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Embryo Survival after Vitrification in Different Ovulation Induction Protocols: A Randomized Prospective Study

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Abstract

Objective: To compare embryo quality and survival after vitrification using two different ovulation induction protocols—one based on highly purified human chorionic gonadotropin (HP-hCG) and the other on a gonadotropin-releasing hormone (GnRH) agonist (triptorelin)—regardless of the risk of ovarian hyperstimulation syndrome (OHSS). **Materials and Methods:** A prospective randomized study was conducted at the MAP Unit of San Filippo Neri Hospital, Rome, Italy, including 57 women undergoing frozen embryo transfer between January 2018 and March 2021. Participants were randomly assigned to receive ovulation induction with either 10,000 IU of HP-hCG or 0.2 mg/mL of triptorelin acetate. Oocytes were retrieved, fertilized via ICSI, cultured to the blastocyst stage, vitrified, and later thawed. Outcome measures included post-thaw embryo survival rate, number and maturity of retrieved oocytes, fertilization rate, blastulation rate, implantation rate, pregnancy rate, and miscarriage rate. **Results:** No significant difference was observed in post-thaw embryo survival between the two groups (HP-hCG: 90%; triptorelin: 91%). The HP-hCG group showed a significantly higher number of mature oocytes retrieved ($p = 0.004$). All other embryological and clinical parameters, including fertilization, blastulation, implantation, pregnancy, and miscarriage rates, were comparable between groups. **Conclusion:** Both ovulation induction protocols yielded similar embryo survival and clinical outcomes following vitrification and thawing. However, the use of HP-hCG resulted in a higher number of mature oocytes retrieved, suggesting it may be more advantageous for patients with a poor ovarian response. Conversely, triptorelin may be preferable for patients at risk of OHSS.

Keywords

Ovulation Induction Protocols, Vitrification, Embryo Survival

1. Introduction

Controlled ovarian stimulation (COS) is a cornerstone of assisted reproductive technologies (ART), designed to maximize the number of oocytes retrieved during a single treatment cycle, thereby enhancing the chances of successful fertilization and pregnancy [1] [2]. The standard goal of COS is to induce multifollicular growth through the administration of exogenous gonadotropins, enabling the retrieval of multiple mature oocytes suitable for *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) [3] [4].

Two primary agents are employed to trigger final oocyte maturation: human chorionic gonadotropin (hCG) [5] and gonadotropin-releasing hormone (GnRH) agonists [6] [7]. hCG has been traditionally favored due to its molecular similarity to luteinizing hormone (LH), which allows it to induce the LH surge necessary for ovulation [8]. However, its longer half-life compared to LH results in prolonged luteotropic activity, which has been strongly associated with the development of ovarian hyperstimulation syndrome (OHSS) [6] [9]—a potentially life-threatening complication characterized by increased vascular permeability, hemoconcentration, and fluid shifts into third spaces, largely mediated by vascular endothelial growth factor (VEGF) [10] [11].

To mitigate OHSS, especially in high-risk patients (e.g., those with polycystic ovary syndrome, low BMI, or elevated AMH levels), the use of GnRH agonists as ovulation triggers has become increasingly prevalent [6] [12]. These agents induce an endogenous flare-up of LH and FSH by initially stimulating the pituitary before causing downregulation of GnRH receptors [13]. The short-lived surge of gonadotropins more closely resembles the physiological hormonal milieu, reduces VEGF expression in the ovary, and results in a lower incidence of OHSS [6] [14] [15].

In parallel, the practice of embryo cryopreservation has gained momentum through the "freeze-all" strategy, where all embryos are vitrified and transferred in subsequent cycles [16] [17]. While this approach further reduces OHSS risk and may improve endometrial receptivity by avoiding the supraphysiologic hormonal environment of fresh transfers, vitrification and warming processes introduce cellular stress [18]. Oxidative stress and potential DNA fragmentation may compromise embryo viability, implantation potential, and pregnancy outcomes [1].

Despite extensive use of both hCG and GnRH agonists in clinical practice, there remains uncertainty as to whether the type of ovulation trigger influences not only immediate embryological outcomes but also post-thaw embryo survival and clinical success. Therefore, it is critical to investigate the impact of different ovulatory agents on embryo quality after vitrification, especially given the growing reliance on cryopreserved embryo transfer in ART programs [19]-[21].

2. Materials and Methods

A prospective randomized trial was conducted at the IVF Unit, Ospedale San Filippo Neri (Rome) on 57 patients undergoing frozen embryo transfer between January 2018 and March 2021. The inclusion criteria were: age between 28 and 42 years, AMH levels between 1 and 5 ng/mL, BMI between 18.5 and 28 kg/m^2, presence of tubal factor infertility, and absence of autoimmune or genetic disorders. To compare embryo survival and clinical outcomes after vitrification in IVF cycles triggered with HP-hCG or GnRH-a in normo-responder women at low-to-moderate risk of ovarian hyperstimulation syndrome (OHSS). The final oocyte maturation trigger was administered when at least three follicles measured ≥18 mm on transvaginal ultrasound. In our center, we also considered serum estradiol levels consistent with mature follicles (typically ~150 - 250 pg/mL per follicle ≥18 mm). Depending on randomization, participants received HP-hCG (10,000 IU, s.c.) or triptorelin (0.2 mg, s.c.). Oocyte retrieval was scheduled 36 ± 2 hours after trigger.

Patients were randomly assigned to two groups:

Group A: Ovulation triggered with 10,000 IU HP-hCG (Gonasi HP®)

Group B: Triggered with 0.2 mg/ml triptorelin acetate (Fertipeptil®)

Stimulation and Laboratory Protocols

COS was performed with combined HP-hMG (Meriofert® 125 IU/day) and recombinant FSH (Puregon® 125 IU/day), alongside GnRH antagonist ganirelix (Orgalutran®). Oocyte retrieval was scheduled 36 hours post-trigger. ICSI was performed exclusively on MII oocytes. Resulting embryos were cultured to the blastocyst stage, vitrified using a closed Cryotop® system, and thawed two hours before transfer.

Outcome Measures

The primary outcome was embryo survival post-thaw, defined as the percentage of viable blastocysts after warming. Secondary outcomes included the mean number of oocytes retrieved and matured, fertilization rate, blastulation rate, implantation rate, pregnancy rate (defined as β-hCG positivity), and miscarriage rate (pregnancy loss following a positive β-hCG test).

Statistical Analysis

Statistical analysis was performed using Student's t-test for continuous variables and the χ^2 test for categorical variables. A p-value < 0.05 was considered statistically significant.

3. Results

Baseline Characteristics

At baseline, the Group A (HP-hCG) and Group B (Triptorelin) showed comparable clinical and demographic characteristics. Specifically, no statistically significant differences were observed in age, body mass index (BMI), anti-Müllerian hormone (AMH) levels, or the duration of ovarian stimulation between the groups (**Table 1**).

Table 1. Baseline characteristics—age, BMI, AMH levels, and duration of stimulation—of patients undergoing controlled ovarian stimulation in the two study groups: Group A (HP-hCG) and Group B (Triptorelin). Data are expressed as mean ± standard deviation.

Variable	Group A (HP-hCG)	Group B (Triptorelin)	p-value
Age (years)	35.5 ± 4.6	34.9 ± 4.1	0.616
BMI (kg/m^2)	22.9 ± 4.0	23.2 ± 4.4	0.277
AMH (ng/mL)	3.06 ± 1.38	3.21 ± 1.46	0.363
Stimulation (days)	11.3 ± 1.6	11.0 ± 2.0	0.371

Ovarian and Embryological Outcomes

A comparison of ovarian and embryonic outcomes was conducted between two treatment groups: Group A (HP-hCG) and Group B (Triptorelin). The analyzed parameters included the mean number of mature and total oocytes retrieved, fertilization rate, blastulation rate, post-devitrification embryonic survival, pregnancy rate, implantation rate, and spontaneous abortion rate (Table 2, Figure 1). The only statistically significant difference between the groups was observed in the mean number of mature oocytes ($p = 0.004$) (Figure 2).

4. Discussion

This randomized prospective study demonstrates that embryo survival rates following vitrification are comparable between patients who underwent ovulation induction with either HP-hCG or a GnRH agonist. Although fertilization, blastulation, implantation, and pregnancy rates were comparable between the two protocols, the number of mature oocytes retrieved was significantly higher in the HP-hCG group.

Table 2. Comparison of ovarian and embryonic outcomes in the two treatment groups: Group A (HP-hCG) and Group B (Triptorelin). Data include mean number of mature and total oocytes retrieved, fertilization rate, blastulation, post-devitrification embryonic survival, pregnancy rate, implantation and spontaneous abortion. Values are expressed as mean ± standard deviation or percentage.

Outcome	Group A	Group B	p-value
Mean mature oocytes	8.11 ± 3.32	5.70 ± 2.81	**0.004**
Total oocytes retrieved	9.93 ± 3.92	8.13 ± 3.65	NS
Fertilization rate	79%	86%	NS
Blastulation rate	84%	77%	NS
Embryo survival rate	90%	91%	NS
Pregnancy rate	56%	57%	NS
Implantation rate	23%	31%	NS
Miscarriage rate	40%	41%	NS

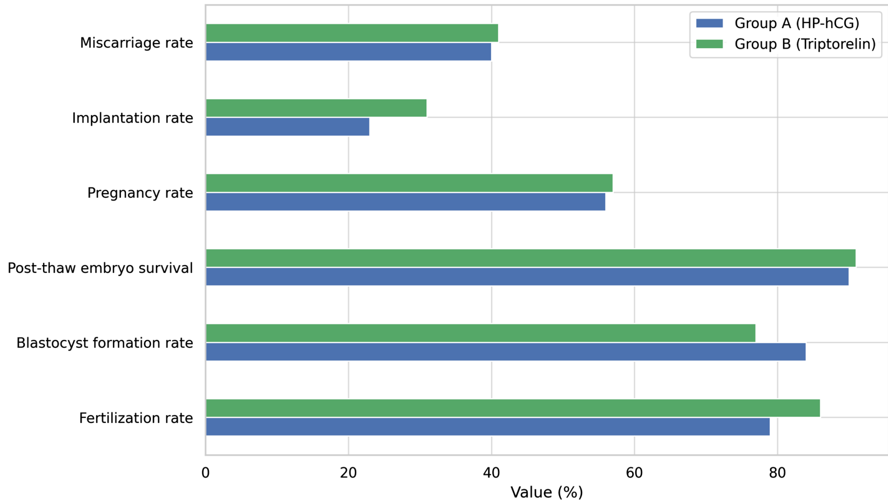

Figure 1. Overview of ovarian and embryological parameters in Group A (HP-hCG) and Group B (Triptorelin).

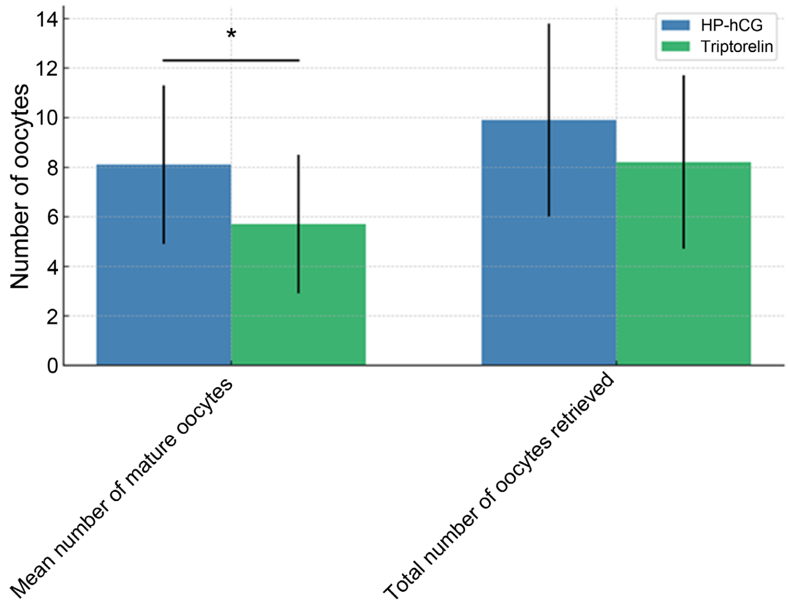

Figure 2. Comparison of oocyte counts between HP-hCG and triptorelin ovulation induction protocols. Error bars represent standard deviations.

The increased maturation rate with HP-hCG may be attributed to its longer half-life and sustained luteotropic activity, which more effectively supports the final stages of oocyte maturation. This may be particularly beneficial in patients categorized as poor responders, who often require an optimized hormonal environment to yield a satisfactory number of mature oocytes. On the other hand, the use of GnRH agonists, which induce a shorter, more physiological LH surge, appears to preserve embryo viability without the prolonged exposure to supraphysiological luteinizing activity, thus reducing the risk of OHSS—a critical consideration for patients with high ovarian reserve or predisposition to this complication [6] [22].

The similarity in post-thaw embryo survival between the two groups is a noteworthy finding. Vitrification techniques, while efficient in preserving embryo morphology, expose cells to osmotic and oxidative stress that may affect their viability and developmental potential. Our findings suggest that the hormonal milieu established at the time of oocyte maturation does not significantly alter the resilience of embryos to cryopreservation-induced stress, at least when vitrification is performed under optimized laboratory protocols.

Moreover, the comparable implantation and pregnancy rates further reinforce the clinical equivalence of these protocols in frozen embryo transfer settings. The lack of significant differences in miscarriage rates also suggests that neither approach compromises embryo competence in a clinically meaningful way. These outcomes align with earlier reports by Eldar-Geva *et al*., 2007 [23] and Humaidan *et al*., [24] who observed similar efficacy of GnRH agonists and hCG in the context of cryopreserved embryo transfers, emphasizing the importance of tailoring ovulation triggers to individual risk profiles rather than applying a one-size-fits-all approach.

Nevertheless, it should be acknowledged that this study is limited by its relatively small sample size and single-center design. Additionally, while the post-thaw survival rate is a crucial parameter, it is only a surrogate marker of embryo competence. Future investigations could incorporate additional markers such as metabolic profiling, mitochondrial DNA content, or time-lapse imaging parameters to provide a more nuanced assessment of embryo quality post-vitrification.

Lastly, while triptorelin presents a safer alternative in terms of OHSS prevention, clinicians must be cautious in ensuring adequate luteal support, as luteal phase deficiency is a well-recognized issue following GnRH agonist trigger. The use of modified luteal support protocols, including low-dose hCG rescue or intensive progesterone supplementation, may be necessary to optimize outcomes in these cases.

5. Conclusions

The present study confirms that both HP hCG and GnRH agonist represent valid and effective strategies for final oocyte maturation in IVF protocols employing embryo vitrification. Although there were no significant differences between the two groups in terms of post-thaw embryo survival and clinical pregnancy rates, patients receiving HP-hCG showed a significantly greater number of mature oocytes retrieved. These findings suggest that the choice of ovulation trigger can impact key biological parameters, especially oocyte quality.

Importantly, these findings highlight the growing relevance of individualized approaches to ovarian stimulation. The variability in ovarian reserve, response to gonadotropins, and risk of complications such as OHSS makes it essential to tailor the stimulation and trigger regimen to each patient's reproductive profile. Based on its longer half-life and strong luteotropic activity, HP-hCG could theoretically offer advantages in women with diminished ovarian reserve or suboptimal ovarian response, by sustaining the final stages of folliculogenesis. However, this hypothesis remains to be confirmed in studies specifically designed for this population [8] [25].

On the other hand, triptorelin—as a GnRH agonist—induces a more physiological LH surge of shorter duration and is thus preferable in high responders or patients at risk of OHSS, where safety is a primary concern [24] [26].

Emerging data suggest that a dual-trigger approach, combining a GnRH agonist with a low-dose h-CG supplementation, may provide synergistic benefits by promoting both endogenous gonadotropin surges and sustained luteal support. This strategy has been associated with improved oocyte maturation, embryo quality, and even clinical pregnancy rates, particularly in borderline or poor responders [20] [27]. Recent prospective trials and meta-analyses support the efficacy of dual-trigger protocols in enhancing IVF outcomes without significantly increasing the risk of OHSS [28] [29]. However, outcomes appear to be dose-dependent; insufficient hCG administration may result in luteal phase deficiency and lower implantation potential, underscoring the importance of proper protocol design [30] [31]. Our findings indicate that HP-hCG and GnRH-a triggers yield comparable post-warming embryo survival and clinical outcomes, yet each presents distinct advantages: HP-hCG was associated with a higher number of mature oocytes, whereas GnRH-a reduces the risk of ovarian hyperstimulation. In this context, the "dual-trigger" approach—combining a GnRH agonist with a low dose of hCG—has been proposed to integrate these benefits by enhancing oocyte competence while maintaining safety. Although our study did not evaluate this protocol, future trials could explore whether dual-triggering optimally balances oocyte maturation and OHSS prevention in patients undergoing vitrified embryo transfer cycles.

Personalized trigger selection—based on biomarkers such as Anti-Müllerian Hormone, Antral Follicle Count, previous stimulation outcomes, and OHSS risk—allows clinicians to optimize both efficacy and safety [32]. The dual-trigger method represents a promising strategy to enhance oocyte competence without compromising embryo quality or increasing OHSS incidence [33]. As reproductive medicine evolves toward more patient-centered paradigms, such flexible and targeted interventions will likely become central to IVF practice.

Further multicenter, randomized studies are warranted to explore the mechanistic underpinnings of dual-trigger effects, refine patient selection criteria, and integrate emerging technologies—such as time-lapse imaging, metabolomic profiling to further personalize and improve outcomes in assisted reproduction.

Author Contributions

A.P. conception and design, F.S. and S.N. data collection and interpretation, F.S. wrote the manuscript. A.P. provided suggestions and revised the manuscript for final submission. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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