Endometrial preparation does not affect the risk of hypertensive disorders of pregnancy in low-risk women undergoing frozen embryo transfer.

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ABSTRACT

INTRODUCTION: Frozen embryo transfer (FET) is associated with a higher risk of hypertensive disorders in pregnancy. The objective of the present study is to evaluate the effect of different protocols of endometrial preparation on the risk of these disorders.

MATERIALS AND METHODS: We conducted a retrospective cohort study on 594 singleton pregnancies achieved by embryo transfer of single frozen-thawed blastocysts. Women with pre-existing risk factors for hypertensive disorders were excluded. Women were divided into two groups according to the endometrial preparation protocol: either natural cycle (n=495) or programming cycle with hormonal replacement therapy (n=97). The primary outcome was the frequency of hypertensive disorders in pregnancy: specifically, gestational hypertension and preeclampsia.

RESULTS: No differences emerged between women following natural cycle and those following programming cycle in the frequency of gestational hypertension (5% vs 4%) and preeclampsia (1.1% vs 1.2%). No impact emerged also after multivariate analyses.

CONCLUSION: Women receiving hormonal replacement therapy have the same risk of gestational hypertension and preeclampsia as women following natural cycles, when considering low-risk singleton pregnancies.

Keywords: frozen embryo transfer; corpus luteum; endometrial preparation; gestational hypertension; preeclampsia.

Introduction

In recent years freezing-thawing techniques have been facing an extraordinary development and have reached a wide diffusion, due to their numerous advantages. Frozen-thawed embryo transfer (FET) enables the storage of supernumerary embryos and facilitates the practice of single embryo transfer. In addition, it allows to prevent ovarian hyperstimulation syndrome (OHSS) and to perform preimplantation genetic testing programs [1].

FET cycle requires synchronization of the embryo development with the endometrium. Three different protocols of endometrial preparation can be considered [2]. The natural ovulatory cycle is based on detection of spontaneous ovulation by ultrasound checks and urine tests for LH surge; the modified natural cycle is characterized by induction of ovulation by the administration of human chorionic gonadotropins once the dominant follicle has reached appropriate development; the programming cycle requires hormonal replacement therapy (HRT) with estrogens, responsible for the endometrial thickening, and progesterone, responsible for endometrial receptivity. To date, there is no evidence supporting a difference among these approaches in terms of chances of pregnancy [2].

However, obstetrics outcomes may differ. The focus has recently been shifted to a possible association between the presence/absence of corpus luteum and the risk of hypertensive disorders in pregnancy (HDP) [3-5]. Albeit not univocal, there is emerging evidence that preeclampsia and gestational hypertension could be more frequent in women transferring frozen embryos in the context of HRT prepared endometrium compared to those treated with natural cycle (Table 1) [6-14]. It has been hypothesized that the absence of the corpus luteum in programming cycles may be responsible for an altered maternal cardiovascular adaptation in early pregnancy, thus increasing the risk of preeclampsia [15]. The corpus luteum produces not only estradiol and progesterone, crucial for implantation,

placentation and pregnancy maintenance, but also relaxin, a potent vasodilator which contributes to cardiovascular and renal adaptation in pregnancy [15-17].

The aim of the present study is to further investigate the possible role of corpus luteum in modulating the risk of HDP in a population of women who are transferred frozen-thawed blastocysts. More specifically, we compared the incidence of these disorders between women treated with natural cycles and those receiving HRT.

Materials and Methods

The present study is a single center, retrospective, cohort study, carried on at the Infertility Unit of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy. All the pregnancies achieved by embryo transfer of frozen-thawed blastocysts between January 2014 and December 2019 were retrospectively reviewed. Patients were identified using Meditex software (Critex GmbH, Regensburg, Germany). Inclusion criteria were as follows: 1) embryo transfer of a single frozen-thawed blastocyst, 2) achievement of a singleton pregnancy, 3) reaching of, at least, 20 gestational weeks. Those women who met all the eligibility criteria and achieved more than one pregnancy during the study period, were included only once. Women who had multiple pregnancies or risk factors for HDP (such as chronic hypertension, previous pregnancy complicated by gestational hypertension or preeclampsia, antiphospholipid antibody syndrome, pregestational diabetes and nephropathies) were excluded. Women included in the study were divided into two groups, according to the endometrial preparation protocol: 1) natural cycle and 2) programming cycle with HRT. The study was accepted by the local Ethical Committee (Comitato Etico Milano Area B, N. 1015_2019). An informed consent was not requested due to its retrospective nature. However, all patients referring to our unit routinely give an informed consent for their

data to be used for research purposes. The study was funded by the 2019 award "*Birth - Better Innovation and Research with Theramex*".

Data were extracted from patients' clinical and biological charts. They included demographic and clinical characteristics as well as details of the in vitro fertilization (IVF) procedures and pregnancy outcome. According to the local Italian legislation, an active follow-up of all pregnancies around the time of the expected date of delivery was systematically performed. Information regarding pregnancy course was collected using a standardized questionnaire and relied on obstetrical charts for women who delivered in our hospital while for those who delivered elsewhere it was obtained through a phone call. Inconsistencies or missing data were solved by additional phone contacts.

The primary outcome was the frequency of HDP (i.e., gestational hypertension and preeclampsia) in women undergoing HRT and in those following a natural cycle. *Preeclampsia* was defined according to criteria of the International Society for the Study of Hypertension in Pregnancy (*ISSHP*), as gestational hypertension accompanied by one or more of the following new-onset conditions at or after 20 weeks gestation: proteinuria, organ dysfunction (including acute kidney injury, liver involvement, neurological complications, hematological complications), uteroplacental dysfunctions (fetal growth restriction, abnormal umbilical artery Doppler waveform analysis, stillbirth). *Gestational hypertension* was conversely defined as isolated persistent de novo hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) that develops at or after 20 weeks gestation. Proteinuria was defined as 24-h urinary protein ≥ 300 mg per day or dipstick testing 2+ [18].

Allocation to natural cycle or HRT was mainly decided based on medical history [19]. Women with regular menstrual cycles and a mean cycle length between 24 and 35 days were scheduled for the natural cycle protocol. Those with irregular cycles as well as those who failed to identify the LH surge in a preceding cycle received HRT. In addition, HRT could be

considered for women with logistic problems such women residents in distant areas. The protocols for the endometrial preparation are reported in detail elsewhere [19]. Briefly, women scheduled for natural cycle were monitored through serial transvaginal ultrasounds, starting on day 6-9 of the menstrual cycle, until a leading follicle of 15-16 mm was evident. Then, urinary sticks for LH surge were performed twice a day. Once the urinary stick turned positive, patients underwent one more ultrasound to check the presence of the corpus luteum and endometrial thickness. The cycle was canceled if ovulation had not occurred or if the endometrial thickness was not appropriate. Embryo transfer was performed 6 days after LH surge. Women scheduled for HRT, specifically, started with 2 patches of 50 mcg transdermal micronized estradiol every three days (Estraderm, Merus Labs Luxco) or 2 tablets of 2 mg estradiol valerate orally daily from day 2 of the menstrual cycle. On day 8 the dose was increased to 3 patches every 3 days or 6 tablets daily. On day 12 ultrasound was performed to check endometrial thickness and to confirm the ovarian inactivation. Once the endometrium reached a trilaminar appearance with a thickness of at least 7-8 mm, embryo transfer was planned and vaginal progesterone (Progeffik, Effick Italia S.p.A) was progressively introduced: 200 mg once a day the first day, 200 mg twice a day the second day, 400 mg twice a day from the third day, until the day of the hCG assessment. Embryo transfer was performed 5 days after the initiation of progesterone. In the case of pregnancy, HRT had to be continued until 12 weeks' gestation.

Data collected were transferred in Statistical Package for Social Science (SPSS 26.0, IL, USA) database for subsequent analyses. Differences among the two groups were compared using Fisher Exact Test, Chi Square Test, Student *t* test or Mann-Whitney test, as appropriate. Shapiro-Wilk test was used to verify the normal distribution. Non-normal variables were compared using non parametric statistics. Continuous variables were reported as mean ± standard deviation (SD) or median [interquartile range (IQR)], while categorical

variables were reported as frequencies and percentages. In order to disentangle an adjusted measure of the association between endometrial preparation and HDP, baseline variables found to differ between study groups were entered into a multivariate logistic model. P values below 0.05 were considered statistically significant.

The primary outcome was the frequency of women developing preeclampsia. The scheduled sample size was at least 300 women. It was calculated stating as clinically relevant a three-fold increased risk in HRT women (30% vs 10% in natural cycle cases) and setting type I and II errors at 0.05 and 0.20, respectively. The expected 10% rate of preeclampsia was obtained from published data [20]. Even if the sample size could be reached by reviewing the last four years of activity (2016-2019), we decided to go back to the beginning of our program of embryo transfer on a natural cycle (up to 2014) because a larger sample size could allow more precise estimates of the association.

Results

On the whole, 594 women were initially selected; 495 women performed natural cycle monitoring, while 97 received HRT. Baseline characteristics of the two groups are shown in Table 2. Women in HRT group showed younger age, higher BMI, longer infertility duration and fewer previous deliveries. Moreover, antral follicle count (AFC) was significantly higher in the HRT group, than in the group performing the natural cycle. As expected, women performing natural cycles had more frequently regular menstrual cycles than women in programming cycle group (94% vs 41%, $p<0.001$), who therefore needed HRT. Among women performing natural cycle, the main indication for cryopreservation was the presence of supernumerary embryos, while women undergoing HRT more frequently had cryopreserved embryos due to enhanced risk of OHSS. Smoking habits, number of previous pregnancies, number of previous IVF cycles and frequency of gynecological surgery did not differ.

Obstetric outcome is summarized in Table 3. No differences emerged in the frequency of HDP. Preeclampsia complicated 6 (1.2%) and 1 (1.1%) pregnancies in the natural cycle group and the HRT group, respectively (p=1.00). Pregnancy-induced hypertension developed in 25 (5%) and 4 pregnancies (4%), respectively (p=1.00). Conversely, gestational diabetes was more frequent in the HRT group than in the natural cycle group. The incidence of placenta previa, mode of delivery (vaginal delivery vs cesarean section), preterm birth frequency and neonatal weight did not differ significantly (Table 3).

Baseline characteristics found to be significantly associated with the study groups were entered into a multivariate logistic model. The adjusted OR of developing preeclampsia was 1.20 (95%CI: 0.12 - 11.69) in women transferring blastocysts in the context of a natural cycle. The adjusted OR of developing gestation hypertension was 0.40 (95%CI: 0.08 - 1.94). The adjusted OR of developing preeclampsia or gestation hypertension was 0.54 (95%CI: 0.15 - 1.98).

Discussion

This retrospective study conducted on 594 cycles of frozen-thawed blastocyst aimed to investigate the effect of the endometrial preparation protocol on the risk of HDP. No differences were observed between women who were transferred within a natural cycle and those who received HRT. Our study does not corroborate previous findings from widespread geographical areas that generally highlighted an increased risk of HDP with HRT compared to natural cycles [6-14] (Table 1).

The absence of the corpus luteum in programming cycles has been proposed as the possible cause of the development of HDP. The corpus luteum plays a crucial role in early pregnancy by producing a multitude of hormones necessary for implantation, placentation and maintenance of pregnancy. In particular, the corpus luteum is responsible for the production

of relaxin, a potent vasodilator that contributes to cardiovascular and renal adaptation in pregnancy [21]. In HRT regimen, ovulation is suppressed, no corpus luteum develops, and this situation may lead to an abnormal vascular response and thus to the onset of preeclampsia.

Our findings are therefore in contrast with the available literature. To explain this inconsistency, it has first to be underlined that results of previous studies were not univocal and incidences of HDP varied widely (Table 1). This could reflect different study populations. In fact, we hypothesize a crucial role of the characteristics of our population in explaining the lack of any association. Women with risk factors for HDP were not included in our study. We excluded women with baseline clinical conditions that could favor preeclampsia, including chronic hypertension, previous pregnancy complicated by gestational hypertension or preeclampsia, antiphospholipid antibody syndrome, pregestational diabetes and nephropathies. Moreover, and most importantly, we excluded multiple pregnancies, an additional important risk factor for HDP. In fact, this latter choice did not significantly shrink the cohort of studied cases because, based on the policy of our Unit, single embryo transfer was systematically performed in women who were transferred thawed blastocysts (only 8 cases of monozygotic twins were excluded). Furthermore, it has to be underlined that our population was at low risk of preeclampsia per se because other risk factors that were not considered in our selection criteria were poorly represented among our population. Women were relatively young (mean age of 34-36 years) and, most importantly, generally normal weight (mean BMI was 21-22 Kg/m^2). Overall, due to the study design (selection criteria that excluded a priori women at high risk), policy (systematic single embryo transfer) and local habits (low weight), our population had an intrinsic low risk of HDP, as reflected by the low rate in both study groups. On these bases, one may speculate that the role of the corpus luteum to prevent HDP could be marginal if not unremarkable in women at low risk. This

interpretation remains speculative and more in-depth investigations on this issue could be important. Indeed, if confirmed, the embryo transfer within a natural cycle would be fundamental only in women with baseline increased risk of preeclampsia. In other words, the emerging recommendation to favor natural cycle transfer to lower the risk of HDP may not be valid for low risk groups.

An alternative explanation for our inconsistent findings could be found in some confounders. Since evidence from the literature was mainly obtained from observational rather than randomized controlled trials, one cannot exclude selection biases in both our study and previous contributions. The considerably different rates of HDP observed in previous studies support this possibility (Table 1). Of utmost relevance here is that the proportion of women treated with natural cycle compared to those receiving HRT varies widely among studies. Noteworthy, the highest rate of natural cycle use for transfer of thawed embryos was documented in our study (about 5:1) (Table 1). In our setting, natural cycle was indeed the first option. Only a minority was scheduled for HRT due to the failure of natural cycle monitoring or because the woman decided on this approach for logistic reasons. To note, in the Japanese study by Saito et al. [8], this proportion was even inverted (1:2). Overall, some selection biases could have occurred among studies and may contribute to explain the controversial findings. For instance, in our study, irregular menstrual cycle counted for 26% of the women in the HRT group versus 1% of the women in the natural cycle group. More specifically, the main cause of irregular menstrual cycle in infertile population is Polycystic Ovary Syndrome (PCOS), accounting for 80% of the cases of anovulatory infertility [22]. Of utmost relevance here is that PCOS is associated with an increased risk of obstetric complications, including in particular gestational diabetes but also HDP and preterm birth [23]. Accordingly, we detected a higher frequency of gestational diabetes among women treated with HRT in our study, thus supporting the likelihood of selection biases occurrence in

non-randomized studies. To note, this bias (more PCOS women among those receiving HRT) was expected to inflate the frequency of preeclampsia among those treated with HRT. The lack of differences tends to reinforce our conclusion.

Some strengths and limitations of our study should be acknowledged. Considering strengths, data was highly accurate because information were retrieved from patients' charts and the number of women lost to follow-up was irrelevant. In our opinion, the retrospective design did not significantly affect the high accuracy of the information. Moreover, based on the policy of our Unit, a pure natural cycle preparation was used, i.e. women were transferred the embryos without receiving any additional treatment. Moreover, we exclusively included women who underwent single blastocyst transfer. These stringent selection criteria should have protected our findings from confounders. On the other hand, one has to recognize that the sample size was relatively small and could thus capture only strong associations. Given the rarity of the events of preeclampsia or gestational hypertension in our population, the statistical power was insufficient to detect mild but potentially clinically relevant differences. In this regard, it has to be highlighted that the frequency of HDP was markedly lower than the previously reported local data of the general population that we used to estimate the sample size [20].

In conclusion, in our study, women receiving HRT had the same risk of preeclampsia and gestational hypertension as those treated with the natural cycle. In our opinion, our results do not fully contradict previous evidence because we studied a population at low risk of preeclampsia. One could infer that, in women without risk factors for preeclampsia and within a policy of stringent single embryo transfer, HRT does not expose women to a significantly enhanced risk of preeclampsia. Even if the natural cycle policy may have a benefit in terms of preventing obstetric complications, our data suggest that in the absence of additional risk factors for preeclampsia, this indication should not be mandatory. Further

evidence is however needed from large randomized controlled trials for a definitive conclusion.

Declaration of Interest Statement

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Data availability statement

The database can be provided on request.

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