothalamic-pituitary-adrenal axis: analysis of 11beta-HSD-1-deficient mice. Endocrinology 2001;142:114–20.
28. Paterson JM, Holmes MC, Kenyon CJ, Carter R, Mullins JJ, Seckl JR. Liver-selective transgene rescue of hypothalamic-pituitary-adrenal axis dysfunction in 11beta-hydroxysteroid dehydrogenase type 1-deficient mice. Endocrinology 2007;148: 961–6.
29. McGill BE, Bundle SF, Yaylaoglu MB, Carson JP, Thaller C, Zoghbi HY. Enhanced anxiety and stress-induced corticosterone release are associated with increased Crh expression in a mouse model of Rett syndrome. Proc Natl Acad Sci 2006;103: 18267–72.
30. Sainsbury A, Schwarzer C, Couzens M, Fetissov S, Sperk G, Hökfelt T, et al. Important role of hypothalamic Y2[NpyR2] receptors in body weight regulation revealed in conditional knockout mice. Proc Natl Acad Sci 2002;99:8938–43.
31. Funder JW. Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. Ann Rev Med 1997;48:231–40.
32. Westphal CH, Muller L, Zhou A, Steiner DF, Lindberg I, Leder P, et al. The neuroendocrine protein 7B2 [Scg5] is required for peptide hormone processing in vivo and provides a novel mechanism for pituitary Cushing's disease. Cell 1999;96: 689–700.
33. Laurent V, Kimble A, Peng B, Pintar JE, Steiner DF, Lindberg I, et al. Mortality in 7B2 [Scg5] null mice can be rescued by adrenalectomy: involvement of dopamine in ACTH hypersecretion. Proc Natl Acad Sci 2002;99:3087–92.
34. Benigni F, Villa P, Demitri MT, Lagunowich L, Panayotatos N, Ghezzi P, et al. Ciliary neurotrophic factor inhibits brain and peripheral tumor necrosis factor production and, when coadministered with its soluble receptor, protects mice from lipopolysaccharide toxicity. Mol Med 1995;1: 568–75.
35. Kronenberg H, Melmed S, Polonsky K, Larsen P. Williams textbook of endocrinology. 11th ed. Philadelphia: Sander Elsevier, 1990: chapters 14 and 15.
36. Kulkarni JN, Mistry RC, Kamat MR, Chinoy R, Lotlikar RG. Autonomous aldosterone-secreting ovarian tumor. Gynecol Oncol 1990;37:284–9.
37. Jackson B, Valentine R, Wagner G. Primary aldosteronism due to a malignant ovarian tumour. Aust N Z J Med 1986;16:69–71.
38. Todesco S, Terribile V, Borsatti A, Mantero F. Primary aldosteronism due to a malignant ovarian tumor. J Clin Endocrinol Metab 1975;41:809–19.