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Relationship between Ovarian Reserve Markers and Body Mass Index in Infertile Women with and without Polycystic Ovary Syndrome: A Retrospective Case–Control Study

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Abstract: This study, carried out on 94 women with polycystic ovary syndrome (PCOS) and 176 controls without it, investigated the influence of body mass index (BMI) on serum levels of antimüllerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and 17ß-estradiol (E2) in infertile patients. Patients were assigned to four subgroups according to age (<35 or $\geq$35 years) and BMI (<25 kg/m^2 or $\geq$25 kg/m^2). Significant differences between PCOS-affected and control women were observed with respect to most of the parameters of interest. In both PCOS-affected and control women, age was inversely correlated with AMH. In the control patients, age was directly correlated with FSH and LH. In women affected by PCOS, no correlation was found between BMI and serum levels of AMH, E2, and LH, except FSH. No correlation was found between BMI and markers of ovarian reserve in control women. BMI was not correlated with AMH in any of the four subgroups considered regardless of the presence of PCOS. An inverse correlation was found only in PCOS-affected women aged $\geq$35 years between a BMI < 25/FSH and a BMI $\geq$ 25/LH. The possible association between BMI and ovulation disorder (OD) was investigated in 96 control women aged $\leq$37 years. In women with OD, the BMI values were significantly higher and FSH and E2 levels were lower than those of patients without OD. Taken together, our data suggest that BMI is not related to hormonal parameters of ovarian reserve, irrespective of the presence of PCOS, and could influence ovulation disorder rather than cause a decrease in ovarian reserve.

Keywords: anti-Müllerian hormone; body mass index; ovarian reserve; ovulation; polycystic ovarian syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women of reproductive age. The combined effect of a complex series of factors including altered oocytes, reduced embryo quality, defective endometrial receptivity, as well as infertility-related complications leads to a general reduced reproductive outcome in affected women [1]. PCOS is characterized by oligo-anovulation, hyperandrogenism, and polycystic ovaries, and is closely associated with obesity. A meta-analysis of cross-sectional and retrospective studies showed that women with PCOS had a higher prevalence of being overweight, obesity, and central obesity compared to women without PCOS [2]. Body mass index (BMI) provides a practically useful population-level measure of overweight and obesity since it is the same for both sexes and for all ages of adults. High BMI values have

been associated with anovulation, abnormal uterine bleeding, gynecological cancers, endometrial hyperplasia, lower spontaneous pregnancy rates, and miscarriage, and have also been associated with poor outcomes among women undergoing in vitro fertilization [3–7]. However, the relationship between BMI and both anti-Müllerian hormone (AMH) and ovarian reserve is still unclear and controversial. In fact, some studies showed a significant inverse correlation between markers of ovarian reserve and obesity [8–10]; conversely, other studies did not find this relationship [11–15]. These discrepancies could be, at least in part, explained by the large variability in age of the subjects included in most of the above studies; this could have influenced both BMI and serum AMH levels. To further clarify this controversial issue, the present study was carried out to investigate the relationship between BMI and serum levels of AMH, follicle stimulating hormone (FSH), luteinizing hormone (LH) and 17ßestradiol (E2), in a cohort of infertile women with and without PCOS, stratified by age and BMI. A secondary objective was to further investigate the role of BMI in ovulation disorder (OD) among young women without PCOS.

2. Materials and Methods

2.1. Subjects and Study Design

This retrospective case–control study was carried out in a cohort of infertile women who attended the Infertility Center of the Policlinico Tor Vergata University Hospital, Section of Gynecology, between 1 October 2016 and 1 December 2019. The study was approved by the Institutional Review Board of the Policlinico Tor Vergata University Hospital on 26 February 2020 (Protocol number: 26/20). Infertility is defined as a lack of conception after at least one year of unprotected intercourse. At the time of their first visit, all patients gave their informed written consent to the use of their own clinical, hormonal, and instrumental data for the research. All women underwent a complete clinical and gynecological examination during which their BMI was calculated. Furthermore, the type of infertility was recorded and defined as follows; it was primary if the woman never conceived and secondary for those incapable of conceiving after at least one previous successful conception. The women in the study then underwent a standardized infertility evaluation, which included at least two semen analyses of the male partner, a hysterosalpingogram and/or laparoscopy, a transvaginal ultrasound performed in the early follicular phase of their cycle, and a hysteroscopy; ovulation was verified using ultrasound and hormonal dosages. Furthermore, serum levels of total and HDL cholesterol, triglycerides, and glucose were detected in order to exclude patients with metabolic syndrome, diabetes, or glucose intolerance.

Exclusion criteria were congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, galactorrhea, hyperprolactinemia, thyroid dysfunctions and other endocrinological disorders, autoimmune diseases, premature ovarian failure (an antral follicle count (AFC) of <5 follicles, AMH of <1.1 ng/mL and/or FSH of >12 mIU/mL), ovarian cysts or tumors, or endometriosis, or previous ovarian and uterine surgery or chemotherapy.

To investigate the effect of BMI and PCOS on markers of ovarian reserve, which is the main objective of the present study, 270 women were included in the study and divided into two major groups: group 1, which included infertile women with PCOS (*n* = 94) and group 2, which included infertile women without PCOS (*n* = 176).

Group 1 was composed of subjects who were diagnosed with PCOS without any other detectable cause of infertility. PCOS is defined by the Rotterdam classification [16], that is, the presence of at least two of the following criteria: oligo-anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovarian morphology (PCO) in the absence of other etiologies. A serum AMH threshold of >4.6 ng/mL was used as an alternative to antral follicle count and/or hyperandrogenism [11]. PCO was defined using a threshold of at least 12 follicles (2–9 mm in diameter) per whole ovary and/or increased ovarian volume (>10 mL).

Group 2 (control patients) included women without PCOS. Their infertility was related to a male factor, tubal infertility, an ovulation disorder (OD), or it was unexplained. Infertil-

ity was defined as unexplained when the above-reported standard fertility evaluation was normal in women with regular cycles. Women were diagnosed with ovulation disorder (OD) when their cycles were abnormally short (<21 days) or long (>35 days), or when they had oligomenorrhea (<8 cycles per year). All women in group 2 had morphologically normal ovaries and none of them had clinical or biochemical hyperandrogenism.

The women in the study were then compared for the following parameters of interest:

1. Hormonal parameters: serum AMH, FSH, LH, Inhibin B, free testosterone, and delta 4-androstenedione;
2. Metabolic biochemical parameters: serum levels of total and HDL cholesterol, triglycerides, and glucose;
3. Ultrasound parameters: The number of follicles in each ovary and the ovarian volume of each ovary.

The women in the study were further stratified into the following subgroups according to their age and BMI:

-   Subgroup A = age < 35 years and BMI < 25 kg/m^2; this was composed of 34 women with PCOS (A1) and 43 control women (A2);
-   Subgroup B = age < 35 years and BMI $\geq$ 25 kg/m^2; this was composed of 34 women with PCOS (B1) and 21 control women (B2);
-   Subgroup C = age $\geq$ 35 years and BMI < 25 kg/m^2; this was composed of 14 women with PCOS (C1) and 70 control women (C2);
-   Subgroup D = age $\geq$ 35 years and BMI $\geq$ 25 kg/m^2; this was composed of 12 women with PCOS (D1) and 42 control women (D2);

To investigate the possible association between BMI and ovulation disorder (OD) by excluding age and PCOS as possible confounder factors—which was another objective of the present study—96 of the 176 women of group 2, aged $\leq$37 years, were also divided into two additional subgroups. The first subgroup included 22 women with OD, and the second included 74 women with regular cycles and documented ovulation.

2.2. Ultrasound Evaluation

All patients underwent pelvic ultrasound and peripheral venipuncture on day 2 or 3 of a spontaneous or progesterone-induced cycle (in women with oligomenorrhea).

The ultrasound device used was Hitachi H21 HI Vision (the probe frequency was 6.5 MHz). Through a slow and careful scan from one margin of the ovary to the other, all follicles between 2 and 9 mm were considered to define the AFC. Ovarian volume was calculated using the following formula: V = 0.526 × length × height × width.

2.3. Hormone and Metabolic Marker Assays

Peripheral blood was collected as previously reported and centrifuged at 3000 rpm, and serum was stored at −80 °C until successive assays were performed.

AMH levels were measured using the AMH Gen II ELISA kit (reference A79765 Beckman Coulter, Inc., Brea, CA, USA). FSH, LH and E$_2$ levels were determined using immunoassay systems (ADVIA Centaur, Siemens Healthcare Diagnostic Inc., Malvern, PA, USA); delta 4-androstenedione was measured using automatic immunoassay systems (Immulite/Immulite 1000, Siemens Medical Solutions Diagnostics, Malvern, PA, USA). Serum levels of free testosterone (FT) and inhibin B were measured using RIA (DIA source Immunoassay SA, Nivelles, Belgium) and using the Inhibin Gen II enzyme linked immunosorbent assay kit (reference A81303, Beckman Coulter, Inc., Brea, CA, USA), respectively. The intra- and inter-assay coefficients of variation were <9% for all assays.

Total cholesterol, HDL-C, triglycerides, and glucose were also measured using standard enzymatic methods (Dimension Vista System, Siemens Healthcare Diagnostic Inc., Malvern, PA, USA).

2.4. Data Handling

The data of interest were reported in a preconceived template. A computerized database was developed that was available for successive analyses. Any collected information was anonymized and deidentified prior to analysis.

2.5. Statistical Analysis

A preliminary power analysis of the study was carried out. With an expected BMI variation = 10% between the two groups (group 1 and group 2), a minimum sample size of 80 women was needed for each group to reach a power of 85% when the alpha and beta values were 0.05 and 0.15, respectively.

The Kolmogorov–Smirnov test was used to check the distribution of the values that resulted to be non-Gaussian. The variances were analyzed via Levene's test and resulted to be non-homogeneous for the vast majority of the variables of interest. Therefore, the data have been reported as medians and ranges and analyzed via nonparametric tests. Specifically, comparisons between two groups were performed using the Mann–Whitney U test; the Kruskal–Wallis, followed by Dunn's test as a post hoc test, was used for multiple comparisons among groups. Correlations were analyzed using the Spearman's rank test. All analyses were carried out using the Windows statistical package for the Social Sciences, version 15.0 (IBM SPSS, Chicago, IL, USA). The significance was established at $p < 0.05$.

3. Results

The main clinical characteristics, as well as the laboratory and ultrasonographic findings of the two groups, are shown in Table 1. The age of the control women was significantly higher than that of the women affected by PCOS; on the contrary, a significantly higher BMI was found in women with PCOS. All serum levels of the assayed hormones were significantly different in women with PCOS than in control women, with the exception of E2. The US findings revealed that the number of antral follicles and ovarian volumes was higher in PCOS-affected patients than in control women. The serum levels of total cholesterol and glucose were similar in the two groups of women, while levels of triglycerides and HDL-C were higher and lower, respectively, in PCOS-affected women than in control women (Table 1).

Table 1. Clinical, ultrasonographic and laboratory characteristics of women in the study.

	Group 1 Women with PCOS (n = 94)	Group 2 Control Women (n = 176)	p *
Age (years)	32 (21–43)	37 (24–47)	<0.00001
BMI (kg/m^2)	24.9 (18.4–43.9)	23.0 (17.2–41.9)	0.03
AMH (ng/mL)	6.1 (1–24.5)	1.6 (0–10)	<0.0001
FSH (mIU/mL)	5.9 (1.1–16.2)	7.6 (0.8–37.5)	<0.00001
LH (mIU/mL)	5.5 (0.6–29.8)	4.8 (0.1–29.3)	0.029
E2 (pg/mL)	43.2 (2.5–123)	41.5 (8–206)	0.51
Inhibin B (pg/mL)	60.5 (8.2–288.3)	46.3 (0.1–189)	<0.00001
Free testosterone (pg/mL)	1.4 (0.2–7.5)	0.7 (0.12–3)	0.007
Androstenedione (ng/mL)	2.7 (0.5–10)	1.7 (0.3–3.5)	<0.00001
Follicle number (2–9 mm) right ovary	17 (0–55)	6 (0–33)	<0.00001
Follicle number (2–9 mm) left ovary	14 (2–45)	6 (0–30)	<0.00001
Right ovarian volume (mL)	9.1(1–51.4)	3.6 (0.3–28.6)	<0.00001
Left ovarian volume (mL)	8.7 (1.2–36.4)	3.0 (0.7–40.3)	<0.00001
HDL-C (mg/dL)	57.0 (28–100)	60.4 (34–102)	0.0002
Total cholesterol (mg/dL)	189.5 (27.4–403)	187.0 (127–267)	0.091
Triglycerides (mg/dL)	90.0 (27–313)	69.5 (34–182)	<0.00001
Glucose (mg/dL)	86.5 (68–121)	86.0 (65–116)	0.34

Values are shown as median (range); * Mann–Whitney U test.

The Spearman's rank correlation analysis carried out on all women in the study revealed that age was inversely correlated with AMH in both PCOS-affected and control women. Furthermore, age was directly correlated with FSH and LH only in control women (Table 2). On the contrary, none of the hormonal parameters was correlated with BMI, except for FSH in the group of women with PCOS (r = -0.25, $p = 0.014$) (Table 2).

Table 2. Spearman's rank correlation between BMI or age and hormonal parameters of ovarian reserve in PCOS-affected and control women.

	Group 1 PCOS Women (*n* = 94)	Group 2 Control Women (*n* = 176)
Age/AMH	r = -0.22 $p = 0.031$	r = -0.36 $p = 0.00001$
Age/FSH	r = 0.19 $p = 0.056$	r = 0.33 $p = 0.00001$
Age/LH	r = -0.13 $p = 0.21$	r = 0.22 $p = 0.003$
Age/E2	r = 0.084 $p = 0.42$	r = 0.08 $p = 0.24$
BMI/AMH	r = -0.01 $p = 0.92$	r = -0.01 $p = 0.83$
BMI/FSH	r = -0.25 $p = 0.014$	r = -0.01 $p = 0.88$
BMI/LH	r = -0.06 $p = 0.56$	r = -0.07 $p = 0.34$
BMI/E2	r = 0.09 $p = 0.37$	r = -0.10 $p = 0.15$

The subgroup analysis of hormonal parameters of ovarian reserve in PCOS-affected women (Group 1) and control women (Group 2), stratified by age and BMI, is shown in Table 3.

Table 3. Hormonal parameters of ovarian reserve in women with and without PCOS stratified in four groups according to age and BMI.

Group 1 Women with PCOS (*n* = 94) Subgroup	A1 [*n* = 34] age < 35 years BMI < 25 kg/m^2	B1 [*n* = 34] age < 35 years BMI $\geq$ 25 kg/m^2	C1 [*n* = 14] age $\geq$ 35 years BMI < 25 kg/m^2	D1 [*n* = 12] age $\geq$ 35 years BMI $\geq$ 25 kg/m^2	*p*-value *
AMH (ng/mL)	6.5 (2.8–16.6)	6.2 (2.1–24.5)	3.8 (1–8.2)	3.9 (1.1–15)	0.02 NS °
FSH (mIU/mL)	6.0 (1.1–10.3)	5.7 (2–9.9)	6.8 (4–16.2)	5.3 (2.8–8.5)	0.03 B1 vs. C1 0.005 °
LH (mIU/mL)	5.4 (0.6–29.8)	6.9 (1.1–22.2)	5.3 (2.1–14.3)	3.9 (1.7–19.5)	0.16
E2 (pg/mL)	37.9 (11.8–99.2)	45.5 (2.5–123)	44.6 (20–74.5)	41.1 (11–60)	0.18

Table 3. *Cont.*

Subgroup	A2 [*n* = 41] age < 35 years BMI < 25 kg/m²	B2 [*n* = 21] age < 35 years BMI ≥ 25 kg/m²	C2 [*n* = 70] age ≥ 35 years BMI < 25 kg/m²	D2 [*n* = 42] age ≥ 35 years BMI ≥ 25 kg/m²	*p*-value *
Group 2 Women without PCOS (*n* = 176)					
AMH (ng/mL)	2.9 (0.3–6.3)	1.6 (0.3–8.9)	1.4 (0–10)	1.5 (0.1–5.7)	0.0001 A2 vs. C2 0.00003 ° A2 vs. D2 0.0003 °
FSH (mIU/mL)	6.4 (0.8–22.6)	7.6 (4.6–16.8)	8.6 (3–37.5)	8.2 (2.5–26.8)	0.0004 A2 vs. C2 0.00003 ° A2 vs. D2 0.003 °
LH (mIU/mL)	4.6 (0.1–12.3)	3.9 (1.1–11.5)	5.1 (1.9–29.3)	5.7 (1.7–22.2)	0.054
E2 (pg/mL)	41.5 (20–206)	35.9 (9–94.9)	46.1 (8.0–194)	39.6 (12.5–149)	0.81

Values are shown as median (range); * Kruskal-Wallis' test; ° Dunn's test; NS = not significant.

The Kruskal–Wallis analysis of variance carried out in the four subgroups (A1, B1, C1, D1) of women with PCOS showed that no differences could be found in E2 and LH levels, while a significant difference was detected in FSH and AMH levels. This difference was confirmed via Dunn's test in the case of FSH—the levels of which were higher in the C1 subgroup than in the B1 subgroup—and disappeared in the case of AMH levels (Table 3, top).

In control women (Group 2), no statistically significant differences between subgroups were found in the serum levels of LH and E2. On the contrary, the levels of AMH and FSH were higher and lower, respectively, in subgroup A2 compared to the subgroups C2 and D2. This finding was confirmed after post hoc analysis (Table 3, bottom). Spearman's correlation was then carried out on the women in the study stratified into subgroups by age and BMI. The following significant correlations were found: In women with PCOS, Age/AMH (subgroup D1, inverse correlation); Age/FSH (subgroup D1, direct correlation); BMI/FSH (subgroup C1, inverse correlation); BMI/LH (subgroup D1, inverse correlation) (Table 4).

Table 4. Spearman's rank correlation between BMI or age and hormonal parameters of ovarian reserve in PCOS-affected women stratified by BMI and age.

	A1 [*n* = 34] age < 35 years BMI < 25 kg/m²	B1 [*n* = 34] age < 35 years BMI ≥ 25 kg/m²	C1 [*n* = 14] age ≥ 35 years BMI < 25 kg/m²	D1 [*n* = 12] age ≥ 35 years BMI ≥ 25 kg/m²
Age/AMH	r = −0.008 p = 0.96	r = 0.21 p = 0.23	r = −0.34 p = 0.22	r = −0.67 p = 0.015
Age/FSH	r = −0.01 p = 0.93	r = 0.10 p = 0.57	r = −0.33 p = 0.90	r = 0.62 p = 0.029
Age/LH	r = 0.11 p = 0.53	r = 0.15 p = 0.39	r = −0.46 p = 0.097	r = −0.04 p = 0.88
Age/E2	r = 0.14 p = 0.40	r = 0.31 p = 0.072	r = 0.34 p = 0.22	r = 0.07 p = 0.82
BMI/AMH	r = −0.5 p = 0.74	r = 0.12 p = 0.50	r = 0.24 p = 0.41	r = −0.13 p = 0.68

Table 4. *Cont.*

	A1 [*n* = 34] age < 35 years BMI < 25 kg/m^2	B1 [*n* = 34] age < 35 years BMI ≥ 25 kg/m^2	C1 [*n* = 14] age ≥ 35 years BMI < 25 kg/m^2	D1 [*n* = 12] age ≥ 35 years BMI ≥ 25 kg/m^2
BMI/FSH	r = −0.03 *p* = 0.85	r = −0.02 *p* = 0.90	r = −0.54 *p* = 0.045	r = −0.35 *p* = 0.27
BMI/LH	r = 0.04 *p* = 0.78	r = −0.13 *p* = 0.43	r = −0.28 *p* = 0.32	r = −0.63 *p* = 0.029
BMI/E2	r = −0.004 *p* = 0.81	r = 0.09 *p* = 0.60	r = 0.27 *p* = 0.34	r = −0.31 *p* = 0.33

In control women, Age/AMH (subgroup C2, inverse correlation); Age/FSH (subgroup C2, direct correlation) (Table 5).

Table 5. Spearman's rank correlation between BMI or age and hormonal parameters of ovarian reserve in control women stratified by BMI and age.

	A2 [*n* = 43] age < 35 years BMI < 25 kg/m^2	B2 [*n* = 21] age < 35 years BMI ≥ 25 kg/m^2	C2 [*n* = 70] age ≥ 35 years BMI < 25 kg/m^2	D2 [*n* = 42] age ≥ 35 years BMI ≥ 25 kg/m^2
Age/AMH	r = −0.01 *p* = 0.93	r = −0.31 *p* = 0.17	r = −0.29 *p* = 0.015	r = −0.12 *p* = 0.44
Age/FSH	r = −0.004 *p* = 0.81	r = −0.031 *p* = 0.89	r = 0.34 *p* = 0.004	r = −0.07 *p* = 0.67
Age/LH	r = 0.17 *p* = 0.28	r = −0.11 *p*= 0.62	r = 0.12 *p* = 0.30	r = 0.04 *p* = 0.81
Age/E2	r = 0.09 *p* = 0.55	r = 0.20 *p* = 0.39	r = −0.18 *p* = 0.14	r = 0.22 *p* = 0.16
BMI/AMH	r = 0.11 *p* = 0.47	r = −0.12 *p* = 0.60	r = 0.18 *p* = 0.14	r = 0.15 *p* = 0.34
BMI/FSH	r = 0.06 *p* = 0.70	r = −0.38 *p* = 0.09	r = −0.22 *p* = 0.07	r = −0.04 *p* = 0.80
BMI/LH	r = 0.032 *p* = 0.84	r = −0.18 *p* = 0.44	r = −0.18 *p* = 0.14	r = 0.17 *p* = 0.29
BMI/E2	r = 0.083 *p* = 0.59	r = −0.18 *p* = 0.44	r = 0.098 *p* = 0.42	r = −0.06 *p* = 0.70

The possible association between BMI and ovulation disorder (OD), excluding age and PCOS as potential confounders, was investigated in 96 women aged ≤37 years, belonging to the control group, of which 22 were with OD and 74 were without OD. In these women, the BMI values were significantly higher and those of FSH and E2 were significantly lower in patients with OD than in patients without OD. There were no statistically significant differences in age, serum AMH, and LH levels between the two groups (Table 6). The Spearman's analysis carried out on these subjects revealed the absence of any correlation between BMI and hormonal parameters of ovarian reserve regardless of the presence of OD (Table S1).

Table 6. Clinical and hormonal parameters in control women with and without ovulation disorders (OD).

	Women with OD (*n* = 22)	Women without OD (*n* = 74)	*p*-Value (*)
Age (years)	35 (24−37)	33 (24−37)	0.14
BMI (kg/m^2)	26.1 (18.7−35.7)	22.1 (17.2−41.9)	0.001
AMH (ng/mL)	1.6 (0.1−6.3)	2.3 (0.1−8.9)	0.106
FSH (mIU/mL)	6.1 (0.8−12.1)	7.1 (3.5−25.0)	0.006
LH (mIU/mL)	4.2 (0.1−9)	4.7 (1.1−29.3)	0.279
E2 (pg/mL)	33.2 (12.5−74)	43.6 (9−206)	0.031

Values are shown as median (range); * Mann–Whitney test.

4. Discussion

PCOS is a common endocrine condition characterized by a dysregulation of the female reproductive axis leading to several features such as hyperandrogenism, anovulation, a polycystic appearance of the ovaries, insulin resistance, and metabolic syndrome that can be present with different rates and combinations in different women. Recent evidence shows that PCOS is much more complex than was previously believed since in women with PCOS other comorbidities and alterations of relevant factors are often coexisting leading to endometrial dysfunction, impaired oocyte development and quality, embryo competence, and defective placentation when pregnancy occurs. All of these abnormalities reduce overall reproductive performance [17,18].

The relationship between BMI and markers of ovarian reserve is complex and still controversial. Previous studies on this issue have shown conflicting results. Casadei et al. [11] found no significant correlation between AMH and BMI in infertile women, regardless of the presence of PCOS. Gorkem et al. [12] evaluated infertile women under 45 years of age assigned to three ovarian reserve groups. They reported that the obesity parameters (BMI and waist circumference) had no relevant effect on the ovarian reserve markers (AMH, E2 and FSH) investigated. Simões-Pereira et al. [13] explored the association between BMI and serum AMH levels in women without PCOS. They reported that AMH was not correlated with BMI and that age was the only variable significantly associated with AMH. Shaw et al. [19] examined premenopausal women, 16% of whom were obese women younger than 45 years, and found no correlation between AMH levels and BMI. Other authors [14,15] concluded that there was no significant association between serum AMH and BMI. On the contrary, other studies reported a significant and strong negative correlation between BMI and AMH in controls and/or in women with PCOS. Moy et al. [10] noted that obesity is inversely correlated with AMH in Caucasian women, but not in other racial groups. Lefebvre et al. [20] reported that no effect of metabolic status was found on serum AMH levels in infertile women without PCOS; conversely, they found an independent, although weak, significant negative correlation between AMH and BMI in women affected by PCOS. Moslehi et al. [21] in a recent systematic review and meta-analysis reported that serum levels of AMH and FSH were significantly lower in obese women than in non-obese women; moreover, they reported that BMI was negatively correlated with AMH in all study populations and with FSH in fertile non-PCOS subgroups. On the other hand, surprisingly, Albu and Albu [22] reported that in a large cohort of infertile women without PCOS, a BMI ≥25 kg/m^2, after adjustment for age, was significantly associated with higher AMH serum levels; in their study, a mild excess of adiposity appeared to be associated with higher AMH serum levels.

The main objective of the present study was to investigate the influence of being overweight on ovarian reserve measures in women with and without PCOS. The analysis of the clinical characteristics of the study women showed that the two populations of women (PCOS-affected and control patients) were markedly different in almost all clinical, hormonal, and metabolic parameters (Table 1). Interestingly, we found that the BMI of women with PCOS was higher than that of control women, who, however, were older.

Taken together, our findings do not support the concept that a high BMI affects ovarian reserve. Indeed, a significant correlation between age and the three main markers of ovarian reserve (AMH, FSH, and LH) was found only in control women, whereas no correlation was found between BMI and all hormonal parameters investigated (Table 2). Substantially similar findings were observed in women with PCOS in which a significant inverse correlation was also detected between age and AMH; this correlation disappeared in the case of FSH and LH. This finding can be explained by the effect of age on AMH levels regardless of the presence of PCOS. With regard to BMI, in PCOS-affected women, the only significant inverse correlation with FSH was found. Low serum FSH levels can be present in women with PCOS and are correlated with anovulation, although this neuroendocrine feature of PCOS remains unclear.

A major strength of the present study is the assignment of women to subgroups established according to age and BMI to eliminate age as a confounding factor in the relationship between BMI and the parameters of ovarian reserve, particularly AMH. Despite the small sample size, which could represent a potential limitation of the study, the results suggest that BMI does not have a negative impact on the ovarian reserve. Several considerations support this concept:

(1) With regard to the PCOS group, (a) the serum levels of FSH in the C1 subgroup, which includes women with high age and low BMI, are higher than those found in the B1 subgroup, which includes women of a young age and high BMI; the finding of high values of serum FSH levels in the subgroup of PCOS-affected women with normal weight and ≥35 years seems to be due to age only; (b) serum levels of AMH, LH, and E2 do not change in any of the subgroups of PCOS-affected women irrespective of BMI (Table 3, top); (c) there is no correlation between BMI and AMH in any of the four subgroups of women considered (Table 4):

The finding of a significant inverse correlation between BMI and FSH in the C1 subgroup of women (of old age and with a low BMI) but not in the D1 subgroup of women (of old age and with a high BMI) and the finding of a significant inverse correlation between BMI and LH in the D1 subgroup of women but not in the C1 subgroup of women argue against a relevant effect of BMI (Table 4).

(2) With regard to the control group, the higher AMH and the lower FSH serum levels in the A2 subgroup of women than in both C2 and D2 subgroups of women are due to age rather than BMI (Table 3, bottom part); this hypothesis is further strengthened by the absence of any correlation between the BMI and the hormonal parameters considered (Table 5).

The present study also explored the association between BMI and OD in control women aged ≤37 years in order to avoid confounding factors such as PCOS and age. BMI values were significantly higher and serum levels of FSH and E2 were significantly lower in women with OD than in women without OD, even if no statistically significant differences were found in serum levels of AMH and LH. Furthermore, no correlation was found between BMI and the hormonal parameters of ovarian reserve irrespective of the presence of OD (SM Table S1).

The relationship between BMI, serum FSH levels, and ovulation disorder in women with and without PCOS is complex and still unclear. Catteau-Jonard et al. [23] reported a significantly higher BMI and lower FSH levels in women with OD compared to those in women without OD, in a cohort of infertile non-PCOS obese women. Being overweight and obesity could be etiological factors of infertility or factors that contribute to infertility; further studies are still needed to fully clarify their role, as well as the role of other factors, such as inflammatory markers and lipids found in follicular fluid, in ovarian function and in ovulation disorder in overweight women [24,25]. Our study showed that BMI does not influence ovarian reserve, but can influence ovulatory phenomena independently of ovarian reserve. The finding that in non-PCOS women with OD the BMI was higher and serum levels of FSH and E2 were lower than in women without OD while AMH and LH levels were similar suggests that being overweight influences reproductive outcomes in a

still unclear manner, possibly affecting follicle selection, ovulation or other still unknown reproductive mechanisms, but not ovarian reserve, which is best represented by AMH. Unlike AMH, FSH is cycle-dependent, although a recent systematic review and meta-analysis showed that serum AMH levels have significant fluctuations throughout the menstrual cycle in healthy women with regular menstrual cycles [26]. Clinical concerns for overweight and obese women are the management and safety of pregnancy. In infertile women with a BMI ≥ 25 kg/m^2, it is necessary to modify lifestyle and diet, as well as to implement exercise to lose weight [27–30]. However, in these women with and without PCOS, a pharmacological intervention with the administration of exogenous FSH is often required to restore ovulation and facilitate pregnancy [31].

In overweight and obese women with PCOS, weight loss was shown to improve reproductive function; however, serum AMH levels did not change, suggesting that weight loss and AMH levels were independently related to improved reproductive function [32]. A recent study shows that in severely obese women with and without PCOS, serum levels of AMH did not change regardless of significant weight loss after 1 year of dietary treatment [33]. In the present study, we considered several but not all parameters that define metabolic syndrome because our main objective was to investigate the possible relationship between ovarian reserve and weight, expressed simply as BMI. The relationship between metabolic syndrome and ovarian reserve is a relevant and still-debated issue that deserves further investigation in future studies.

In conclusion, BMI does not appear to affect markers of ovarian reserve. The influence of BMI on fertility may be more complex than expected and may be related to ovulation disorder rather than decreased ovarian reserve.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/reprodmed4030018/s1, Table S1: Spearman's rank correlation between BMI or age and hormonal parameters of ovarian reserve in control women stratified by the presence of ovulatory disorders (OD).

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References

1. Palomba, S. Is fertility reduced in ovulatory women with polycystic ovary syndrome? An opinion paper. *Hum. Reprod.* **2021**, *36*, 2421–2428. [CrossRef]
2. Lim, S.S.; Davies, M.J.; Norman, R.J.; Moran, L.J. Overweight, obesity and central obesity in women with polycystic ovary syndrome: A systematic review and meta-analysis. *Hum. Reprod. Update.* **2012**, *18*, 618–637. [CrossRef]
3. Gesink Law, D.C.; Maclehose, R.F.; Longnecker, M.P. Obesity and time to pregnancy. *Hum. Reprod.* **2007**, *22*, 414–420. [CrossRef]
4. Van der Steeg, J.W.; Steures, P.; Eijkemans, M.J.; Habbema, J.D.; Hompes, P.G.; Burggraaff, J.M.; Oosterhuis, G.J.; Bossuyt, P.M.; van der Veen, F.; Mol, B.W. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum. Reprod.* **2008**, *23*, 324–328. [CrossRef]
5. Practice Committee of the American Society for Reproductive Medicine. Obesity and reproduction: A committee opinion. *Fertil. Steril.* **2021**, *116*, 1266–1285. [CrossRef]

6. Epplein, M.; Reed, S.D.; Voigt, L.F.; Newton, K.M.; Holt, V.L.; Weiss, N.S. Risk of complex and atypical endometrial hyperplasia in relation to anthropometric measures and reproductive history. *Am. J. Epidemiol.* **2008**, *168*, 563–570. [CrossRef]

7. Boots, C.; Stephenson, M.D. Does obesity increase the risk of miscarriage in spontaneous conception: A systematic review. *Semin. Reprod. Med.* **2011**, *29*, 507–513. [CrossRef]

8. Yang, J.H.; Chou, C.H.; Yang, W.S.; Ho, H.N.; Yang, Y.S.; Chen, M.J. Iron stores and obesity are negatively associated with ovarian volume and anti-Müllerian hormone levels in women with polycystic ovary syndrome. *Taiwan J. Obstet. Gynecol.* **2015**, *54*, 686–692. [CrossRef]

9. Bernardi, L.A.; Carnethon, M.R.; de Chavez, P.J.; Ikhena, D.E.; Neff, L.M.; Baird, D.D.; Marsh, E.E. Relationship between obesity and anti-Müllerian hormone in reproductive-aged African American women. *Obes. (Silver Spring)* **2017**, *25*, 229–235. [CrossRef] [PubMed]

10. Moy, V.; Jindal, S.; Lieman, H.; Buyuk, E. Obesity adversely affects serum anti-müllerian hormone (AMH) levels in Caucasian women. *J. Assist. Reprod. Genet.* **2015**, *32*, 1305–1311. [CrossRef]

11. Casadei, L.; Fanisio, F.; Sorge, R.P.; Collamarini, M.; Piccolo, E.; Piccione, E. The diagnosis of PCOS in young infertile women according to different diagnostic criteria: The role of serum anti-Müllerian hormone. *Arch. Gynecol. Obstet.* **2018**, *298*, 207–215. [CrossRef] [PubMed]

12. Gorkem, U.; Kucukler, F.K.; Togrul, C.; Gulen, Ş. Obesity Does not Compromise Ovarian Reserve Markers in Infertile Women. *Geburtshilfe Frauenheilkd.* **2019**, *79*, 79–85. [CrossRef] [PubMed]

13. Simões-Pereira, J.; Nunes, J.; Aguiar, A.; Sousa, S.; Rodrigues, C.; Sampaio Matias, J.; Calhaz-Jorge, C. Influence of body mass index in anti-Müllerian hormone levels in 951 non-polycystic ovarian syndrome women followed at a reproductive medicine unit. *Endocrine.* **2018**, *61*, 144–148. [CrossRef] [PubMed]

14. Sahmay, S.; Usta, T.; Erel, C.T.; Imamoğlu, M.; Küçük, M.; Atakul, N.; Seyisoğlu, H. Is there any correlation between amh and obesity in premenopausal women? *Arch. Gynecol. Obstet.* **2012**, *286*, 661–665. [CrossRef]

15. Halawaty, S.; ElKattan, E.; Azab, H.; ElGhamry, N.; Al-Inany, H. Effect of obesity on parameters of ovarian reserve in pre-menopausal women. *J. Obstet. Gynaecol. Can.* **2010**, *32*, 687–690. [CrossRef]

16. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Ferti.l Steril.* **2004**, *81*, 19–25. [CrossRef]

17. Palomba, S.; Daolio, J.; La Sala, G.B. Oocyte competence in women with polycystic ovary syndrome. *Trends Endocrinol. Metab.* **2017**, *28*, 186–198. [CrossRef] [PubMed]

18. Palomba, S.; Piltonen, T.T.; Giudice, L.C. Endometrial function in women with polycystic ovary syndrome: A comprehensive review. *Hum. Reprod. Update.* **2021**, *27*, 584–618. [CrossRef] [PubMed]

19. Shaw, C.M.; Stanczyk, F.Z.; Egleston, B.L.; Kahle, L.L.; Spittle, C.S.; Godwin, A.K.; Brinton, L.A.; Dorgan, J.F. Serum antimüllerian hormone in healthy premenopausal women. *Fertil. Steril.* **2011**, *95*, 2718–2721. [CrossRef] [PubMed]

20. Lefebvre, T.; Dumont, A.; Pigny, P.; Dewailly, D. Effect of obesity and its related metabolic factors on serum anti-Müllerian hormone concentrations in women with and without polycystic ovaries. *Reprod. Biomed. Online* **2017**, *35*, 325–330. [CrossRef]

21. Moslehi, N.; Shab-Bidar, S.; Ramezani Tehrani, F.; Mirmiran, P.; Azizi, F. Is ovarian reserve associated with body mass index and obesity in reproductive aged women? A meta-analysis. *Menopause.* **2018**, *25*, 1046–1055. [CrossRef]

22. Albu, D.; Albu, A. The relationship between anti-Müllerian hormone serum level and body mass index in a large cohort of infertile patients. *Endocrine.* **2019**, *63*, 157–163. [CrossRef]

23. Catteau-Jonard, S.; Brunel, A.; Dumont, A.; Robin, G.; Pigny, P.; Dewailly, D. Serum FSH level is lower in dysovulating than in ovulating non-PCOS obese women, independently of body mass index. *Ann. Endocrinol.* **2019**, *80*, 225–228. [CrossRef]

24. Ruebel, M.L.; Cotter, M.; Sims, C.R.; Moutos, D.M.; Badger, T.M.; Cleves, M.A.; Shankar, K.; Andres, A. Obesity Modulates Inflammation and Lipid Metabolism Oocyte Gene Expression: A Single-Cell Transcriptome Perspective. *J. Clin. Endocrinol. Metab.* **2017**, *102*, 2029–2038. [CrossRef] [PubMed]

25. Gonzalez, M.B.; Lane, M.; Knight, E.J.; Robker, R.L. Inflammatory markers in human follicular fluid correlate with lipid levels and Body Mass Index. *J. Reprod. Immunol.* **2018**, *130*, 25–29. [CrossRef]

26. Khodavirdilou, R.; Pournaghi, M.; Rastgar Rezaei, Y.; Hajizadeh, K.; Khodavirdilou, L.; Javid, F.; Hamdi, K.; Shahnazi, M.; Nouri, M.; Fattahi, A.; et al. Does Anti-Müllerian hormone vary during a menstrual cycle? A systematic review and meta-analysis. *J. Ovarian Res.* **2022**, *15*, 78. [CrossRef] [PubMed]

27. Collins, G.G.; Rossi, B.V. The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. *Fertil. Res. Pract.* **2015**, *1*, 11. [CrossRef]

28. Gaskins, A.J.; Chavarro, J.E. Diet and fertility: A review. *Am. J. Obstet. Gynecol.* **2018**, *218*, 379–389. [CrossRef] [PubMed]

29. Vitek, W.S.; Hoeger, K.M. Worth the wait? Preconception weight reduction in women and men with obesity and infertility: A narrative review. *Fertil. Steril.* **2022**, *118*, 447–455. [CrossRef]

30. Hart, T.L.; Petersen, K.S.; Kris-Etherton, P.M. Nutrition recommendations for a healthy pregnancy and lactation in women with overweight and obesity – strategies for weight loss before and after pregnancy. *Fertil. Steril.* **2022**, *118*, 434–446. [CrossRef]

31. Casadei, L.; Puca, F.; Emidi, E.; Manicuti, C.; Madrigale, A.; Piccione, E. Sequential low-dose step-up and step-down protocols with recombinant follicle-stimulating hormone in polycystic ovary syndrome: Prospective comparison with step-down protocol. *Arch. Gynecol. Obstet.* **2012**, *286*, 1291–1297. [CrossRef] [PubMed]

32. Thomson, R.L.; Buckley, J.D.; Moran, L.J.; Noakes, M.; Clifton, P.M.; Norman, R.J.; Brinkworth, G.D. The effect of weight loss on anti-Müllerian hormone levels in overweight and obese women with polycystic ovary syndrome and reproductive impairment. *Hum. Reprod.* **2009**, *24*, 1976–1981. [CrossRef] [PubMed]
33. Kataoka, J.; Larsson, I.; Lindgren, E.; Kindstrand, L.O.; Schmidt, J.; Stener-Victorin, E. Circulating Anti-Müllerian hormone in a cohort-study of women with severe obesity with and without polycystic ovary syndrome and the effect of a one-year weight loss intervention. *Reprod. Biol. Endocrinol.* **2022**, *20*, 153. [CrossRef] [PubMed]