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Fresh blastocyst transfer as a clinical approach to overcome the detrimental effect of progesterone elevation at hCG triggering: a strategy in the context of the Italian law

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ABSTRACT

Objective: To retrospectively evaluate whether fresh day-5 embryo transfer could overcomes the detrimental effect of subtle progesterone elevations at hCG administration on pregnancy outcomes in women undergoing ovarian hyperstimulation for IVF/ICSI cycles.
Study design: Retrospective study of 204 infertile patients aged 23–44 years who underwent IVF/ICSI treatment and fresh blastocyst transfer under the Italian law (embryos cryopreservation cannot be planned in advance). Women were divided into those with a progesterone level <1.5 ng/ml and those with a progesterone concentration ≥1.5 ng/ml at hCG triggering. The clinical pregnancy rate (CPR) after blastocyst transfer was the primary outcome.
Results: Age, body mass index (BMI), antral follicle count, anti-Mullerian hormone (AMH) and FSH values, mean number of stimulation days, ratio of GnRH agonist and antagonist cycles and total dose of gonadotrophins administered did not differ between the two groups. Serum estradiol and number of retrieved oocytes were significantly increased in the group with elevated progesterone and a significantly higher number of oocytes was used in this group. Fertilization rate, percentage of top quality embryos, and number of transferred blastocysts were similar in the two groups. The CPR was significantly higher in women with progesterone levels <1.5 ng/ml at hCG (50%) compared with women with progesterone concentration ≥1.5 ng/ml (33.3%) (odds ratio = 2.00, 95% confidence interval 1.07–3.75).
Conclusions: A fresh blastocyst transfer does not completely overcome the detrimental effect of progesterone rise at hCG on IVF/ICSI pregnancy outcomes.

1. Introduction

Modest increases in serum progesterone levels are detected on the day of hCG administration in controlled ovarian stimulation (COS) cycles for IVF and ICSI/embryo transfer with an incidence as high as 35% using GnRH agonist protocols [1,2] and 38% using GnRH antagonists [3–5]. The topic has recently gained much attention in relation to the recent overwhelming literature reporting a negative impact of this "subtle" elevation on cycle outcomes. Very large clinical studies have recently revealed that a premature progesterone rise at the end of COS is associated with lower pregnancy and implantation rates [6–13], but the mechanism leading to this detrimental effect is unclear.

Elevated progesterone levels may impair endometrial receptivity [14] or affect oocyte/embryo quality [15]. Another theory has proposed that a high follicular progesterone level tends to advance the endometrium leading to an asynchronous dialogue between the endometrium and the embryo [16]. Given this limited knowledge of the biological basis underlying the adverse effects of a progesterone rise on pregnancy outcomes, the choice of the best clinical strategy to adopt in order to overcome the problem is still debated.

In general, most of the studies propose cryopreservation of pronuclear or cleavage stage embryos in patients with progesterone levels higher than 1.5–2.0 ng/ml on the day of hCG administration [10,11]. Conversely, the efficacy of the extending culture to day 5 and blastocyst transfer as a strategy to allow the

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endometrium to recover from the damage induced by high progesterone levels is more controversial. In a prospective cohort study, Papanikolau et al. failed to observe any effect of the progesterone rise on the clinical pregnancy rate following a fresh blastocyst transfer [17]. In contrast, two retrospective studies demonstrated that even day-5 embryo transfers were associated with a significantly lower live birth rate in patients with a subtle progesterone rise at ovulation induction, especially when pituitary down-regulation was obtained by GnRH agonists [11,18].

In Italy, the Law 40/2004 regulating assisted reproductive technology procedures does not allow embryo cryopreservation to be planned in advance even for clinical reasons. This has the ultimate aim of avoiding unnecessary and exaggerated embryo freezing. In cases of a progesterone elevation above a detrimental threshold level, a strategy of embryo cryopreservation cannot be decided at oocyte retrieval. Other approaches, for instance oocyte preservation, may be arranged. In our centre, we have decided to proceed favouring extended culture and fresh blastocyst stage transfer as a potentially reasonable option in the context of the Italian situation, based on literature indicating that pregnancy outcomes derived from blastocyst transfer are not affected by the detrimental effect of progesterone rise [17]. The aim of this study, therefore, was to retrospectively evaluate the association between elevated serum progesterone level on the day of hCG administration and IVF/ICSI outcomes after fresh day-5 embryo transfer.

2. Materials and methods

2.1. Recruited population and clinical procedures

This retrospective, single-centre cohort study was conducted from January 2012 to December 2012 at the University-affiliated IVF unit of the San Raffaele Scientific Institute, Milano. All the ICSI/IVF cycles were performed in accordance with the guidelines of the Italian Law 40/2004. Although the Constitutional Court in 2009 has declared the autonomous responsibility of the physician in selecting the best suitable treatment for the patients with their full consent, the veto still exists on embryo cryopreservation when, at the moment of oocyte retrieval, there are strong clinical reasons to postpone the transfer.

Based on an internal audit of our IVF unit performed in 2011, a progesterone level $\geq$1.5 ng/ml on the day of hCG administration was found to have a detrimental effect on pregnancy outcome with fresh day-3 embryo transfer in our centre (clinical pregnancy 14.5% for progesterone at hCG $\geq$ 1.5 ng/ml vs 26.2% for progesterone at hCG $<$ 1.5 ng/ml). This finding was in line with the data in the literature [7,8,17]. To solve this problem, we could not propose in advance cryopreservation of pronuclear or cleavage stage embryos to our patients with high progesterone at hCG since, based on the guidelines of the Italian law, a strategy of embryo cryopreservation in order to postpone the transfer to a subsequent non-stimulated cycle cannot be decided a priori. Therefore, in cases of a progesterone elevation above a detrimental threshold level, we have opted for fresh blastocyst transfer based on specific laboratory criteria for extended culture to blastocyst stage.

In 2012, a total of 912 IVF or ICSI cycles with controlled ovarian stimulation (COS) were performed in our centre. In 681 cycles, strategy for embryo transfer was decided based on embryo evaluation on day 3. The overall presence of a progesterone level at hCG $\geq$1.5 ng/ml in this cohort of 681 cycles was 17.9% (n = 122). Of these 122 cases with an elevated progesterone at hCG, an extended culture to the blastocyst stage following strictly the laboratory criteria was decided in 60 patients (49.1%). In the remaining cases in whom our laboratory criteria for extended culture to blastocyst could not be satisfied (n = 62), the choice to proceed with a cleavage stage transfer or to freeze embryos was taken in discussion with the patient on the same day (day 3) based on embryo number and quality. The outcome of these latter strategies in the presence of a 'high' progesterone is well described in the literature [7,8,17] and was not related to the aim of the present study.

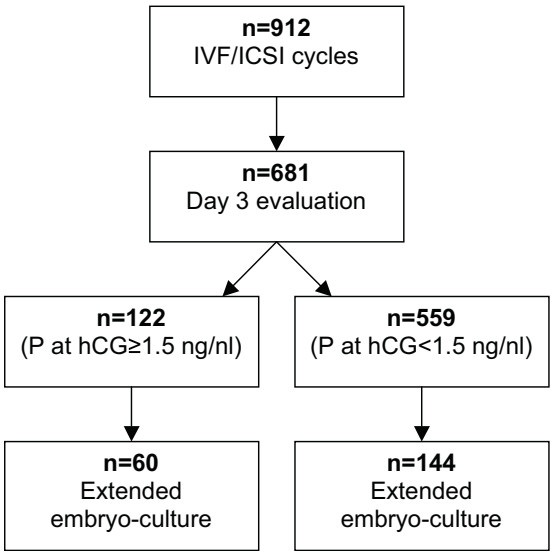

Fig. 1. Flowchart of the subjects included in the study.

These 60 patients with elevated progesterone at hCG, in whom an extended culture to the blastocyst stage was decided, were compared to a cohort of 144 women without a progesterone rise who underwent extended culture and transfer on day 5 in the same time period. Thus, a total of 204 women undergoing IVF or ICSI cycle with controlled ovarian stimulation (COS) and fresh day-5 embryo transfer were included in the present study (Fig. 1). No other inclusion/exclusion criteria were applied on baseline characteristics of patients. All women routinely provided informed consent for their clinical data and anonymised records to be used for researches purposes. Local Institutional Review Board approvals for the use of clinical data for research studies were obtained.

2.2. Controlled ovarian stimulation

Controlled ovarian stimulation was performed according to the standard clinical practice. Either GnRH agonist or GnRH antagonist daily protocol was used for pituitary down-regulation, and ovarian stimulation was carried out according to one of the following: (a) recombinant FSH (rFSH) alone; (b) rFSH combined with recombinant LH (rLH); (c) highly purified human menopausal gonadotrophin alone (HP-hMG). Both the initial dose and dose adjustments during treatment were chosen on a case-by-case basis according to patients' characteristics and response to gonadotrophin. Triggering of ovulation was performed with HP-human chorionic gonadotrophin (hCG) when one or more follicles had reached a diameter $\geq$17–18 mm. Serial determinations of serum oestrogen (E_2) and progesterone levels were performed during the treatment. The first (basal) determination was carried out before the beginning of gonadotrophin administration, while the last assessment of E_2 and progesterone levels was obtained on the day of hCG administration. Oocytes were retrieved after about 35 h after hCG administration.

2.3. ICSI/IVF and embryo culture

ICSI and conventional IVF were performed in a standard way [19]. Cumulus-corona-oocyte complexes were collected and washed in HTF medium with HEPES (Sage In Vitro Fertilization, Inc. Trumbull, CT, USA) supplemented with 5 g/l of human serum