Adiponectin and its receptors modulate granulosa cell and cumulus cell functions, fertility, and early embryo development in the mouse and human

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Objective: To study the expression and function of adiponectin and its receptors in mouse and human follicle cells and in early embryo development.
Design: Whole ovaries, granulosa cells, and cumulus-oocyte complexes isolated from immature mice before and during hormone-induced ovulation were used to analyze the expression of adiponectin, its receptors, and ovulation-related genes; human cumulus cells and granulosa cells were isolated from patients undergoing in vitro fertilization (IVF) procedures.
Setting: Multicenter.
Patient(s): Women in IVF programs in Japan and the United States.
Intervention(s): None.
Main Outcome Measure(s): Expression of adiponectin receptors and fertility.
Result(s): Adiponectin expression is absent/low in mouse and human granulosa cells and cumulus cells. Adiponectin receptors are hormonally regulated in mouse granulosa and cumulus cells in vivo and in culture. Adiponectin differentially alters the expression of *Adipor1*/*Adipor2* as well as genes related to steroidogenesis, ovulation, and apoptosis in cumulus cells versus granulosa cells. Adiponectin enhances oocyte maturation and early embryo development in mouse and human IVF procedures.
Conclusion(s): Adiponectin can modulate not only follicle growth but also embryo development in mice and humans. (Fertil Steril® 2012;98:471–9. ©2012 by American Society for Reproductive Medicine.)
Key Words: Adiponectin, *Adipor1*, *Adipor2*, adiponectin receptors, cumulus cells, granulosa cells, IVF, oocyte

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Adipose tissue is now known to be an important part of the endocrine system and releases potent hormones known as adipokines, such as leptin and adiponectin, that regulate energy homeostasis, lipid metabolism, and neuroendocrine functions (1, 2). Although adiponectin has previously been thought to be expressed exclusively by adipocytes

(1), recent reports have indicated that adiponectin and its receptors *Adipor1* and *Adipor2*, may be expressed in ovarian cells (3–5). These observations raised the intriguing possibility that adiponectin might be regulated by pituitary and/or ovarian factors and exert endocrine or paracrine functions within the ovary (6).

Several reports in different species, including humans, have indicated that adiponectin can modulate granulosa cell steroidogenesis (3, 4, 7) and the expression of genes associated with ovulation (6, 7). There is also evidence that the functions of the two adiponectin receptors in granulosa cells may differ (8). Reducing *ADIPOR1* led to apoptosis of human KGN granulosa cells whereas reduction of *ADIPOR2* decreased steroidogenesis. Mutant mouse models indicate that depletion of adiponectin or adiponectin receptors decreases insulin sensitivity without reported changes in fertility whereas overexpression of adiponectin leads to increased insulin sensitivity and infertility or subfertility (6, 9–12). However, the mechanisms by which adiponectin modulates fertility and whether the disruption of *ADIPOR1/R2* causes any changes in ovarian function have not yet been addressed. Of clinical relevance, adiponectin levels are reduced in women with polycystic ovarian syndrome (PCOS) compared with fertile women (13, 14), which is possibly associated with elevated androgens (15, 16), obesity, and altered adipose tissue functions (17). Altered responses of ovarian cells to insulin and insulin-like growth factor 1 (IGF-1) in PCOS patients may be linked, in part, to obesity and reduced levels of serum adiponectin (18).

Collectively, these observations provide evidence that adiponectin might impact metabolic homeostasis in granulosa and cumulus cells, thereby modulating the expression of factors that control steroidogenesis, ovulation and apoptosis. Among the many transcription factors that are regulated by the insulin/IGF and follicle-stimulating hormone (FSH) pathways, FOXO1 and FOXO3 are expressed in mouse and human granulosa cells and appear be linked to granulosa cell metabolism and apoptosis as well as steroidogenesis (19–25). Furthermore, FOXO1 has been shown to increase the expression of adiponectin in adipose cells (26) and *Adipor2* in hepatic cells (27), providing evidence that FOXO1/3 may also modulate the response of granulosa cells to adiponectin by similar or different mechanisms.

Although some effects of adiponectin have been analyzed in granulosa cells, less is known about the role of the adipokine on cumulus cells and oocyte quality or with the preimplantation embryo (28–31). Therefore, we analyzed whether adiponectin alone or in conjunction with pituitary hormones could alter not only mouse granulosa cell functions but also cumulus-oocyte complex (COC) functions in culture and if this was associated with changes in the expression of specific genes or oocyte quality, including fertilization and early stages of embryo development. We studied whether there were any correlations among the levels of the adiponectin receptors (*ADIPOR1* and *ADIPOR2*) and *FOXO1* or *FOXO3* in human granulosa cells or cumulus cells collected from in vitro fertilization (IVF) patients and checked the fertility outcome of these patients.

MATERIALS AND METHODS

Animals

Immature (age 24 days) C57BL/6 female mice were housed under a 16:8-hour light:dark schedule in the Center for Comparative Medicine at Baylor College of Medicine and Hiroshima University, and they were provided food and water ad libitum. Animals were treated in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee at Baylor College of Medicine and Hiroshima University. For in vivo studies, immature mice were treated with equine chorionic gonadotropin (eCG, 4 IU, intraperitoneally; Calbiochem/EMD) to stimulate follicular development. Forty-eight hours later (designated 0 hours), the mice were injected with human chorionic gonadotropin (hCG, 5 IU; Green Park Pharmacy) to stimulate ovulation (16 hours) and luteinization (48 hours) (21). Whole ovaries (WO), granulosa cells (GC), and COCs were isolated.

Granulosa Cell Culture and Treatment with Adiponectin

Immature mice were injected intraperitoneally with 4 IU eCG and were killed 24 hours later (21). Granulosa cells were cultured in serum-free medium alone (controls) or were treated with adiponectin alone (globular, 20 μg/mL; R&D Research), forskolin alone (10 μM; Calbiochem), or adiponectin and forskolin. Cells were used to prepare total RNA or protein. Media samples were saved for analyses of progesterone. Granulosa cells were also transfected with adenoviral vectors expressing GFP (control) or FOXOA3, a stable, active form of FOXO1, as described previously elsewhere (21).

Cumulus-Oocyte Complex (COC) Isolation and Culture

Unexpanded COCs were isolated by needle puncture from the preovulatory follicles of eCG-primed mice (48 hours) and were cultured (50/well) in defined medium with FSH or eCG, with or without adiponectin (globular, 20 μg/mL) for 16 hour (32). Some COCs were then placed in a 50-μL drop of human tubal fluid (HTF) medium for IVF and embryo development (32). Spermatozoa were collected from the cauda epididymis of adult mice into 500 μL of HTF medium. After 60 minutes, the spermatozoa were introduced into the fertilization medium at a final concentration of 1,000 spermatozoa/μL. Twelve hours after insemination, the oocytes were washed thoroughly five times, and were then examined for formation of pronuclei under a phase-contrast microscopy. The gametes were further cultured for an additional day in the developing medium (KSOM + AA; Millipore) to check the cleavage rate and development to blastocyst stage (32).

Messenger RNA Extraction and Real-Time RT-PCR

Total RNA was prepared from WO, GCs, and COCs using the Qiagen Kit. Real-time polymerase chain reaction (RT-PCR) analysis was performed using the Rotor-Gene 6000