ORIGINAL ARTICLE

sciendo

10.2478/AMB-2024-0013

BODY MASS INDEX AS A FACTOR INFLUENCING OOCYTE COMPETENCE AND IMPLANTATION POTENTIAL – DISTRIBUTION AND RESULTS IN 4,882 WOMEN UNDERGOING FERTILITY TREATMENT

I. Antonova[1], D. Ivanov[2], G. Yaneva[2], N. Magunska[3], D. Duylgerova-Nikolova[1,5], M. Yunakova[1,4], A. Shterev[1,3]

[1]IVF department, Ob/Gyn "Dr Shterev Hospital" – Sofia, Bulgaria
[2]Department of Biology, Faculty of Pharmacy, Medical University „Prof. dr. Paraskev Stoyanov" – Varna, Bulgaria
[3]Department of Health Care, Faculty of Public Health and Health Care, University of Ruse „Angel Kanchev" – Ruse, Bulgaria
[4]Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University – Sofia, Bulgaria
[5]IBIR – Bulgarian Academy of Science – Sofia, Bulgaria

Abstract. Introduction: *The large amount of excess adipose tissue affects negatively every single step of the assisted reproductive treatment process – from the controlled ovarian hyperstimulation (COH) to obstetric complications and health consequences for the fetus. A number of scientific data show correlation between impaired clinical pregnancy rates (CPR) and obese patients maintaining high body mass index (BMI). The debate whether high proportion of body fat negatively affects oocyte quality or only impacts endometrial receptivity remains open.* ***Aim:*** *The aim of this study is to investigate the specific effect of the different BMI range in patients undergoing assisted reproductive technology (ART) treatment with reference to oocyte quality, fertilization capacity and implantation potential.* ***Materials and methods:*** *We analyzed a total of 4,882 women from partner couples who underwent ART treatment. Height and weight data were entered at the start of the in vitro fertilization (IVF) procedure. According to their BMI the female patients were divided in four distinct groups: in group 1 (underweight and BMI below 18.5 kg/m²) – 498 women, group 2 (normal range from 18.5 to 24.9 kg/m²) – 3021 women, group 3 (overweight with BMI between 25 – 29.9 kg/m²) – 789 women and group 4 (obese with BMI over 30 kg/m²) – 484 women.* ***Results:*** *Distribution of the women in different BMI groups indicates that most of the Bulgarian patients undergoing fertility treatment are in the normal range. No association between BMI and ovarian response was found. A total of 33,205 oocytes were obtained after the COH. The mean numbers of the retrieved oocytes were similar (6,85 in group 1; 6,83 in group 2, 6,77 in group 3 and 6,67 in group 4) per woman and the fertilization rate was comparable in the four groups as it varies between 74-75%. Proportional categories of good, fair and bad embryos at cleavage stage were found. On the contrary, according to CPR, statistical difference between normal weight group (34,38%) versus overweight (29,6%) and obese groups (28,80%) (p < 0,05) was found.* ***Conclusion:*** *No correlation was found between the number of the retrieved oocytes and BMI values. Statistically lower pregnancy rates were reported in overweight women with BMI ≥ 25 kg/m² group and further onwards*

in the obesity group (BMI ≥ 30 kg/m²), which may be associated with impaired endometrial receptivity as a result of abnormally high levels of adipose tissue.

Key words: *BMI, obesity, fertility treatment, pregnancy rate, assisted reproduction*

Corresponding author: *Irena Antonova, MD, Ob/Gyn "Dr Shterev Hospital", 25 Hristo Blagoev str, 1330, Sofia, Bulgaria, tel: +35988712765,1 e-mail: irendreaming@gmail.com*

Received: *21 September 2023;* ***Accepted:*** *3 November 2023*

INTRODUCTION

Overweight and obesity have been one of the biggest public health challenges in the recent years. According to the World Health Organization (WHO) European Regional Report 2022 [1], overweight and obesity affect over 60% of the population in Europe and 23% of women and 20% of men suffer from varying degrees of obesity. In addition to numerous negative health complications, such as cardiovascular disorders [2], metabolic dysfunctions, respiratory problems, carcinomas, and psycho-emotional disturbances, excess weight is also associated with various reproductive difficulties. In women, regardless of ovarian reserve status, the increased amount of adipose tissue negatively affects fertility. Lower chances of spontaneous pregnancy [3], ovulatory dysfunction, higher consumption of gonadotropins in COH, prolonged stimulation, impaired implantation rates, and higher risk of miscarriage after ART were found.

One of the largest studies of how obesity affects assisted reproductive treatment outcomes is based on approximately half million cycles, with data taken from the US National Registry [4]. The results strongly confirm that obese women have significantly lower chance for achieving both clinical pregnancy and a live birth, as the risk of spontaneous abortion is statistically higher for them compared to the other groups [5].

Although an association between increased BMI and oocyte quality has been found in some reports [6], the direct negative effect on oocytes is of unclear mechanism at this stage. In recent years, there have been publications grounding a link between obesity, inflammatory processes and increased oxidative stress [7] in the ovary, which affects the quality of the oocytes produced [8]. A reduced amount of retrieved oocytes per unit of gonadotropins consumed and technical difficulties in performing the follicular puncture were also found. It has been suggested that the reduced successful outcomes of assisted reproductive treatment are more likely due to alterations in endometrial gene regulation than to impaired oocyte quality [9].

MATERIALS AND METHODS

Population

This study includes 33 205 oocytes retrieved from 4,882 Bulgarian female patients who underwent IVF cycles of assisted reproduction using own fresh gametes. In all of the cases measurements of the height and weight were entered at the start of the in vitro fertilization procedure. The data is calculated in BMI ratio. BMI is expressed in kg/m^2, resulting from mass in kilograms and height in metres (Fig. 1).

$$\text{BMI} = \frac{\text{Weight (in kilograms)}}{\text{Height}^2\text{ (in meters)}}$$

Fig. 1. BMI metric calculation formula

Study design

The aim of the present study was to investigate the specific effect of excess amounts of adipose tissue on oocytes, their quality and fertilization capacity, as well as to study the potential effect over the implantation potential of the endometrium.

For the purpose of the following research, all patients were divided into 4 groups according the international BMI WHO classification – group 1 underweight (BMI < 18.49 kg/m^2), (n = 498); group 2 normal range (BMI 18.5-24.9 kg/m^2), (n = 3021); group 3 overweight (BMI 25-29.9 kg/m^2) (n = 789) and group 4 obese (BMI > 30 kg/m^2) (n = 484).

The following research was performed as a retrospective cohort study for 9 years in the period from January 2013 to December 2021. Patients who underwent ART treatment in Ob/gyn „Dr. Shterev" Sofia, Bulgaria were included.

All medical data was extracted using hospital computer software – JOYSTICK healthcare management system („Smart Software Systems" – certified with ISO/IEC 27001:2017 and ISO 9001:2015).

Statistical analysis

Analysis was performed using GraphPad 8 software. Tests for determining the parametric and non-parametric tests for correlation analysis (Pearson product-moment); for comparison of mean values of samples (Student's t-test; Mann–Whitney U test; Kruskal-Wallis test by ranks) and for comparison of relative shares (Pearson's chi-square test) were included in the analysis of the data.

RESULTS

Distribution in the groups

The preparation of patients for assisted reproduction begins with entering data on their height and weight in order to estimate the optimal dose of gonadotropic hormone medication to be administered for controlled ovarian hyperstimulation.

Distribution of the patient according to their BMI measurement was: in group 1 (BMI below 18.5 kg/m^2) – 498 women, group 2 (18.5 to 24.9 kg/m^2) – 3021 women, group 3 (25-29.9 kg/m^2) – 789 women and group 4 (over 30 kg/m^2) – 484 women. The allocation ratio percentage in the different groups, demonstrated in figure 2, shows that the majority of Bulgarian patients' BMI fell within the normal weight range. Less than 10% of all women treated by assisted reproduction methods are affected by varying degrees of obesity.

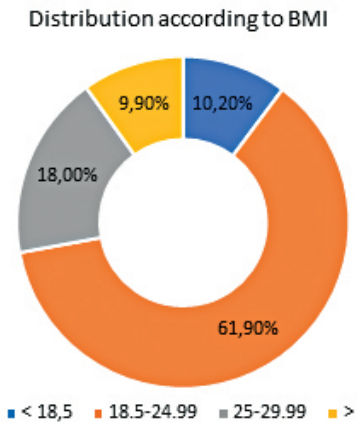

Fig. 2. Distribution of the patients according their BMI status

Oocyte retrieval

Suitable stimulation protocol aimed at the recruitment, activation and growth of optimal number of follicles, by means of administration of follicle-stimulating hormone (FSH) containing medications according to different schemes was performed. Ovulation timing was achieved by adding human choriogonadotropin (CHG) hormone or agonists of gonadoliberin (αGnRH) [10], with which the oocyte completes metaphase II (MII) of the second meiotic division [11]. After an interval of 34-36 hours from the triggering of ovulation, a follicular pick-up was conducted [12]. In a manipulation room under short-term intravenous anesthesia, retrieval of the follicular contents was performed using a vaginal transducer and the test tubes were transferred to the laboratory for evaluation and processing of the cumulus cells. The total number of eggs obtained by women in different groups depending on their BMI is shown in table. 1.

Table 1. Distribution of total oocyte number in BMI groups

total number oocytes					
BMI	n	min.	max.	average	SE
< 18.5 kg/m^2	3406	0	43	6,85	0,26
18.5-24.9 kg/m^2	20620	0	45	6,83	0,1
25-30 kg/m^2	5950	0	36	6,77	0,19
> 30 kg/m^2	3229	0	28	6,67	0,23

The non-parametric Kruskal-Wallis test showed no statistical differences in the mean number of oocytes retrieved by BMI group (Fig. 3).

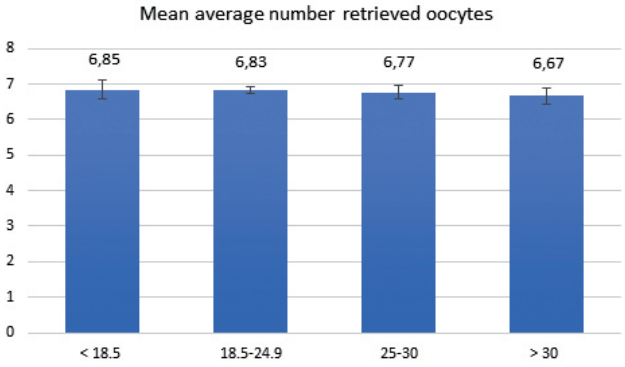

Fig. 3. Distribution of mean average number of the retrieved oocytes according to BMI

Mature oocytes

Between 2 and 5 hours after oocyte pick-up (OPU), [13] all oocytes were denudated from the surrounding cumulus-oocyte complexes and only mature MII gametes were fertilized via classic in vitro fertilization or intra-cytoplasmic sperm injection (ICSI) [14].

Regarding the mean number of MII oocytes suitable for fertilization, multiple comparison analysis with Dunn's test also showed no significant differences.

Analyzing the data on the percentage ratio of mature oocytes to the total population of obtained oocytes, a tendency to decrease their proportions was observed

in the obesity group 4 (Fig. 4), but no statistically significant difference was found.

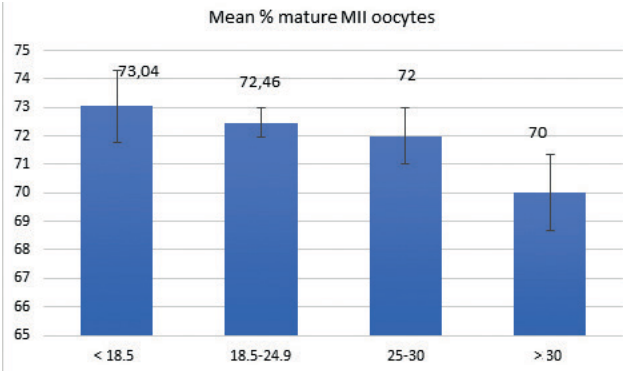

Fig. 4. Distribution of mean ratio MII oocytes among BMI groups

Fertilization

Fertilization check was performed at 18th hour after manipulation procedure and all zygotes showing two visible pronuclei were assessed [15].

Data for cancellation rate after follicular puncture was also under review. The percentage of cycles in which not a single oocyte was harvested, or there were no mature eggs obtained varied between 9-11% in the different groups and was not found to be significant.

Fertilization rate was also comparable in the separate BMI categories and was in the range between 74-75% (74% in group 1, 75% in group 2, 75% in group 3 and 74% in group 4).

Embryo quality

At cleavage stage we measured the embryo quality according to the Istanbul consensus on embryo assessment [16]. All available embryos were distributed into 3 main groups according to their characteristics shown during the cleavage stage – number and size of the cells, fragmentation, multinucleation. After comparison between all BMI groups, they exhibited similar proportions of good, fair and bad embryos (Fig. 5) with no statistical difference.

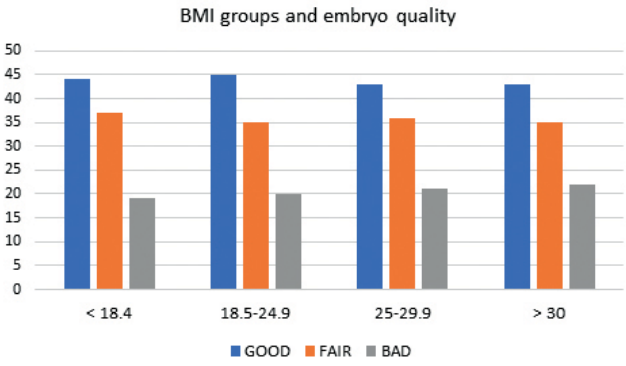

Fig. 5. Distribution of embryos with good, fair and bad quality in BMI groups

Embryo transfer

After incubating the preimplantation embryos at optimal culture conditions (37C° and 6.0 CO_2) between 2 and 5 days [17], embryo transfer of the top-quality embryos was performed [18, 19]. All spare embryos with good characteristics were cryopreserved [20] via vitrification method [21]. In cycles with high risk of ovarian hyperstimulation, "freeze all" strategy was applied and all good embryos were cryopreserved [22] and used in consequent menstrual cycle for frozen embryo transfer (FET). In all cases where no fertilization was registered or no developing embryos were available, treatment cycle was cancelled due to lack of viable embryos for transfer or freezing.

The analysis of the final results of the in vitro cycles in different BMI groups are demonstrated in table. 2.

Table 2. Results from cycle fertility treatment in BMI groups

GROUP BMI	< 18,5 kg/m²	18,5-24,9 kg/m²	25-29,9 kg/m²	> 30 kg/m²
Cancelled procedure				
number	113	595	175	106
%	23%	20%	20%	22%
Embryo transfer				
number	292	1825	535	293
%	58%	60%	61%	60%
Embryo transfer and freezing				
number	45	342	83	51
%	9%	11%	9%	11%
Freeze all				
number	48	259	86	34
%	10%	9%	10%	7%

An important indicator on analyzing the data results in the ART cycles is the cancellation rate, namely – at what percentage rate the performed procedures did not reach transfer and/or freezing due to incompetent gametes, fertilization failure or lack of developing embryos. Cancellation rate with no eligible embryos was similar in different BMI categories (23% for group 1, 20% for group 2 and 3, 22% in group 4) with no significance shown.

Pregnancy rates

In order to establish a potential pregnancy, a blood sample was examined on the 10th day after the embryo transfer and the βHCG values were recorded [23, 24]. 2 weeks after the positive blood test result, an ultrasound scan was performed to identify a gestational sac(s) [25].

When analyzing the frequency of achieved pregnancies (number of clinical pregnancies per embryo transfers), significant differences were found in some of the groups (Fig. 6).

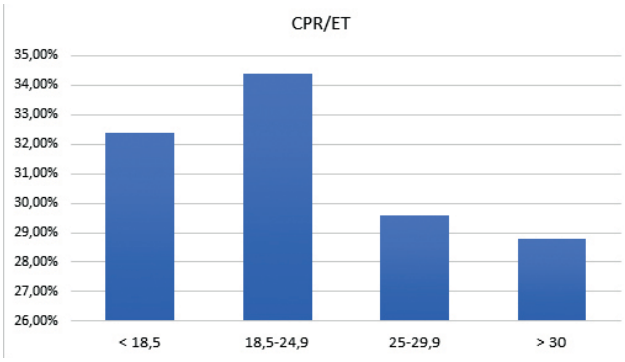

Fig. 6. Pregnancy rate per embryo transfer in BMI groups

In group 1 positive results were established in 32,37% of the patients, resulting in 109 clinical pregnancies. Highest values were recorded in the normal weight group 2 – 745 pregnancies and 34,38% CPR/ET. In the group with overweight women 183 positive blood tests and 29,60% successful cycles were registered. Lowest numbers occurred in the obese group – only 28,80% implantations from 344 embryo transfers [26, 27].

In a comparative analysis between individual groups, no statistical difference was reported between the group of women with underweight (group 1) and normal weight (group 2) ($p = 0.4714$). The percentage of clinical pregnancies was significantly lower between group 2 and the overweight group (group 3) ($p < 0.05$), as well as between group 2 and the obese group (group 4) ($p < 0.05$) [5, 28]. Although multiple comparison by Dunn's test did not find a statistical difference between groups 3 and 4, a clear inverse correlation was observed between BMI values and percentage of successful implantations.

DISSCUSION

When analyzing the metrical proportions of the patients distributed into different categories according to their BMI, it is notable that the majority of Bulgarian patients maintain an optimal weight. This is a positive trend, different from the data published by the WHO, which alarmingly alerted that as high as 39% of the world's population over the age of 18 is overweight (1.9 billion people) and 13% is obese (650 million people) [29, 30]. This is an important indicator for the Bulgarian population, considering that the figures for Europe regarding overweight and obesity are even higher [1]. Among the women treated by ART there are also differences compared to the Bulgarian cohort – over 40% of the patients in the largest American study [4] were overweight and obese, while in the present study less than 30% of the Bulgarian female patients fell into these categories.

The indicators that directly demonstrate the quality and fertilization potential of the oocytes – average number of retrieved oocytes, proportion of MII, fertilization index, do not show a direct correlation in connection to BMI values of the patients. In the present study a significant difference in relation to the percentage of achieved successful pregnancies in the different groups was found. The main outcomes drawn by the present study are that increased levels of fat tissue do not directly affect oocyte quality. Adipose tissue has a major role as an endocrine gland and adipose-specific cytokines (adipokines) [31], as well as non-adipose specific cytokines determine accurate regulation of the hypothalamus-pituitary-gonadal axis and affect endometrial implantation molecular process.

CONCLUSIONS

Our study shows that the endometrium receptiveness is the one that is negatively influenced by excessive amounts of adipose tissue due to molecular and endocrinological pathophysiological disruptions. This could be a suitable predictive model for implantation capacity as there is a clear cut-off range in BMI values. Weight-loss counseling before fertility treatment and appropriate nutritional diet with calorie deficit, as well as good physical activity, could be beneficial for the successful outcome of the fertility treatment.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Organization WH. WHO European regional obesity report 2022: World Health Organization. Regional Office for Europe; 2022.
2. Powell-Wiley TM, Poirier P, Burke LE, et al. Obesity and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2021;143(21):e984-e1010.
3. Creanga AA, Catalano PM, Bateman BT. Obesity in pregnancy. New England Journal of Medicine. 2022; 387(3):248-59.
4. Kawwass JF, Kulkarni AD, Hipp HS, et al. Extremities of body mass index and their association with pregnancy outcomes in women undergoing in vitro fertilization in the United States. Fertility and Sterility. 2016; 106(7):1742-50.
5. Sciorio R, Bellaminutti S, Tramontano L, et al. Impact of obesity on medically assisted reproductive treatments. Zygote. 2022; 30(4):431-9.
6. Gonzalez MB, Robker RL, Rose RD. Obesity and oocyte quality: significant implications for ART and emerging mechanistic insights. Biology of Reproduction. 2022; 106(2):338-50.

7. Snider AP, Wood JR. Obesity induces ovarian inflammation and reduces oocyte quality. Reproduction. 2019; 158(3):R79-R90.
8. Lainez NM, Coss D. Obesity, neuroinflammation, and reproductive function. Endocrinology. 2019; 160(11):2719-36.
9. Comstock IA, Diaz-Gimeno P, Cabanillas S, et al. Does an increased body mass index affect endometrial gene expression patterns in infertile patients? A functional genomics analysis. Fertility and Sterility. 2017; 107(3):740-8.e2.
10. Shrestha D, La X, Feng HL. Comparison of different stimulation protocols used in vitro fertilization: a review. Annals of translational medicine. 2015; 3(10).
11. Orvieto R. Triggering final follicular maturation-hCG, GnRH-agonist or both, when and to whom? Journal of ovarian research. 2015; 8:1-6.
12. D'Angelo A, Panayotidis C, Amso N, et al. Recommendations for good practice in ultrasound: oocyte pick up. Human Reproduction Open. 2019;2019(4):hoz025.
13. Maggiulli R, Cimadomo D, Fabozzi G, et al. The effect of ICSI-related procedural timings and operators on the outcome. Human Reproduction. 2020; 35(1):32-43.
14. Bridges PJ, Jeoung M, Kim H, et al. Methodology matters: IVF versus ICSI and embryonic gene expression. Reproductive biomedicine online. 2011; 23(2):234-44.
15. Berger DS, Hoff HS. Fertilization Assessment in IVF and ICSI. Principles of IVF Laboratory Practice: Laboratory Set-Up, Training and Daily Operation. 2023:207.
16. Medicine ASiR, Embryology ESIGo. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting†. Human Reproduction. 2011; 26(6):1270-83.
17. Kermack AJ, Fesenko I, Christensen DR, et al. Incubator type affects human blastocyst formation and embryo metabolism: a randomized controlled trial. Human Reproduction. 2022; 37(12):2757-67.
18. 18. Glujovsky D, Retamar AMQ, Sedo CRA, et al. Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology. Cochrane database of systematic reviews. 2022(5).
19. Clua E, Rodriguez I, Arroyo G, et al. Blastocyst versus cleavage embryo transfer improves cumulative live birth rates, time and cost in oocyte recipients: a randomized controlled trial. Reproductive BioMedicine Online. 2022; 44(6):995-1004.
20. Korkmaz C, Yıldız ÜG, Fidan U, et al. Investigation of transfer results of human embryos that were vitrified and thawed at the cleavage, morula and blastocyst stages. Zygote. 2020; 28(3):191-5.
21. Schiewe MC, Anderson RE. Vitrification: the pioneering past to current trends and perspectives of cryopreserving human embryos, gametes and reproductive tissue. Journal of Biorepository Science for Applied Medicine. 2017:57-68.
22. Nagy ZP, Shapiro D, Chang C-C. Vitrification of the human embryo: a more efficient and safer in vitro fertilization treatment. Fertility and sterility. 2020; 113(2):241-7.
23. Ojeda Varela M, Aguilar Prieto J, Táboas Lima E, et al. P-212 βhCG concentration in peripherical maternal blood after single embryo transfer, ongoing pregnancy rates and morphokinetics. Human Reproduction. 2023; 38(Supplement_1):dead093. 571.
24. Linh PK. Predictive value of βhCG measured on day 11 after blastocyst embryo transfer for early pregnancy outcome. Tạp chí Phụ sản. 2023;21(1):80-7.
25. Ouyang Y, Qin J, Lin G, et al. Reference intervals of gestational sac, yolk sac, embryonic length, embryonic heart rate at 6-10 weeks after in vitro fertilization-embryo transfer. BMC Pregnancy and Childbirth. 2020; 20(1):1-10.
26. Fedorcsák P, Storeng R, Dale PO, et al. Obesity is a risk factor for early pregnancy loss after IVF or ICSI. Acta obstetricia et gynecologica Scandinavica. 2000; 79(1):43-8.
27. Sermondade N, Huberlant S, Bourhis-Lefebvre V, et al. Female obesity is negatively associated with live birth rate following IVF: a systematic review and meta-analysis. Human reproduction update. 2019; 25(4):439-51.
28. Rittenberg V, Seshadri S, Sunkara SK, et al. Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis. Reproductive biomedicine online. 2011; 23(4):421-39.
29. Lobstein T, Brinsden H, Neveux M. World obesity atlas 2022. 2022.
30. Mahase E. Obesity: No European country is on track to halt rising levels by 2025, WHO warns. BMJ: British Medical Journal (Online). 2022; 377:o1107.
31. Silvestris E, de Pergola G, Rosania R, et al. Obesity as disruptor of the female fertility. Reproductive Biology and Endocrinology. 2018; 16(1):22.