The levels of hepatocyte growth factor in serum and follicular fluid and the expression of c-Met in granulosa cells in patients with polycystic ovary syndrome

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Objective: To evaluate the levels of hepatocyte growth factor (HGF) in follicular fluid (FF) and the expression of c-Met in granulosa cells (GCs) with respect to the quality of the oocyte and embryo both in patients with polycystic ovary syndrome (PCOS) and in the normal ovary during controlled ovarian hyperstimulation cycles.
Design: Prospective controlled study.
Setting: University hospital.
Patient(s): Fifty-nine women undergoing IVF treatment (of whom 21 had PCOS and 38 were in the control group).
Intervention(s): A total of 168 FF samples were collected at the time of oocyte retrieval. The HGF levels were measured by ELISA, and the mRNA expression of c-Met in GCs was detected by real-time polymerase chain reaction.
Main Outcome Measure(s): The predictive values of HGF levels in serum and FF and the mRNA expression of c-Met in GCs for successful fertilization and oocyte-embryo quality.
Result(s): The levels of HGF in serum and FF and the c-Met expression in GCs were similar between the PCOS and control groups. Granulosa cells of fertilized oocytes (2PN) had a significantly higher level of c-Met expression than that in oocytes that failed to fertilize. The mean HGF level in FF was significantly higher in the grade 1 embryos than in the grades 2–4 embryos.
Conclusion(s): This study suggests that HGF/c-Met signaling may be a crucial determinant of fertilization success. (Fertil Steril® 2013;99:264–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: HGF, c-Met, PCOS, quality of oocyte, quality of embryo

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Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome that is characterized by the hypersecretion of LH, ovarian hyperandrogenism, polycystic ovaries, hyperinsulinemia from insulin resistance, and reduced fecundity (1). Hyperandrogenism and hyperinsulinemia affect follicular microenvironment, resulting in reduced ovarian development and the development of immature oocytes. Moreover, in PCOS cases, significant abnormalities are observed at the earliest stages of folliculogenesis. However, it is unclear which molecules are responsible for the abnormal regulation during early folliculogenesis. It has been reported that some growth factors and sex steroids may have a role in aberrant folliculogenesis in PCOS (2).

A number of growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, insulin-like growth factor-I, and epidermal growth factor, localize within growing follicles and regulate important aspects of folliculogenesis (3, 4). Recent animal studies suggested

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that hepatocyte growth factor (HGF)- and c-Met-mediated epithelial mesenchymal mechanisms are important for follicle development (5–7).

HGF, originally identified as a fibroblast-derived cell motility factor for epithelial cells, is a heparin-binding glycoprotein that consists of a 60-kDa alpha chain and a 30-kDa beta chain linked by disulphide bonds (8). The HGF receptor, identified as c-Met, is a member of the receptor protein tyrosine kinase family (9). The activation of HGF/c-Met signaling is important for the regulation of cell cycle progression, cell survival, cellular motility and invasion, and morphogenesis (10). In the ovary, HGF is produced by mesenchymal-derived theca cells, and it stimulates the proliferation of epithelial granulosa cells (GCs) during follicular development (10). HGF mediates angiogenesis and increases capillary density and blood flow through its direct actions on endothelial cells (11). The granulosa cell layer is avascular, but the theca cell layer is a rich vascular network in mammalian ovarian follicles (12). In the ovary, the development of the vascular network is associated with follicular development (3). The expression of both HGF and c-Met in mammalian ovaries has been reported in several models, including human, pig, rat, cow, and mouse models (10, 13, 14). The HGF level was measured in follicular fluid (FF) for the first time by Osuga et al. (13). In their study, it was demonstrated that mRNA expression of HGF and its receptor, c-Met, were found in the crude GC subpopulations of human preovulatory follicles. Ito et al. (14) showed that HGF significantly increased P production in human GCs but that HGF did not alter the stimulatory effects of hCG on P production. Ito et al. proposed that HGF and hCG may exert stimulatory effects through the same pathway. In 2003, Kawano et al. (15) evaluated the effects of HGF with regard to oocyte maturation. The concentration of HGF in FF was significantly increased in mature oocytes compared with that in immature oocytes. Nevertheless, there is no published study on the role of aberrant HGF and/or c-Met expression during the development of immature oocytes in patients with PCOS.

In the present study, we evaluated the levels of HGF in FF and the expression of c-Met in GCs in patients with PCOS. The results were used to determine the relationship between the expression levels of HGF/c-Met and oocyte-embryo quality during controlled ovarian hyperstimulation cycles.

MATERIALS AND METHODS

Subjects and Assays

This prospective controlled study was performed in the IVF center of the Obstetrics and Gynecology Department and in the laboratories of the Medical Biology and Genetics Department of the Dokuz Eylul University Medicine School, Izmir, Turkey, between August 2009 and February 2010. The study was approved by the Human Investigation Review Committee of the Medicine School, and an informed written consent was obtained from each participant. Fifty-nine patients younger than 35 years of age and undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment were enrolled in the study. Patients were categorized into two groups before treatment, namely, those with normal ovaries ($n = 38$) and those with PCOS ($n = 21$) according to the Rotterdam Criteria (16). All women had at least 1 year of infertility symptoms, with a mean of 6.48 years. The causes of infertility were anovulatory PCOS (35.60%), male factors (32.20%), and unexplained factors (32.20%). The study included 168 oocytes and their FF samples: 80 fertilized oocytes and 76 embryos.

Ovarian Stimulation Treatment

The same ovulation induction protocol was used for all patients. Gonadotropin-releasing hormone analog (GnRH-a; Buserelin, Suprafact) was injected SC (500 μg/day) for pituitary desensitization. GnRH-a treatment was started on the 20th day of the previous cycle. After menstrual bleeding, down-regulation was detected by the absence of a follicle <14 mm diameter and an endometrial thickness of <5 mm. The induction of ovulation with recombinant FSH (Puregon, Schering Plough or Gonal-F, Serono) was initiated with 150–300 IU per day, and the dose of GnRH-a was decreased to 200 μg/day. On day 6 of the cycle, follicular growth was evaluated by a transvaginal ultrasound (7.5 MHz endovaginal probe, Siemens) examination. When at least two follicles of an 18 mm maximum diameter were present, 10,000 IU hCG (Pregnyl, Organon) was administered. Oocyte retrieval was performed by a transvaginal ultrasound-guided follicle aspiration, 36 hours after hCG administration.

A 10-mL blood sample was collected from each patient before starting FSH administration (day 0), on the day of hCG administration, and on the day of oocyte retrieval. The blood samples were immediately centrifuged (3,000 *g* for 10 minutes) at room temperature, and the supernatant was separated and stored at -80°C until assays were performed.

During oocyte pickup (OPU), all follicle retrievals were performed by the same clinician (B.G.); the leading follicles were measured before oocyte retrieval, and the puncture started from the biggest follicle. After the aspiration of the last drop of the first follicle, the needle was removed from the ovary and flushed with warm heparinized saline before the aspiration of the next follicle; this procedure was done in the first three follicles in each patient in the study protocol. Follicle aspirates that were not clear and that were contaminated with blood were excluded. The oocytes were isolated from the aspirate and examined separately in four-well dishes. The maturation of oocytes was microscopically evaluated and classified according to the nuclear maturation (mature shows metaphase II [MII] oocytes, immature shows MI or a germinal vesicle, and degenerated shows degenerated oocytes). After the isolation of oocytes, the aspirated FF samples were immediately centrifuged (2,000 *g* for 5 minutes), and the supernatants were separated and stored at -80°C until assay. The pellets were separated, 1 mL RNAlater solution was added to each sample, and the samples were stored at -80°C until assays were performed.

The standard ICSI procedure, as described by Van Steirteghem et al., was used (17). The quality of oocytes and embryos was assessed according to Veeck's criteria (18). Fertilization was assessed 18 hours after ICSI for the appearance of two distinct pronuclei and two polar bodies. Quality scores of