



Association between parental anthropometric measures and the outcomes of intracytoplasmic sperm injection cycles

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Received: 20 July 2018 / Accepted: 27 November 2018 / Published online: 5 December 2018
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Abstract

Purpose To study the association between body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR), and response to controlled ovarian stimulation, semen quality, and intracytoplasmic sperm injection (ICSI) outcomes.

Methods This prospective observational study included data from 402 couples undergoing ICSI between January/2010–September/2016. Participants had their weight, height, waist, and hip measured before ICSI treatment.

Results Gonadotropin dose was positively associated with maternal WHR. Maternal WC and WHR negatively influenced the estradiol level, number of follicles, oocytes, and mature oocytes. Maternal BMI negatively influenced oocyte yield. Semen volume was negatively influenced by male BMI. Sperm concentration was negatively associated with WHR and WHtR. Progressive sperm motility was negatively influenced by all anthropometric measures (AMs). The TMSC was negatively affected by all AM but WC. Significant differences were observed in all ICSI outcomes except pregnancy and miscarriage rates, favoring women with AM below the cut off values compared to women above the cut off. It was observed that men with AM below the cut off values showed significantly higher rates of fertilization, embryo and blastocyst development, implantation, and pregnancy than men above the cut off. When combined maternal and paternal AM were analyzed, significant differences were observed in fertilization, embryo and blastocyst development and implantation rates, favoring couples with normal BMI, WC, and WHR.

Conclusions Lower AMs have a protective effect on fertility. Awareness and counseling on how to minimize its impact in both partners could improve ICSI outcomes.

Keywords Anthropometric measures · Infertility · Intracytoplasmic sperm injection · Lifestyle

Introduction

The importance of abdominal fat mass was recognized by the World Health Organization (WHO) Expert Consultation on Obesity, in 1997 [1]. Presently, waist circumference (WC) is used in defining central obesity [2], which is significantly associated with the metabolic syndrome [3].

In 2008, the waist-to-hip ratio (WHR), which is a more precise measure of central adiposity, was recommended by the WHO in defining the metabolic syndrome [4]. Similarly,

the waist-to-height ratio (WHtR) has also emerged as a predictor of intra-abdominal fat [5]. The WHR and WHtR appear to be stronger independent risk factors than body mass index (BMI) for increased risk of cardiovascular diseases in men [6, 7] and women [7, 8], as they diverge substantially within a slight range of BMI [4].

The WC, WHR, and WHtR have also been used for the assessment of obesity, which is a gradually predominant health condition in modern society [9]. The negative associations between obesity and male and female fertility are well documented [10–16]. However, the majority of previous studies have used the BMI to estimate overall adiposity, without taking body fat distribution into account.

Few studies have explored the association between other anthropometric measures (AMs) and infertility. In men, it has been demonstrated that increasing waist circumference negatively impacts ejaculate volume [17] and

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sperm count [17–20], increasing hip circumference (HC) negatively impacts sperm count and motile sperm count [18, 19], and increasing WHtR negatively impacts sperm concentration [20]. In women, increasing WHR was positively associated with irregular menstruation, oligomenorrhea, and hirsutism [21] and negatively associated with the odds of conception after intrauterine insemination (IUI) [22].

To date, the association between parental AM and human-assisted reproduction has never been investigated. To investigate the association between maternal BMI, WC, WHR and WHtR, and ovarian response to controlled ovarian stimulation and oocyte quality; paternal BMI, WC, WHR and WHtR, and semen quality; and maternal and paternal BMI, WC, WHR and WHtR, and the outcomes of intracytoplasmic sperm injection (ICSI) cycles.

Material and methods

Study design

This prospective observational study included data from 402 couples undergoing their first ICSI cycle between January/2010 and September/2016.

All male and female participants had their weight, height, waist, and hip measured before the ICSI treatment. The effects of (i) maternal BMI, WC, WHR, and WHtR, on ovarian response to COS; (ii) paternal BMI, WC, WHR, and WHtR on semen quality; and (iii) maternal and paternal BMI, WC, WHR, and WHtR on ICSI outcomes were investigated.

Anthropometric parameters measures

Before the beginning of the ICSI treatment, all male and female patients who consented to participate were physically examined to measure individual anthropometric parameters. All measures were performed by the same nutrition professional. Collected data consisted of participants' weight, height, WC, and HC. Weight was measured in kilograms using a scale, with patients wearing minimal clothing. Height was measured in meters with a stadiometer. Both WC and HC were measured in centimeters using a measuring tape, according to the WHO STEPwise Approach to Surveillance [23]. The WC was measured at the approximate midpoint between the lower margin of the last palpable rib and the top of the iliac crest, and the HC around the widest portion of the buttocks. The WHR was calculated by dividing the WC by the HC. The WHtR was calculated by dividing the WC by the height. Patients were also asked regarding smoking and physical activity habits.

Controlled ovarian stimulation

Recombinant FSH (Gonal-F; Serono, Geneva, Switzerland) and GnRH antagonist (Cetrotide; Serono, Geneva, Switzerland) were administered for controlled ovarian stimulation (COS). Hormonal dosages were determined according to (i) patients' antral follicle count performed prior to the start of COS, and (ii) serum estradiol levels and (iii) follicular development monitoring with ultrasound scans, which were performed in each visit during the stimulus. Recombinant hCG (Ovidrel; Serono, Geneva, Switzerland) was administered to trigger final follicular maturation. Oocyte retrieval was performed 35 h later.

Laboratorial procedures

Andrology laboratory

All semen samples were collected in the laboratory by masturbation. After liquefaction for 30 min, semen samples were evaluated for sperm count, motility, and morphology. Sperm count and motility assessment were performed by following the instructions of the counting chamber manufacturer (Leja@ slide, Gynotec Malden, Nieuw-Vennep, the Netherlands). The total sperm count is the end concentration, expressed as $\times 10^6$ spermatozoa. Prewash total motile sperm count (TMSC) was calculated by multiplying the ejaculate volume by the sperm concentration/mL by the percentage of motile sperm in the neat sample. Sperm samples were prepared using a two-layered density gradient centrifugation technique (50 and 90% isolate, Irvine Scientific, Santa Ana, CA, USA).

Embryology laboratory

Oocyte morphology was assessed after chemical and mechanical removal of cumulus cells, using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under $\times 400$ magnification, just before sperm injection. Oocytes that had released the first polar body (mature oocytes) were used for ICSI. Fertilization was confirmed approximately 16 h post ICSI. Embryos were maintained in a 50- μ L drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement covered with paraffin oil in a humidified atmosphere under 6% CO₂ at 37 °C for 5 days. The embryo morphology was assessed on the mornings of days 2, 3, and 5 of embryo development.

High-quality embryos were defined as those with 4 cells on day 2 or 8–10 cells on day 3, < 15% fragmentation, symmetric, and mononucleated blastomeres, and absence of cytoplasmic inclusions and/or dimorphisms in the perivitelline space and zona pellucida.

On day 5 of development, embryos that reached the blastocyst stage were considered when (i) the blastocoel was greater than half the volume of the embryo; (ii) the blastocoel completely filled the embryo; (iii) the blastocyst was expanded; (iv) blastocyst hatching occurred; and (v) blastocyst hatched.

Embryo transfers were performed on day 5 of embryo development. Two to three embryos were transferred per patient, depending on maternal age and embryo quality.

Clinical follow-up

A pregnancy test was performed 10 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed when the fetal heartbeat was detected. Pregnancy rates were calculated per transfer. Miscarriage was defined as clinical pregnancy loss before 20 weeks.

Statistical analyses

The sample size calculation revealed that a sample of at least 314 subjects had 95% power to detect a 20% effect with a significance level (α) of 5% (two-tailed). The calculation was performed using G*Power 3.1.7. Data are expressed as mean \pm standard deviation for continuous variables, and percentages for categorical variables.

Generalized linear models (GzLMs) with adjustment for potential confounders were used to investigate the association between:

1. Maternal AM and ovarian response to COS (number of follicles, retrieved oocytes, and mature oocytes)—adjusted for maternal age, physical activity, smoking habit, total dose of FSH administered, and estradiol level on the day of hCG trigger
2. Paternal AM and semen parameters (semen volume, sperm count per mL, total sperm count, TMSC, and sperm progressive motility)—adjusted for male age, ejaculatory abstinence interval, physical activity, and smoking habit.

In a second analysis, the influences of maternal and paternal AM on the outcomes of ICSI were investigated independently and combined. For the independent analysis, female and male partner were divided into normal and abnormal groups each, corresponding to below and above cut off value, respectively, for each AM investigated. For the combined analysis, couples were grouped into normal category, when both partners fitted below the cut off value for the corresponding AM, or abnormal category, when at least one partner fitted above the cut off value for the corresponding AM. The cut off values used were as follows: BMI > 24.9 [24], female WHR \geq

0.85, male cut off ≥ 0.90 [4], female WC > 80 cm, male WC > 94 cm [4], and WHtR > 0.5 [25].

Data was analyzed by GzLM followed by Bonferroni post hoc test, adjusted for maternal age, paternal age, total dose of FSH administered, estradiol level, number of obtained oocytes, sperm concentration and motility, physical activity, and smoking habit.

Potential confounders were selected when the literature revealed a strong association between the variable and the dependent variable. Since BMI and measures of central adiposity (WC, WHR, WHtR) are correlated with each other [26], BMI was included as a covariate in all the regression models in which those AM of central adiposity acted as independent variables. When BMI acted as independent variable, anthropometric measures were not included as covariates.

Continuous variables were analyzed using linear regression models with either gamma with log link distribution (dose of FSH and estradiol level) or normal distribution (oocyte yield, semen volume, sperm concentration/mL, total sperm concentration, progressive sperm motility, and TMSC), count (number of follicles, retrieved oocytes, mature oocytes, and obtained embryos), and rate variables (fertilization rate, high-quality embryos rate on days 2 and 3, blastocyst development rate, and implantation rate) were analyzed using Poisson log-linear models, and categorical variables were analyzed using logistic regression models (pregnancy and miscarriage). The Akaike Information Criteria (AIC) were used to compare different models and to determine which model best fitted the data. The results are expressed as unstandardized regression coefficients (B), or exponentiation of B coefficient [Exp(B)], with 95% confidence intervals (CIs) and *p* values. The α adopted was 5%. The analysis was performed using SPSS Statistics 21 (IBM, New York, New York, USA).

Results

Patient's demographics are shown in Table 1.

The association between maternal AM and the ovarian response to COS is shown in Table 2. The total dose of FSH administered was positively associated with WHR (B: 0.639; CI: 0.072–1.207; *p*: 0.027). Significant negative associations were observed between the maternal WC and WHR and the estradiol level on the day of hCG trigger (WC = B: -0.022 ; CI: -0.040 to -0.004 ; *p* 0.017 and WHR = B: -2.385 ; CI -4.188 to -0.582 ; *p* 0.010), the number of follicles (WC = B -0.014 ; CI -0.019 to -0.009 ; *p* < 0.001 and WHR = B -0.61 ; CI -1.14 to -0.7 ; *p* 0.026), the number of obtained oocytes (WC = B: -0.010 ; CI -0.017 to -0.004 ; *p* 0.001 and WHR = B: -1.451 ; CI -2.116 to -0.785 ; *p* < 0.001), and the number of mature oocytes (WC = B -0.873 ; CI -1.691 to -0.055 ; *p* 0.036 and WHR = B: -0.010 ; CI: -0.018 to $-$

Table 1 Descriptive analysis of patient's demographics, ovarian response to COS, semen quality, and ICSI outcomes

Demographics (<i>n</i> = 402)	Female	Male
	Mean + SD	Mean + SD
Age (years)	35.9 ± 4.0	37.4 ± 5.6
Body weight (kg)	66.2 ± 12.6	89.7 ± 16.4
Height (meters)	1.63 ± 0.03	1.8 ± 0.1
BMI	24.8 ± 4.3	28.5 ± 4.1
Waist circumference (cm)	87.4 ± 10.9	101.9 ± 12.4
Hip circumference (cm)	103.8 ± 9.8	108.1 ± 8.3
WHR	0.8 ± 0.1	0.9 ± 0.1
WHtR	53.2 ± 6.5	57.0 ± 6.4
Ovarian response to COS	Mean + SD	
Total dose of FSH administered (IU)	2568.1 ± 631.1	
Estradiol level on the day of hCG trigger (pg/mL)	1804.5 ± 1549.4	
Follicles (<i>n</i>)	15.7 ± 12.0	
Retrieved oocytes (<i>n</i>)	10.9 ± 8.9	
Oocyte yield (%)	68.3	
Mature oocytes (<i>n</i>)	8.5 ± 7.3	
Semen quality	Mean + SD	
Semen volume	2.91 ± 1.59	
Sperm concentration/mL	44.53 ± 51.72	
Total sperm concentration (million × 10 ⁶)	118.50 ± 152.89	
Progressive sperm motility (%)	38.70	
Total motile sperm count (%)	177.95	
ICSI outcomes	Mean + SD	
Injected oocytes/patient (<i>n</i>)	8.1 ± 6.2	
Fertilization rate (%)	73.4	
Obtained embryos/patient (<i>n</i>)	7.2 ± 5.7	
High-quality embryos on day 2 (%)	47.3 ± 25.1	
High-quality embryos on day 3 (%)	47.1 ± 27.8	
Blastocyst development rate (%)	52.8	
Transferred embryos (<i>n</i>)	1.9 ± 1.1	
Implantation rate (%)	27.0	
Pregnancy rate (%; n/N)	31.3 (106/339)	
Miscarriage rate (%; n/N)	16.0 (17/106)	

SD standard deviation, *kg* kilogram, *BMI* body mass index, *cm* centimeter, *WHR* waist-to-hip ratio, *WHtR* waist-to-height ratio, *COS* controlled ovarian stimulation, *ICSI* intracytoplasmic sperm injection, *SD* standard deviation, *IU* international unit, *hCG* human chorionic gonadotropin

0.002; *p* 0.011). The BMI was negatively associated with oocyte yield ($B = -0.533$; $CI = -0.970$ to -0.097 ; *p* 0.017).

The association between paternal AM and semen quality is shown in Table 3. Semen volume was negatively associated with the BMI ($B = -0.099$; $CI = -0.164$ to -0.035 ; *p* 0.003). The total sperm concentration was negatively associated with the WHR ($B = -3.627$; $CI = -7.200$ to -0.054 ; *p* 0.047), and WHtR ($B = -5.328$; $CI = -11.228$ to -0.262 ; *p* 0.012). The progressive sperm motility was negatively influenced by all AMs ($BMI = B = -1.523$; $CI = -2.309$ to -0.736 ; *p* < 0.001; $WC = B = -0.807$; $CI = -1.452$ to -0.162 ; *p* 0.014; $WHR = B = -1.575$; $CI = -30.82$ to -0.068 ; *p* 0.040; and $WHtR = B = -1.262$; $CI = -2.483$ to -0.041 ; *p* 0.023). The TMSC was negatively affected by all

AMs but WC ($BMI = B = -11.655$; $CI = -21.201$ to -2.109 ; *p* 0.017; $WHR = B = -5.971$; $CI = -10.713$ to -1.230 ; *p* 0.014; and $WHtR = B = -15.040$; $CI = -29.548$ to -0.532 ; *p* 0.042).

Tables 4, 5, and 6 show the results for the comparisons of ICSI outcomes between patients and couples according to AM cut-offs. It was observed that women with normal BMI showed higher fertilization rate ($76.5\% \pm 0.9$ vs. $71.7\% \pm 1.1$, *p* < 0.001), number of embryos (6.3 ± 0.2 vs. 5.0 ± 0.2 , *p* < 0.001), high-quality embryo rate on day 2 ($23.3\% \pm 0.5$ vs. $15.3\% \pm 0.5$, *p* < 0.001), high-quality embryo rate on day 3 ($24.3\% \pm 0.6$ vs. $22.5\% \pm 0.7$, *p* 0.041), and implantation rate ($12.6\% \pm 0.3$ vs. $10.1\% \pm 0.3$, *p* < 0.001) compared to women with abnormal BMI. Significant differences were

Table 2 GzLM results for the association between maternal anthropometric measures and ovarian response to COS

varian response (<i>n</i> = 402)	BMI			WC ^c			WHR ^c			WHtR ^c		
	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>
Dose of FSH (IU) ^a	0.004	L - 0.001 U 0.010	0.111	-0.003	L - 0.008 U 0.003	0.320	0.639	L 0.072 U 1.207	0.027	-0.004	L - 0.014 U 0.005	0.372 ^d
Estradiol level (pg/mL) ^a	-0.002	L - 0.021 U 0.017	0.833	-0.022	L - 0.040 U - 0.004	0.017	-2.385	L - 4.188 U - 0.582	0.010 ^d	-0.024	L - 0.055 U 0.007	0.131
Follicles (n) ^b	0.002	L - 0.003 U 0.005	0.599	-0.014	L - 0.019 U - 0.009	<0.001	-0.61	L - 1.14 U - 0.07	0.026 ^d	0.003	L - 0.001 U 0.008	0.117
Retrieved oocytes (n) ^b	0.007	L - 0.001 U 0.015	0.089	-0.010	L - 0.017 U - 0.004	0.001	-1.451	L - 2.116 U - 0.785	<0.001 ^d	-0.009	L - 0.020 U 0.003	0.132
Oocyte yield (%) ^b	-0.533	L - 0.970 U - 0.097	0.017	0.266	L - 0.158 U 0.690	0.219	2.740	L - 43.961 U 49.441	0.908	0.363	L - 0.406 U 1.131	0.355
Mature oocytes (n) ^b	0.010	L - 0.112 U 0.132	0.870	-0.873	L - 1.691 U - 0.055	0.036	-0.010	L - 0.018 U - 0.002	0.011	-0.004	L - 0.018 U 0.009	0.541

The interpretation for the regression coefficient is for a 1 unit change. When the independent variable is a proportion, a 1 unit change is the same as 100%, e.g., if a dependent variable goes down by 1.4 units when the independent variable (measured in proportions) increases by 1 unit, it also implies that Y goes down by 0.014 (1.4/100 = -0.014) units when X increases by 0.01 (1/100 = 0.01) units

GzLM generalized linear model, BMI body mass index, WC waist circumference, WHR waist-to-hip ratio, WHtR waist-to-height ratio, COS controlled ovarian stimulation, B unstandardized regression coefficient, CI confidence interval, L lower, U upper, FSH follicle stimulating hormone, IU international unit

^a Adjusted for maternal age, physical activity, and smoking habit

^b Adjusted for maternal age, physical activity, smoking habit, total dose of FSH administered, and estradiol level on the day of hCG trigger

^c Adjusted for BMI

^d Significant before adjustment for BMI

observed between males with normal and abnormal BMI regarding high-quality embryo rate on day 2 (28.1% ± 0.9 vs. 17.4% ± 0.4, *p* < 0.001, respectively), blastocyst development rate (52.7% ± 1.3 vs. 45.0% ± 0.6, *p* < 0.001, respectively), and implantation rate (18.4% ± 0.7 vs. 10.0% ± 0.2, *p* < 0.001, respectively). Additionally, couples in which both partners had normal BMI showed significantly higher fertilization rate (80.0% ± 2.1 vs. 73.6% ± 0.8, *p* 0.005), high-quality embryo rate on day 2 (30.7% ± 1.2 vs. 17.7% ± 0.4, *p* < 0.001), blastocyst development rate (53.5% ± 1.9 vs. 46.4% ± 0.6, *p* < 0.001), and implantation rate (25.0% ± 1.1 vs. 9.5% ± 0.2, *p* < 0.001) than couples in which at least one partner had abnormal BMI (Table 4).

Women with normal WC showed higher fertilization rate (76.9% ± 1.4 vs. 72.7% ± 0.9, *p* 0.011), number of embryos (6.3 ± 0.3 vs. 5.4 ± 0.2, *p*: 0.007), high-quality embryo rate on day 2 (25.0% ± 0.8 vs. 20.3% ± 0.5, *p* < 0.001), high-quality embryo rate on day 3 (25.2% ± 1.0 vs. 22.4% ± 0.6, *p* 0.024), blastocyst development rate (51.4% ± 1.1 vs. 44.4% ± 0.7, *p* < 0.001), and implantation rate (17.3% ± 0.5 vs. 8.6% ± 0.3, *p* < 0.001) than those with abnormal WC. Men with normal WC showed higher rates of high-quality embryos on days 2 (26.4% ± 1.0 vs. 20.1% ± 0.5, *p* < 0.001) and 3 (26.4% ± 1.1 vs. 23.1% ± 0.6, *p* 0.013), blastocyst development (55.0% ± 1.5 vs. 45.8% ± 0.8, *p* < 0.001), implantation (13.2% ± 0.7 vs. 9.1% ± 0.3, *p* < 0.001), and pregnancy (41.2 vs. 22.1%, *p* 0.021), compared to men with abnormal WC. Couples with

normal WC showed significantly higher rates of fertilization (88.3% ± 2.6 vs. 72.9% ± 0.9, *p* < 0.001), high-quality embryos on day 3 (30.2% ± 1.7 vs. 22.9% ± 0.5, *p* < 0.001), blastocyst development (64.6% ± 2.3 vs. 45.7% ± 0.7, *p* < 0.001), and implantation (19.8% ± 1.1 vs. 9.4% ± 0.3, *p* < 0.001), compared to couples in which at least one partner had abnormal WC (Table 5).

Women with normal WHR showed higher fertilization rate (77.3% ± 1.3 vs. 72.8% ± 1.0, *p* 0.008), number of embryos (6.1 ± 0.2 vs. 5.2 ± 0.2, *p* 0.003), high-quality embryo rate on day 3 (25.0% ± 0.7 vs. 20.9% ± 0.7, *p* < 0.001), blastocyst development rate (48.9% ± 0.8 vs. 44.2% ± 1.0, *p* < 0.001), and implantation rate (11.5% ± 0.4 vs. 10.0% ± 0.4, *p* 0.005) compared to women with abnormal WHR. Men with normal WHR showed higher fertilization rate (77.0% ± 1.6 vs. 72.2% ± 1.0, *p* 0.016), high-quality embryo rate on days 2 (33.9% ± 1.1 vs. 16.6% ± 0.5, *p* < 0.001) and 3 (32.1% ± 1.2 vs. 21.0% ± 0.5, *p* < 0.001), blastocyst development rate (63.8% ± 1.6 vs. 42.7% ± 0.7, *p* < 0.001), implantation rate (18.5% ± 0.7 vs. 6.7% ± 0.2, *p* < 0.001), and pregnancy rate (41.7 vs. 21.0%, *p* 0.016), than those with abnormal WHR. Couples with normal WHR showed significantly higher rates of fertilization (76.7% ± 0.9 vs. 65.9% ± 1.9, *p* < 0.001), high-quality embryos on days 2 (41.0% ± 1.7 vs. 17.9% ± 0.4, *p* < 0.001) and 3 (27.7% ± 1.4 vs. 23.2% ± 0.5, *p* 0.003), blastocyst development (54.4% ± 1.8 vs. 47.3% ± 0.7, *p* < 0.001) and implantation (18.3% ± 0.9 vs. 8.4% ± 0.3, *p* < 0.001),

Table 3 GzLM results for the association between paternal BMI and anthropometric measures, and semen quality

Semen parameters (<i>n</i> = 402)	BMI			WC*			WHR*			WHHR*		
	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>	<i>B</i>	95% <i>CI</i>	<i>p</i>
Volume	-0.099	L -0.164 U -0.035	0.003	0.006	L -0.012 U 0.024	0.528	-1.463	L -3.169 U 0.244	0.093**	-0.013	L -0.045 U 0.019	0.419**
Sperm concentration (mL)	-0.074	L -1.609 U 1.461	0.925	-0.200	L -1.350 U 0.950	0.733	-23.84	L -59.67 U 11.99	0.192	-0.652	L -2.816 U 1.512	0.555
Total sperm concentration (million × 10 ⁶)	-2.756	L -6.738 U 1.226	0.175	-2.397	L -6.012 U 1.219	0.194	-3.627	L -7.200 U -0.054	0.047**	-5.328	L -11.228 U -0.262	0.012**
Progressive sperm motility (%)	-1.523	L -2.309 U -0.736	<0.001	-0.807	L -1.452 U -0.162	0.014**	-1.575	L -30.82 U -0.068	0.040**	-1.262	L -2.483 U -0.041	0.023**
Total motile sperm count (%)	-11.655	L -21.201 U -2.109	0.017	-0.037	L -0.095 U 0.020	0.201**	-5.971	L -10.713 U -1.230	0.014**	-15.040	L -29.548 U -0.532	0.042**

Adjusted for male age, ejaculatory abstinence interval, physical activity, and smoking habit

GzLM generalized linear model, BMI body mass index, WC waist circumference, WHR waist-to-hip ratio, WHHR waist-to-height ratio, B unstandardized regression coefficient, CI confidence interval, L lower, U upper

*Adjusted for BMI; **Significant before adjustment for BMI

compared to couples in which at least one partner had abnormal WHR (Table 6). The remainder ICSI outcomes were not significantly associated with any AM. None of the comparisons were statistically significant for WHtR (data not shown).

Discussion

The objective of this study was to investigate possible associations between maternal and paternal AM and the outcomes of ICSI. Our results demonstrated that higher maternal AM positively impact the total dose of FSH administered and negatively impact the estradiol level on the day of hCG trigger, the number of follicles, the number of obtained oocytes, oocyte yield, and number of mature oocytes. We also observed negative associations between paternal AM and semen volume, total sperm concentration, progressive sperm motility, and TMSC.

As for ICSI outcomes, when maternal AM were analyzed independently of paternal AM, significant differences were observed in all outcomes excepting pregnancy and miscarriage rates, favoring women with AM below the cut off values compared to women above the cut off. When paternal AM were analyzed independently of maternal AM, it was observed that men with AM below the cut off values showed significantly higher rates of fertilization, embryo and blastocyst development, implantation and pregnancy than men above the cut off. When combined maternal and paternal AM were analyzed, significant differences were observed in fertilization, embryo and blastocyst development and implantation rates, favoring couples with normal BMI, WC, and WHR.

Several studies corroborate our findings, suggesting that excess body weight is associated with alterations in the female reproductive system and increases the risk of women infertility [27, 28]. Previous studies have demonstrated a positive association between the total dose of FSH administered and increasing BMI [29–32], and inverse associations between BMI and the number of retrieved oocytes [29, 33, 34], and the oocyte yield [34].

As for ICSI outcomes, previous studies have also showed a negative effect of increasing body fat on fertilization [31, 33] and the number of obtained embryos [29, 31, 34]. Amsiejene, Drasutiene (35) also found no relation between pregnancy outcomes and WHR. On the other hand, there are several studies showing that obesity negatively impacts pregnancy outcomes in IVF treatments [29, 30, 33, 35–37]; however, we failed to demonstrate such association. Additionally, maternal overweight has been shown to increase the time to conception, and the adverse pregnancy outcomes such as diabetes, hypertension, and premature delivery [27].

The mechanism through which excess body weight exerts its detrimental effect upon female fertility is still to be

Table 4 GzLM results for the comparison of ICSI outcomes between female, male and couples with normal and abnormal BMIs

ICSI outcomes (<i>n</i> = 402)	Female			Male			Couple		
	Normal	Abnormal	<i>p</i> value	Normal	Abnormal	<i>p</i> value	Normal ^a	Abnormal ^b	<i>p</i> value
Fertilization rate	76.5 ± 0.9	71.7 ± 1.1	0.001	72.7 ± 1.5	73.6 ± 0.8	0.611	80.0 ± 2.1	73.6 ± 0.8	0.005
Obtained embryos/patient (n)	6.3 ± 0.2	5.0 ± 0.2	<0.001	5.5 ± 0.3	5.8 ± 0.2	0.387	6.4 ± 0.4	5.7 ± 1.6	0.122
High-quality embryos rate on day 2	23.3 ± 0.5	15.3 ± 0.5	<0.001	28.1 ± 0.9	17.4 ± 0.4	<0.001	30.7 ± 1.2	17.7 ± 0.4	<0.001
High-quality embryos rate on day 3	24.3 ± 0.6	22.5 ± 0.7	0.041	24.6 ± 1.0	22.8 ± 0.5	0.090	25.3 ± 1.3	23.3 ± 0.5	0.147
Blastocyst development rate	47.8 ± 0.7	46.4 ± 0.9	0.234	52.7 ± 1.3	45.0 ± 0.6	<0.001	53.5 ± 1.9	46.4 ± 0.6	<0.001
Implantation rate	12.6 ± 0.3	10.1 ± 0.3	<0.001	18.4 ± 0.7	10.0 ± 0.2	<0.001	25.0 ± 1.1	9.5 ± 0.2	<0.001
Pregnancy rate	28.7	24.7	0.508	27.3	26.8	0.951	40.7	23.4	0.153
Miscarriage rate	13.4	6.5	0.362	7.2	12.3	0.560	10.4	11.0	0.955

Values are means ± standard error, unless otherwise noted. Adjusted for maternal and paternal ages, total dose of FSH administered, estradiol level, number of obtained oocytes, sperm concentration and motility, physical activity, and smoking habit

GzLM generalized linear model, ICSI intracytoplasmic sperm injection, BMI body mass index (cut off > 24.9)

^a Both female and male partner

^b Either female or male partner

elucidated. Studies in animal models and humans highlight an adverse effect in hypothalamus–pituitary–ovarian (HPO) axis, and a snowballing impairment of several processes, such as ovulation, oocyte maturation, endometrial development, uterine receptivity, implantation, and maintenance of pregnancy.

Since the adipose tissue is a significant site of steroid production and metabolism, both sexual hormone secretion and bioavailability can be disturbed by excess weight. In fact, central obesity deeply reduces sex hormone-binding globulin (SHBG) serum concentrations, and one study showed that serum SHBG is inversely related to WHR [38]. Low SHBG levels result in higher circulating free testosterone, dihydrotestosterone, and androstenediol, leading to increased hormonal metabolic clearance and subsequent hyperandrogenism, which

are particularly evident in women with central obesity [9]. The consequent negative effect of hyperandrogenism upon follicular development and ovulation are well known.

Additionally, excess weight exerts its negative impact on female reproduction by disturbing insulin and leptin levels. Central obesity creates a condition of hyperinsulinemia and insulin resistance [9]. Insulin plays important roles in the ovarian steroidogenesis, upregulation of lutein hormone (LH) receptor [39], enhancement of gonadotrophic cells sensitivity to GnRH [39], and modulation of sex steroid bioavailability via inhibition of SHBG synthesis [38]. Leptin is a messenger protein secreted by the adipose tissue, which stimulates the HPO axis under normal physiologic concentrations. However, excess weight elevates leptin serum concentration,

Table 5 GzLM results for the comparison of ICSI outcomes between female, male and couples with normal and abnormal WC

ICSI outcomes (<i>n</i> = 402)	Female			Male			Couple		
	Normal	Abnormal	<i>p</i> value	Normal	Abnormal	<i>p</i> value	Normal ^a	Abnormal ^b	<i>p</i> value
Fertilization rate	76.9 ± 1.4	72.7 ± 0.9	0.011	74.5 ± 1.7	73.3 ± 1.0	0.587	88.3 ± 2.6	72.9 ± 0.9	<0.001
Obtained embryos/patient (n)	6.3 ± 0.3	5.4 ± 0.2	0.007	5.6 ± 0.3	5.6 ± 0.2	0.901	6.5 ± 0.5	5.6 ± 0.2	0.099
High-quality embryos rate on day 2	25.0 ± 0.8	20.3 ± 0.5	<0.001	26.4 ± 1.0	20.1 ± 0.5	<0.001	22.5 ± 1.2	21.1 ± 0.4	0.268
High-quality embryos rate on day 3	25.2 ± 1.0	22.4 ± 0.6	0.024	26.4 ± 1.1	23.1 ± 0.6	0.013	30.2 ± 1.7	22.9 ± 0.5	<0.001
Blastocyst development rate	51.4 ± 1.1	44.4 ± 0.7	<0.001	55.0 ± 1.5	45.8 ± 0.8	<0.001	64.6 ± 2.3	45.7 ± 0.7	<0.001
Implantation rate	17.3 ± 0.5	8.6 ± 0.3	<0.001	13.2 ± 0.7	9.1 ± 0.3	<0.001	19.8 ± 1.1	9.4 ± 0.3	<0.001
Pregnancy rate	29.5	27.0	0.718	41.2	22.1	0.021	45.0	26.3	0.133
Miscarriage rate	5.6	14.5	0.205	6.7	16.4	0.226	5.3	12.6	0.319

Values are means ± standard error, unless otherwise noted. Adjusted for body mass index, maternal and paternal ages, total dose of FSH administered, estradiol level, number of obtained oocytes, sperm concentration and motility, physical activity, and smoking habit

GzLM generalized linear model, ICSI intracytoplasmic sperm injection, WC waist circumference (female cut off > 80 cm, male cut off > 94 cm)

^a Both female and male partner

^b Either female or male partner

Table 6 GzLM results for the comparison of ICSI outcomes between female, male, and couples with normal and abnormal WHR

ICSI outcomes (<i>n</i> = 402)	Female			Male			Couple		
	Normal	Abnormal	<i>p</i> value	Normal	Abnormal	<i>p</i> value	Normal ^a	Abnormal ^b	<i>p</i> value
Fertilization rate	77.3 ± 1.3	72.8 ± 1.0	0.008	77.0 ± 1.6	72.2 ± 1.0	0.016	76.7 ± 0.9	65.9 ± 1.9	< 0.001
Obtained embryos/patient (n)	6.1 ± 0.2	5.2 ± 0.2	0.003	5.7 ± 0.3	5.6 ± 0.2	0.842	5.6 ± 0.2	5.7 ± 0.4	0.883
High-quality embryos rate on day 2	21.4 ± 0.6	21.4 ± 0.6	0.983	33.9 ± 1.1	16.6 ± 0.5	< 0.001	41.0 ± 1.7	17.9 ± 0.4	< 0.001
High-quality embryos rate on day 3	25.0 ± 0.7	20.9 ± 0.7	< 0.001	32.1 ± 1.2	21.0 ± 0.5	< 0.001	27.7 ± 1.4	23.2 ± 0.5	0.003
Blastocyst development rate	48.9 ± 0.8	44.2 ± 1.0	< 0.001	63.8 ± 1.6	42.7 ± 0.7	< 0.001	54.4 ± 1.8	47.3 ± 0.7	< 0.001
Implantation rate	11.5 ± 0.4	10.0 ± 0.4	0.005	18.5 ± 0.7	6.7 ± 0.2	< 0.001	18.3 ± 0.9	8.4 ± 0.3	< 0.001
Pregnancy rate	27.8	26.0	0.777	41.7	21.0	0.016	38.4	24.2	0.177
Miscarriage rate	9.5	15.5	0.510	16.7	11.5	0.608	4.7	15.3	0.174

Values are means ± standard error, unless otherwise noted. Adjusted for body mass index, maternal and paternal ages, total dose of FSH administered, estradiol level, number of obtained oocytes, sperm concentration and motility, physical activity, and smoking habit

GzLM generalized linear model, ICSI intracytoplasmic sperm injection, WHR waist-to-hip ratio (female cut off ≥ 0.85, male cut off ≥ 0.90)

^a Both female and male partner

^b Either female or male partner

negatively affecting GnRH secretion [40–42], ovarian steroidogenesis [43–45], folliculogenesis regulation [46], and perifollicular blood flow [47, 48].

A snowball effect may also occur due to gonadotropin resistance in women with excess weight, which may lead to reduced oocyte competence [49]. As initial embryonic development is mainly dependent on the oocyte machinery, one might expect that poor oocyte quality would affect embryo development, leading to reduced implantation potential.

We also observed that excess weight negatively impacts semen quality. In fact, sperm motility and count were negatively influenced by all AM, suggesting that semen quality is highly affected by both body fat amount and distribution. In accordance, Fejes, Koloszar (18) found that male weight was inversely correlated to sperm count and TMSC, and AMs were inversely correlated to semen volume, sperm count, total motile sperm cell number, and sperm motility. Hammiche, Laven (19) observed that BMI is inversely related to sperm motility, ejaculate volume, sperm concentration, and TMSC. In addition, an inverse relation was also observed between WC and sperm concentration and TMSC. Sermondade, Faure (15), in a collaborative meta-analysis, observed that overweight and obesity were associated with an increased prevalence of azoospermia or oligozoospermia.

Data from the Longitudinal Investigation of Fertility and the Environment study showed that both BMI and WC are inversely related to ejaculate volume and sperm count [17]. A more recent study showed that the total sperm count and sperm concentration are negatively affected by increasing BMI, WC, WHtR [20]. However, one study revealed that AM could not predict male semen quality [50]. A recent meta-analysis demonstrated that increasing male BMI was

associated with a significant reduction in clinical pregnancy rate and live birth rate in IVF cycles [16].

The etiology of reduced fertility in men with excess body weight is multifactorial. As it happens in women, the excess weight deregulates the hypothalamus–pituitary–gonadal axis and the endocrine profile, altering the production and regulation of insulin, SHBG, leptin and inhibin B, and testosterone/estradiol ratio, ultimately resulting in abnormal semen parameters [51]. Accordingly, previous studies demonstrated that BMI and all AM and derived ratios are inversely correlated with testosterone and serum SHBG levels, and testosterone/estradiol ratio [18, 52–56].

In addition, the excessive adiposity chronically inflames the body and it has been hypothesized that there is an increase in the formation of reactive oxygen species, which can induce damage to DNA, and stress the testicular environment [57]. In fact, positive correlations were observed between BMI and sperm oxidative stress [58, 59] and negative correlations between obesity and sperm DNA integrity [60, 61].

The British Fertility Society advises clinicians to counsel patients to aim for a normal BMI before starting infertility treatment [62]. In fact, weight loss is the first-line therapy for obese women pursuing infertility treatment, and several evidences support that weight loss in both overweight and obese women improves ovulatory frequency, pregnancy rates, and cost-effectiveness per pregnancy [62–67]. However, the effect of weight loss on semen quality and male fertility potential remains controversial. Despite that there is evidence of short-term improvement, the long-term effect of weight loss in male fertility is still to be elucidated [51]. According to the results of this study, male fat distribution has a significant negative impact on male fertility and ICSI outcomes,

which calls for prompt attentiveness and recognition in the field of reproductive medicine.

The strength of our study relies on the number of couples included in the analysis and in the assessment of the combination between maternal and paternal AM. The major limitation is the fact that we did not take eating habits, stress, medication and environmental exposure, co-pathologies, and others into account, which makes it difficult to infer that a particular factor is the sole cause of reduced outcomes. Additionally, under-reporting in self-reporting of lifestyle factors is not uncommon and may have biased some of the results. Also, the chosen sample population is not representative of the general population, as we deal with a population of sub-fertile couples.

Conclusions

Our findings suggest that lower AM have a protective effect on female and male fertility. It is important that both partners with excess weight attending IVF centers are given the appropriate advice and support to control body weight through sustainable and healthy lifestyle modification. Awareness about these factors and counseling on how to minimize its impact in each partner could increase couples' response to IVF treatment and the chance of a more favorable outcome.

Compliance with ethical standards

A written informed consent was obtained in which patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local Institutional Review Board (410/2010).

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