

ORIGINAL ARTICLE

EFFECT OF ESTRADIOL LEVELS ON PREGNANCY OUTCOME IN OBESE WOMEN

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Background: The incidence of obesity in women of reproductive age group has encountered problems of infertility with frequent referral to reproductive clinics. In order to know whether increase in body mass index (BMI) is associated with poor reproductive outcome, current study was aimed to relate women's BMI, estradiol (E2) levels, and clinic pregnancy rates after intra cytoplasmic sperm injection (ICSI). **Methods:** Quasi experimental design of 323 women was conducted from June 2010 till August 2011. Women were grouped on the basis of BMI; Group A, BMI <18 (underweight); Group B, BMI 18–22.9 (normal weight); Group C, BMI 23–25.9 (overweight); and Group D, BMI \geq 26 (obese). Procedure of ICSI commenced by down regulation of ovaries followed by controlled ovarian stimulation. Oocyte pickup was done 36 hours after ovulation induction by hCG, eggs fertilised *in vitro* were graded and only blastocysts were transferred. Serum samples of basal E2, peak E2 (day of hCG administration) and mid-luteal E2 (7 days after egg collection) measured by ELISA were compared in all BMI groups. Pregnancy outcome of these was categorised as: no conception β -hCG <5 mIU/ml, preclinical abortion with β -hCG >5 mIU/ml, no cardiac activity on transvaginal scan (TVS) and clinical pregnancy with β -hCG >5 mIU/ml and cardiac activity on trans-vaginal scan. **Results:** Obese women with BMI \geq 26 had lowest basal, peak and mid-luteal E2 with minimum clinical pregnancy rates compared to rest of the groups. **Conclusion:** A high BMI is associated with decreased E2 levels in all phases of ovarian cycle and has a negative impact on pregnancy outcome.

Keywords: Intra-cytoplasmic sperm injection, Body mass Index, Estradiol, Obesity, Infertility

INTRODUCTION

Obesity has emerged as an explosive global epidemic, prevalence of which has increased in the West as well as developing countries.¹ The incidence of obesity in women of reproductive age group has encountered problems of an ovulation, menstrual disturbances, delayed conception and frequent referral to reproductive clinics.² Assisted Reproductive Clinics (ARCs) have reported an increase in incidence of infertility in obese women with untoward effects of treatment outcomes.

In vitro fertilisation (IVF) or Intra-cytoplasm sperm injection (ICSI) is a highly developed practice of treatment in ARC where pregnancy fails to occur by conventional procedures. During this process, women undergo a treatment plan of at least 6 weeks with frequent transvaginal scans (TVS), blood tests, hormone analysis and regular checkups. This treatment is extensive, expensive and scrupulous with maximum success rates up to 25–30%, therefore ARCs make their level best to carry out all such measures that attempt to improve success rates. It has thus become a clinical practice to select best treatment plans, avoid possible risk factors and advise women to maintain normal body mass index (BMI) at the time of booking in ARC.²

During treatment, implantation of embryo occurs as a result of successful intercellular interactions between developing embryo and receptive endometrium of a hormonally primed uterus.³ Estradiol (E2) produced by the granulosa cells of the ovaries during reproductive years' initiate hypertrophy and hyperplasia of endometrial epithelia and plays a role in endometrial

synchronisation and blastocyst implantation.⁴ Inadequate uterine receptivity mainly due to disturbance in E2, accounts for 1/3 of unsuccessful outcomes.⁵

Keeping in mind increased incidence of obese women reporting to reproductive clinics² and inappropriate preparation of endometrial lining by E2⁶ being one the factors in failure of implantation, this study is meant to find a correlation between obesity, E2 levels and reproductive outcome.

MATERIAL AND METHODS

The study protocol was approved by Basic Research Cell of Karachi University. It was a quasi experimental design conducted from June 2010 till August 2011 after approval from Ethical Review Board of 'Islamabad Clinic Serving Infertile Couples'. Convenience sampling of 323 consented couples; women age 18–41, duration of infertility more than 2 years, both ovaries present with no morphological abnormalities, normal ovulatory cycle (25–35 days), basal FSH (day 2) serum level <10 mIU/ml, selected for long protocol with Gonadotrophin releasing hormone (GnRH) agonist, stimulated with injection of recombinant follicle stimulating hormone (rFSH) and kept on progesterone support with 400 mg pessary daily were included.

Women aged >41, FSH >10 mIU/ml, presence of polycystic ovaries (diagnosis of PCOS was made by presence of two of the three criteria: polycystic ovaries, Oligo- and anovulation, and clinical or biochemical evidence of hyper-androgenism) GnRH antagonist therapy, short down regulation with GnRH agonist, ICSI with sperm retrieval by testicular biopsy and frozen

embryo transfers were excluded. Basal estradiol on day 2 was taken and screening for infectious diseases was done in all patients.

Weight was recorded in Kg±100 g, and height to nearest 0.5 Cm. BMI was calculated as weight (Kg)/square of the BH (m²). Subjects were grouped per BMI classification for South Asian population as Group A: BMI <18 (underweight), Group B: BMI 18–22.9 (normal weight), Group C: BMI 23–25.9 (overweight), and Group D: BMI ≥26 (obese).⁷

Included women were down regulated with long protocol of GnRH agonist. Controlled ovarian stimulation (COS) by gonadotrophin from 2nd was started from 3rd day of cycle for 14 days. Ovulation induction (OI) with intramuscular injection of hCG was done with measurement of maximum follicles 18–20 mm by TVS. Serum sample on this day was preserved for peak E2 estimation. Eggs were retrieved 36 hours after OI by vaginal ultrasound probe (OPU, oocyte pick up) with 16G adapter and double lumen oocyte aspiration needle on 14th, 15th or 16th day of COS. ICSI of eggs was done and embryos kept in incubator till their fertilisation and development till blastocysts. Embryo transfer (ET) of blastocysts was done 7 days after OI by Sims-Wallace Embryo Replacement Catheter under ultrasound guidance. Samples of mid-luteal E2 were taken 7 days after OPU and all E2 samples (Basal, peak and mid luteal) analysed by ELISA. Luteal support was maintained by progesterone vaginal pessaries twice a day from the day of OPU.

Single serum β-hCG measurement was performed on specimens obtained by peripheral venipuncture 14 days after egg collection as the outcome marker. TVS was performed at 5 weeks gestation to identify clinical pregnancy from preclinical abortion. On the basis of β-hCG and TVS, results were analysed as non-pregnant (β-hCG <5 mIU/ml), Preclinical abortions or biochemical pregnancy (β-hCG >5 mIU/ml without

any cardiac activity) and clinical pregnancy with β-hCG >5 mIU/ml with any cardiac activity.

Data were analysed using SPSS-15. Pregnancy outcome as non-pregnant, preclinical abortion and clinical pregnancy in all BMI groups was expressed in terms of frequencies and percentages. ANOVA was applied for comparison of basal E2, peak E2 and mid-luteal E2 in all groups, and *p*<0.05 was considered significant.

RESULTS

Out of 323, 41 couples were excluded; 1 (2%) ectopic pregnancy, 13 (32%) failed to respond while in 27 (66%) women embryos were transferred before blastocysts maturation. Results of 282 showed that 21 (7%) women were underweight (BMI<18 Kg/m²), 78 (28%) were normal weight (BMI 18–22.9 Kg/m²), 56 (20%) were overweight (BMI 23–25.99 Kg/m²) and 127 (45%) were obese (BMI ≥ 26 Kg/m²). Duration of infertility in BMI groups was not significant, however 5 year duration was found maximum in Group D. Female cause for infertility was found the most in group D and A; 8 (36.1%) and 28 (22.05%) respectively compared to group B (22, 28.21%) and group C (28, 22.05%).

Basal E2, peak E2 and mid-luteal E2 was highest in BMI 18–22.9 (Group C) compared to rest of the groups. Basal E2 levels were significantly low in Group D compared to group B (*p*=0.0008). Peak E2 was highest in group C and was the least in Group D (*p*=0.0000932). Mid-luteal E2 levels differed in all groups, women of group C had significantly high levels compared to group D (*p*=000672). (Table-1).

Maximum clinical pregnancies (26, 46.43%) were acquired in group C with highest basal, peak and mid-luteal E2, and maximum failures of conception were seen in group D (68, 53.54%) with low E2 levels. (Table-2).

Table-1: Implantation factors in various groups

Variable	Group A (Underweight) BMI <18 (Kg/m ²)	Group B (Normal) BMI 18–22.9 (Kg/m ²)	Group C (Overweight) BMI 23–25.99(Kg/m ²)	Group D (Obese) BMI ≥26 (Kg/m ²)	<i>p</i>
Basal Estradiol (pg/ml)	265.993±32.983	235.66±17.942	251.633±20.081	177.153±11.176	0.000871
Peak estradiol (pg/ml)	1150.557±168.155	897.465±85.929	1028.727±106.196	598.012±57.546	0.0000932
Mid-luteal estradiol (pg/ml)	692.81±114.05	594.025±63.009	637.654±68.813	381.774±38.041	0.000672
No. of transferred embryos	1.571±0.111	1.654±0.065	1.696±0.076	1.591±0.054	0.652649
Endometrial Lining	9.524±0.94	8.167±0.393	8.732±0.44	8.646±0.29	0.408784

Table-2: Pregnancy outcome in groups

Body Mass Index	Group	Non-pregnant, n=120	Preclinical abortion, n=61	Clinical pregnancies, n=101
Under weight (<18)	A	7 (6%)	4 (6%)	10 (10%) ^{*o}
Normal weight (18–22.9)	B	30 (25%)	19 (31%)	29 (29%)
Over weight (23–25.99)	C	15 (12%)	15 (2%)	26 (26%) ^{*o}
Obese (≥26)	D	68 (57%)	23 (38%)	36 (35%) ^{oo}

**p*<0.01 in CP group compared to non-pregnant group, ^o*p*<0.01 in CP group compared to preclinical abortion, ^{oo}*p*<0.001 in CP group compared to preclinical abortion

DISCUSSION

Many stimulation cycles in assisted reproduction are associated with failed pregnancy despite the transfer of apparently healthy and morphologically normal embryos.⁴ The detrimental effect of BMI on delay in conception is attributed to its effects on the ovary as well as endometrium.⁸ The fact is revealed in our study with

maximum number of study group comprising of obese women with BMI ≥26. This result is supported by Esinler *et al* who correlated multiple endocrine and metabolic alterations in obese women giving rise to infertility.^{8,9} The recruitment of sufficient number of follicles during ovarian stimulation is required for favourable response to ovarian stimulation. Failure of the

ovaries to respond to gonadotrophin stimulation indicates poor ovarian reserve which is depicted by low basal E2, associated with poor IVF outcome.¹⁰ Our study shows that 42% obese women had significantly low basal E2 and maximum number of this group failed to conceive.

In the follicular phase, as a result of growth of follicles serum E2 concentration progressively increase which causes endometrium hyperplasia of both glandular and stromal components.¹¹ It also induces the production of specific proteins, growth factors and the receptors of oestrogen and progesterone.¹¹ The association between peak E2 level (on the hCG administration day) and IVF-ICSI outcome was evaluated by many studies.¹² In our study a lower pregnancy rate was achieved in obese women who had a low peak E2. Many other studies suggested that with the higher peak E2 levels, higher pregnancy rates were achieved.¹²

Quality of the embryos and endometrial receptivity are one of the most important factors in the foeto-maternal cross-talk which are required for implantation process and the maintenance of early pregnancy. Luteal estradiol has a permissive function to play in this regard to maintain requisite progesterone receptors during decidualisation. Although, there is no consensus on the obligatory contribution of luteal phase E2 to the implantation process and the maintenance of early pregnancy yet mid-luteal decline in E2 and progesterone levels in ICSI cycles were associated with failure of conception.¹¹ In our study, low mid-luteal E2 in obese was found who were reported to have low clinical pregnancy rates.

The inverse correlation between BMI and estradiol level¹³ is attributed to its detrimental effect on ovulatory function, endometrial proliferation, sex hormone binding globulin and hence follicle-stimulating hormone levels.^{14,15} Maximum number of patients who could not conceive in our study had a high BMI, less basal, peak and mid-luteal E2. The important determinants of foeto-maternal cross-talk for desired implantation are number of blastocysts and receptive endometrium endorsed to optimal levels of hormones and growth factors. There was no significant difference in blastocysts number in all BMI groups and endometrial lining yet serum E2 was significantly low in the group which failed to conceive. This may prove the hypothesis that low E2 level associated with a high BMI in different phases of ovarian cycle is responsible for decreased chances of conception. The study, however, is limited with respect to evaluation and comparison of other hormones and growth factors which may affect reproductive outcome in obese women.

CONCLUSION

Maximum number of women who underwent ICSI were obese with BMI ≥ 26 . They had low basal, peak and mid-luteal E2 and majority were not able to conceive which suggests a correlation of raised BMI, decreased E2 and poor reproductive outcome. There is need of counselling obese women with emphasis that reduction in weight can improve E2 levels and hence chances of successful conception.

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