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Polycystic ovary syndrome and maternal obesity affect oocyte size in in vitro fertilization/intracytoplasmic sperm injection cycles

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Abstract

In order to determine the impact of maternal metabolic state on oocyte development in women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), we retrospectively analyzed a cohort of women with PCOS undergoing IVF/ICSI from 2008-2009 in a university-based fertility center. We determined that women with PCOS and obesity have smaller oocytes compared to controls, and that when further subdivided by BMI, both PCOS and obesity influence oocyte size independent of one another.

Polycystic ovary syndrome (PCOS), clinically characterized by hyperandrogenism and ovarian dysfunction (1), is the most common endocrinopathy in reproductive age women (2), and nearly half of women with PCOS are obese (3). Both PCOS and obesity are associated with specific reproductive health complications, including lower clinical pregnancy rates in assisted conception cycles (4-6). For those that achieve a pregnancy, there is evidence that they are at increased risk of miscarriage (4, 7-9), pregnancy complications (10-11), and decreased overall live birth rates (6, 12). Mechanisms responsible for these outcomes are unclear, but may involve metabolism-induced changes in the oocyte.

Evidence that abnormal maternal physiology affects oocytes and results in abnormal pregnancy outcomes can be found in animal models of maternal diabetes. Oocytes from diabetic, insulin resistant, and obese mice show delayed maturation, smaller size, and increased granulosa cell apoptosis (13-17). These findings are linked to adverse embryonic and fetal outcomes including delayed embryonic development, growth restriction, anatomic defects, and smaller fetuses (14-15, 18-20). If similar metabolic conditions in humans also negatively impact oocyte quality, the adverse reproductive sequelae of PCOS and obesity may be partially explained.

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Although maturation and embryogenesis are linked to oocyte size in bovine, canine, and goat models (21-25), the relationship between oocyte growth and competence remains to be established in human oocytes. Thus our objective was to determine the impact of the metabolic changes associated with PCOS and obesity on oocyte size in women undergoing IVF.

A retrospective cohort study was performed comparing women with PCOS to controls undergoing in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) at the Reproductive Endocrinology and Infertility Clinic at Washington University over a consecutive 16 month period from 2008-2009. The diagnosis of PCOS was confirmed using the Rotterdam criteria, which includes at least two of the following: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, or polycystic ovaries on ultrasound; other endocrinopathies were excluded (26). Only PCOS patients undergoing ICSI were included since oocyte measurements were obtainable only after exposure to hyaluronidase per ICSI protocol. Patients undergoing IVF with the sole diagnosis of male factor infertility were identified as control subjects. This classification was an attempt to minimize the effect that other diagnoses might have on oocyte quality (i.e. diminished ovarian reserve, endometriosis). Cycles involving donor oocyte, frozen embryo transfers and preimplantation genetic diagnosis were excluded.

Medical records of identified subjects were reviewed to collect data including age, total gonadotropin dose, days of stimulation, peak estradiol level, total number of oocytes retrieved (immature and mature oocytes), fertilization rates, number of embryos transferred, mean oocyte diameter, BMI and pregnancy outcome. BMI was defined by WHO criteria (< 18.5 kg/m^2 = underweight; 18.5-24.9 kg/m^2 = normal weight; ≥ 25-29.9 kg/m^2 = overweight; ≥30 kg/m^2 = obese).

Patients underwent controlled ovarian hyperstimulation following a standard long luteal-phase GnRH agonist protocol as previously described (5). As part of the standard IVF/ICSI cycle, retrieved oocytes were placed into culture media [IVC-Two (InVitro Care, San Diego, CA) with 5% HAS (Irvine Scientific, Santa Ana, CA)] and exposed to hyaluronidase. Oocyte maturity (MII stage) was defined as germinal vesicle break down (GVBD) with presence of a polar body. Oocytes with a germinal vesicle (GV) or GVBD without a polar body (MI stage) were considered immature.

An Olympus IX71 -inverted microscope (Olympus America, Inc, Melville, NY) equipped with ZILOS laser and software (Hamilton-Thorne Research, Beverly, MA) was used to measure the diameter of each mature oocyte, excluding the zona pellucida. The software allowed for the measurement of computer captured digital images using built-in tools which automatically calculate the mean and standard deviation of each measurement. The average of three measurements was expressed as the mean oocyte diameter (Supplemental Figure 1). Magnification was calibrated weekly with an external slide per manufacturer's instructions. Data were compared using Students t-test or chi-squared analysis using Excel (Redmond, WA), Epi-Info software (Centers for Disease Control and Prevention, Atlanta, GA), or STATA 10 (College Station, TX). A p-value of <0.05 was considered significant. The study protocol was approved by the institutional review board at Washington University.

We identified 13 women with PCOS and 32 controls whose oocytes were measured during their IVF/ICSI cycle. Forty-eight IVF cycles were assessed; a total of 452 mature oocytes were measured, 127 from the PCOS group and 325 from the control group. Patients' age ranged from 23-42 years; there was no significant difference in age, duration of stimulation, average gonadotropin dose, or average peak estradiol levels. On average, the number of oocytes retrieved/cycle was not significantly different between the two groups. The ICSI

fertilization rate was significantly higher in the PCOS versus the control group (81% versus 64%, p = 0.0007) (Table 1A). Five patients in the control cohort underwent testicular sperm extraction (TESE), which may partly account for the lower fertilization rate (68%). The percent of patients undergoing Day 3 embryo transfer and the number of embryos transferred/cycle were similar between groups. The PCOS cohort had a significantly higher mean BMI than the male factor cohort (32.65±7.97 versus 24.25±3.08 kg/m^2, p=0.002). None of the women with PCOS were diabetic.

The oocyte characteristics of the PCOS and control groups are shown in Table 1A. Total percentage of mature oocytes retrieved and the average number of mature (MII) oocytes measured/cycle were similar between the PCOS and control groups. The mean mature oocyte diameter in the PCOS group was significantly smaller than the mean mature oocyte diameter in the control group (114.17±5.25 versus 116.05±4.3 μm, p=0.0004). Regarding oocyte size variability, the inter/intra-patient standard deviations (SD) were similar to or less than the SD of the mean oocyte size (SD between and within PCOS patients was 3.21μm and 4.35μm respectively; SD between and within controls was 2.91μm and 3.11μm respectively). One patient with PCOS was on metformin at the time of oocyte retrieval; her average oocyte size was 116.06 μm (range 112.7-119.1 μm).

To further distinguish the effects of obesity from PCOS, a subgroup analysis was performed. A comparison of oocytes from obese (n=195) versus non-obese (n=257) women revealed significantly smaller oocytes in the obese group (114.95 versus 115.96 μm, p = 0.02). Furthermore, the oocytes from the obese control patients (n=105) were significantly smaller than the non-obese control oocytes (n=220) (114.95 versus 116.58 μm, p=0.004). Oocytes from non-obese PCOS (n=37) women were significantly smaller than oocytes from the non-obese control cohort (n=220) (112.27 versus 116.58 μm, p=0.003) (Table 1B). The total percentage of mature oocytes retrieved from the non-obese PCOS patients (70.8%) was significantly lower than the percentage of mature oocytes from non-obese control patients (82.7%; p =0.03). The clinical pregnancy and live birth rates/cycle in the PCOS group were not different than the control group.

Our data demonstrate that women with PCOS have smaller oocytes versus controls, similar to what is seen in diabetic, insulin resistant, and obese mouse models (15, 17). The subgroup analysis revealed smaller oocytes in the obese control patients compared to the non-obese control infertility patients suggesting that obesity affects oocyte size independent of PCOS. We also show that PCOS affects oocyte size independent of obesity, based on the fact that the oocytes from non-obese PCOS patients are smaller than oocytes from non-obese control patients.

Prior studies examining oocyte maturity and quality show that the number of mature oocytes is significantly decreased in morbidly obese women (10), and the number of good quality oocytes is significantly lower in overweight women (27) compared to normal weight controls. The effects of obesity on oocyte developmental competence can be seen in murine models of diet induced obesity. In mice fed a high fat diet, fewer embryos undergo normal early embryonic developmental progression (20), and smaller oocytes and decreased maturity are associated with abnormal fetal development including significantly decreased size (measured by crown rump length) versus control mice (15). However, little is currently known about how oocyte size influences maturation and embryogenic potential in humans.

The optimal human oocyte size is unclear from the available literature. Diameter measurements in unstimulated cycles range from 81-140μm (28-30), while stimulated mature oocyte diameters range from 104-121μm (29-30). Although a larger diameter (105-106μm) is associated with higher rates of in-vitro maturation in unstimulated immature

oocytes (28-29), size appears unrelated to fertilization or embryo development in stimulated mature oocytes (30). Further examination is needed to determine if oocyte size correlates with fetal outcomes in women with PCOS/obesity, and to further assess possible underlying mechanisms.

There are several limitations to this retrospective study. Many PCOS patients at our center were excluded from oocyte measurement because they were not undergoing ICSI. Also, oocytes were not individually followed to track characteristics of a particular oocyte to fertilization, or embryo morphology, and few patients undergo single embryo transfer in our center, so pregnancy outcomes could not be related to specific oocyte characteristics. Lastly, although statistically significant differences were noted in oocyte size, the clinical significance is unclear given the small patient number, thus it is difficult to make any final conclusions regarding pregnancy rates and pregnancy outcomes between the PCOS/obese and control cohorts.

The precise mechanism responsible for poorer outcomes in women with PCOS and obesity patients is unclear, though altered physiology related to energy metabolism may play a role. An elevated BMI is associated with changes in the preovulatory follicular fluid environment including increased levels of insulin, triglycerides, and androgens (31), and decreased HCG levels (32). Alterations in the intrafollicular hormonal environment, as seen in PCOS and obesity, may impact oocyte size as we have shown here. Furthermore, we found a larger average oocyte diameter in one of the PCOS patients treated with metformin, indicating the need for further examination of how metformin impacts oocyte size and competence. This is particularly relevant given the current clinical and basic science data demonstrating improved reproductive outcomes after treatment with metformin (7, 33-37). In summary, oocytes from women with both PCOS and obesity are smaller than controls; their affect on oocyte size appears independent of one another. The clinical implications of this warrant future study.

Figure 1: Photomicrograph of MII oocyte demonstrating 3 measurements used to calculate mean oocyte diameter (μm). 400X

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1A

Patient IVF/ICSI Cycle Characteristics

	PCOS (n=13; 14 cycles)	Controls (n=32; 34 cycles)	p value
Patient/IVF Cycle Characteristics			
Day 3 Embryo Transfer (%)	78.6	79.4	0.95
Embryos Transferred/Cycle	2.43 ± 0.65	2.38 ± 0.7	0.83
ICSI Fertilization rate (%)	81	64	0.0007
Oocyte Characteristics			
Number of oocytes measured	127	325	
M2 oocytes collected (%)	80.7	82.3	0.61
M2 oocytes measured/cycle	9.07 ± 5.42	9.56 ± 3.59	0.76
Mean oocyte diameter (μm)	114.17 ± 5.25 (range 87.57-122.43)	116.05 ± 4.30 (range 102 – 145.93)	0.0004

M2= mature oocyte

Table 1B

Oocyte Characteristics Non-Obese

	PCOS Non-Obese (n=5)	**Control Non-Obese (n=21; 22 cycles)**	**p value**
Number of oocytes measured	37	220	
M2 oocytes collected (%)	70.8	82.7	0.03
M2 oocytes measured/cycle	7.4 ± 4.39	10 ± 3.63	0.27
Mean oocyte diameter (μm)	112.27 ± 8.04 (range 87.57-120.6)	116.58 ± 3.76 (range 106.53-128)	0.003

M2= mature oocyte