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Research Article

Fertilization and Implantation Rates in Egg Donation IVF Cycles: A Comparison of Three or More Thawed Oocytes

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Abstract

Italian assisted reproduction is regulated by Law No. 40/2004, which limits embryo creation and, following Constitutional Court ruling No. 162/2014, authorizes heterologous oocyte donation under strict ethical criteria. These regulations prohibit the creation of supernumerary embryos, emphasizing the need for efficient and ethically aligned In Vitro Fertilization (IVF) practices.

Objective: In this context, the present study aims to demonstrate that fertilizing three oocytes per cycle in donor- oocyte IVF is sufficient to achieve reproductive success equivalent to that obtained with larger oocyte numbers, confirming that Italy's ethical limitation aligns with both clinical efficacy and economic sustainability.

Methods: A retrospective study of 317 thawing cycles using donor oocytes was performed between 2020 and 2025 at the MAP Units, San Filippo Neri Hospital and Sant' Anna, Rome. Patients were divided into two groups: Group 1 (3 Oocytes, n=114) and Group 2 (6 Oocytes, n=203). Outcomes measured: (1) Embryo Development (≥1 Viable Embryo) and (2) Pregnancy (≥1 Positive Outcome). Statistical analyses used z-tests for proportions and Wilson 95% Confidence Intervals (CI). All patients provided written informed consent in accordance with the Declaration of Helsinki and institutional ethical guidelines. The study protocol was approved by the local ethics committee of San Filippo Neri Hospital (Protocol No. 2020-ART-014).

Results: Embryo development rates were 91.2% and 97.0% (p=0.036 (statistically significant but clinically modest)), and pregnancy rates 21.9% and 37.4% (p=0.008 (statistically significant but clinically modest)) for 3 and 6 oocytes, respectively.

Conclusions: In donor-oocyte IVF, fertilizing up to three oocytes achieves outcomes clinically comparable to cycles with higher oocyte numbers. This supports the Italian ART model, which harmonizes clinical efficacy, ethical compliance, and economic sustainability under Law 40/2004 and Ministerial Decree 12 April 2023-Update of Essential Levels of Care (LEA) for Medically Assisted Procreation [1].

Introduction

Assisted Reproductive Technologies (ART) have profoundly transformed the management of infertility by enabling controlled ovarian stimulation [2], In Vitro Fertilization (IVF), and embryo culture. Over the past decades, continuous improvements in laboratory techniques, cryopreservation, and embryo selection have increased treatment success rates while reducing complications such as ovarian hyperstimulation syndrome and multiple pregnancies. Despite these advances, the number of oocytes retrieved and fertilized per cycle remains a crucial parameter in balancing efficiency, safety, and ethical acceptability in ART practice. Large multicenter analyses, including that of Sunkara and colleagues (2011), demonstrated that the relationship between oocyte number and live birth rate is nonlinear: outcomes improve with increasing oocyte yield up to an optimal threshold, beyond which the benefit plateaus or even declines. Excessive stimulation may thus result in the generation of supernumerary embryos without additional clinical advantage, raising medical, logistical, and ethical concerns regarding embryo storage and disposal. Italy represents a distinctive case in the international landscape of reproductive medicine, where ART is tightly governed by bioethical and legal principles. The cornerstone of this regulatory framework is Law No. 40 of 2004, which established rigorous rules for medically assisted procreation, originally prohibiting heterologous gamete donation and limiting the number of oocytes that could be fertilized in each cycle to three. All resulting embryos were required to be transferred simultaneously, effectively preventing cryopreservation and the creation of surplus embryos. Although several constitutional court rulings have progressively modified this strict framework, the fundamental principle of embryo protection remains central to Italian reproductive policy. The Constitutional Court's landmark decision No. 162/2014 lifted the prohibition of heterologous donation [3], allowing the use of donor gametes while reaffirming the ethical obligation to avoid the excessive production of embryos. As a result, Italian ART practice continues to emphasize proportionality of treatment, quality over quantity, and respect for the embryo's moral and legal status.

Beyond its ethical foundations, the Italian system integrates an explicit economic governance dimension. The Ministerial Decree (D.M.) No. 272 of 25 November 2024 [4] introduced a standardized regional reimbursement of €2,400 per oocyte procurement cycle, thereby embedding economic accountability into ART practice. This policy reflects the broader European shift toward sustainable healthcare systems, but Italy stands out for aligning financial incentives with ethical imperatives. Under this framework, clinical strategies that minimize waste, prevent embryo surplus, and maintain high success rates are not only ethically justified but also economically rewarded. The Italian model thus provides a unique environment in which reproductive medicine operates under the combined lenses of bioethics, clinical efficiency, and public cost-effectiveness. The use of donor oocytes-permitted in Italy since 2014-adds another dimension to this discussion. Donor oocyte cycles offer a biologically stable setting for evaluating the true impact of oocyte number on reproductive outcomes. Because donors are typically young and healthy, the variability in oocyte quality is limited, and the influence of maternal age is minimized. Consequently, heterologous IVF cycles allow a clearer assessment of whether limiting fertilization to three oocytes compromises success rates. In addition, donor cycles often produce a higher number of mature oocytes per retrieval, increasing the risk of creating supernumerary embryos that are not used, stored for long periods, or eventually discarded-an ethically sensitive issue under the principles of Law 40/2004. Demonstrating that comparable outcomes can be achieved by fertilizing only three oocytes would therefore confirm that Italy's ethical restrictions do not hinder clinical efficacy but rather promote a responsible and sustainable approach to assisted reproduction.

In this context, the present study evaluates embryo development and pregnancy outcomes in donor- oocyte IVF cycles performed under the Italian regulatory framework [5], comparing cycles in which three or fewer oocytes were fertilized with those involving more than three. The goal is to determine whether limiting fertilization compromises reproductive success or whether, in the setting of standardized donor oocyte quality, comparable outcomes can be achieved within the ethical and economic boundaries established by national law. This work aims to provide empirical evidence supporting the principle that reproductive medicine can simultaneously achieve ethical integrity, economic sustainability, and clinical excellence, aligning medical innovation with societal and legislative responsibility.

Materials and Methods

Study Design and Population

A retrospective cohort analysis of 317 thawing cycles involving donor oocytes was conducted at the MAP Units, San Filippo Neri Hospital and Sant'Anna, Rome.

Cycles were stratified into:

a) **Group 1:** 3 fertilized oocytes (n=114)

b) **Group 2:** 6 fertilized oocytes (n=203)

All oocytes originated from screened, vitrified donor cohorts obtained via certified biobanks.

Outcomes

a) **Embryo Development Rate:** proportion of cycles yielding ≥1 viable embryo.

b) **Pregnancy Rate:** proportion of cycles achieving at least one clinical pregnancy.

Statistics

Group proportions were compared using z-tests for independent samples. Confidence intervals were calculated via the Wilson method (α=0.05).

Results

A total of 317 donor-oocyte thawing IVF cycles were analyzed. The fertilization rate, defined as the number of normally fertilized

oocytes (2PN) divided by the number of inseminated MII oocytes, was 91.2% in Group 1 and 97.0% in Group 2 (Table1) (Figure 1). No significant difference was observed between the two groups (p =0.07 Mann–Whitney U test).

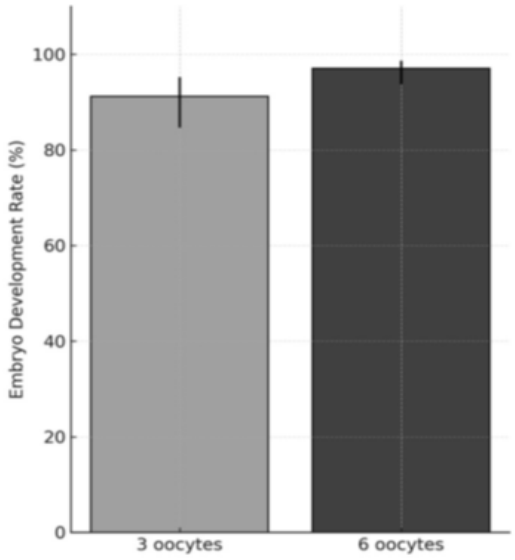

Figure 1: Embryo development rate % in 3 oocytes vs 6 oocytes.

Table 1: The fertilization rate in Group 1 (3 oocytes) and in Group 2(6 oocytes).

Group	N cycles	Successes (≥1embryo)	Probability of success	95% CI
1	114	104	0.912	[0.846–0.952]
2	203	197	0.97	[0.937–0.986]

Pregnancy, defined as a positive β-hCG with ultrasound confirmation, occurred in 25 of 114 cycles (21.9%) in Group 1 and 76 of 203 cycles (37.4%) in Group 2 (Table2) (Figure 2). The difference between the two groups was statistically significant (p=0.008). When the fertilization rate was normalized for the number of inseminated oocytes per cycle, the apparent difference between the two groups substantially decreased. The normalized fertilization rate was 0.070±0.012 for the 3-oocyte group and 0.065±0.010 for the 6-oocyte group (p=0.41, Mann– Whitney U test). This indicates that, when corrected for the initial number of oocytes, the efficiency of fertilization per available oocyte remained stable across groups. The slightly higher absolute fertilization rate in the 6- oocyte group (97.0% vs 91.2%) therefore reflects a quantitative effect due to greater oocyte availability rather than a qualitative improvement in fertilization capacity.

Table2: The pregnancy rate in Group 1 (3 oocytes) and in Group 2(6 oocytes).

Group	N cycles	Pregnancies	Pregnancy Rate
1	114	25	1. 219
2	203	76	0.374

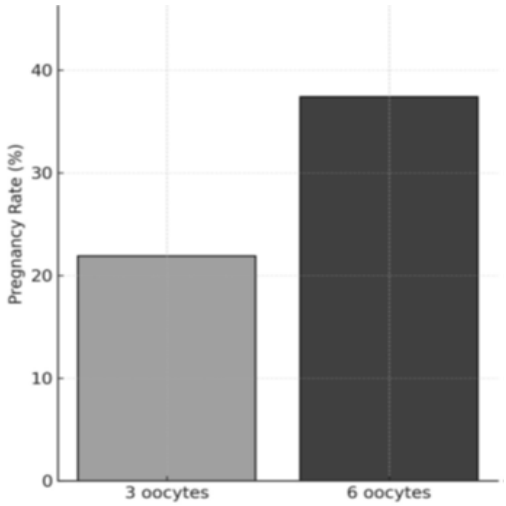

Figure 2: The pregnancy rate % in 3 oocytes vs 6 oocytes.

Discussion

This study demonstrates that fertilizing up to three donor oocytes per cycle yields high rates of embryo development and pregnancy, with outcomes that are comparable to those obtained when a higher number of oocytes are used. Both groups achieved embryo development rates above 90%, confirming that even a limited fertilization strategy ensures adequate embryological success in donor-oocyte IVF. These findings align with the study objective: to show that three oocytes are sufficient to achieve satisfactory results under the Italian ethical and economic framework. The absolute difference in embryo development rate between the two groups was 5.8%, which reached statistical significance but remains clinically modest. When both groups exceed the 90% efficiency threshold, such a difference does not imply a relevant impact on treatment success. The pregnancy rate was higher in the 6-oocyte group (37.4% vs 21.9%), but this increase mainly reflects the availability of more embryos for transfer rather than a true improvement in biological efficiency. Importantly, the competence of embryos-derived from donor oocytes of uniform quality-was maintained across groups.

When considering fertilization efficiency relative to the number of available oocytes, the apparent difference between the groups becomes negligible. The normalized fertilization rate (0.070 vs 0.065) indicates that each mature oocyte had a comparable likelihood of normal fertilization regardless of the total oocyte number per cycle. This normalization effect supports the concept that reproductive efficiency depends primarily on oocyte competence rather than oocyte quantity. Therefore, the modest difference in absolute fertilization rates reflects the greater pool size in the six-oocyte group rather than a superior biological performance. From a clinical standpoint, this observation confirms that limiting fertilization to three oocytes maintains a high per-oocyte efficiency, providing sufficient embryological yield without reducing success potential. From an ethical and policy perspective, this finding further validates the proportional-use principle underpinning Italian Law No. 40/2004, demonstrating that a conservative fertilization strategy can achieve optimal biological outcomes while preventing unnecessary embryo surplus.

Our results are consistent with previous reports indicating that beyond a certain threshold, increasing the number of fertilized oocytes does not proportionally improve outcomes. Large registry studies, such as those by Sunkara, et al. [6] and Polyzos, et al. [7], have shown that while live birth rates initially increase with oocyte yield, they plateau once enough embryos is achieved. Similar observations in donor-oocyte programs (Hipp, et al., 2025) [8] demonstrate that fertilizing large numbers of oocytes leads to embryo surplus without additional live birth benefit. From an ethical and policy perspective, these findings reinforce the rationale of Italian Law No. 40/2004, which emphasizes embryo protection and the proportional use of reproductive resources. Following the Constitutional Court ruling No. 162/2014, which legalized heterologous donation, this ethical balance has become even more relevant. Limiting fertilization to three oocytes avoids the creation of supernumerary embryos-whose storage and disposition remain ethically sensitive-while maintaining excellent reproductive outcomes.

Economically, the results support the adequacy of the €2,400 reimbursement established by Ministerial Decree (D.M.) 272/2024 for oocyte procurement cycles. If comparable pregnancy outcomes can be achieved with only three fertilized oocytes, then the current reimbursement framework is both clinically justified and cost-effective. This convergence between ethical restraint, biological

efficiency, and economic sustainability exemplifies the Italian ART model as a balanced and replicable approach to responsible reproductive medicine. Finally, our data align with the arguments advanced by Connolly, et al. [9], who framed ART as a long- term public investment rather than a short-term expenditure, and by Katz, et al. [10], who highlighted the importance of optimizing, rather than intensifying, reproductive interventions. Similarly, Myers, et al. [11] emphasized that efficient protocol adjustment can reduce resource use without compromising pregnancy rates. Collectively, these studies, together with the present findings, support a principle of clinical optimization over procedural escalation: achieving success with fewer biological inputs and within ethical and financial boundaries [12].

Conclusion

In donor-oocyte IVF cycles performed under the Italian regulatory framework, fertilizing three oocytes per cycle is sufficient to achieve embryo development and pregnancy rates comparable to those obtained with larger oocyte numbers. The minor statistical difference observed in embryo development does not translate into a clinically relevant disparity. These findings validate the ethical and economic coherence of the Italian ART model, confirming that high reproductive efficiency can coexist with limitations designed to prevent embryo surplus and ensure responsible healthcare spending. Within the context of Law No. 40/2004 and Ministerial Decree No. 272/2024, this study supports the principle that ethical proportionality and cost sustainability are compatible with excellent clinical outcomes in assisted reproduction. Italy's approach may serve as a model for other healthcare systems seeking to balance bioethics, medical efficacy, and economic responsibility in ART policy.

Acknowledgements

None.

Conflicts of Interest

None.

References

1. Italian Parliament. Law No. 40/2004: Regulations on medically assisted procreation. Official Gazette.
2. Esteves SC, Roque M, Sunkara SK (2019) Individualized controlled ovarian stimulation in expected poor responders: rationale, protocol design, and clinical outcome. Reprod Biol Endocrinol 17(1): 75.
3. Constitutional Court of Italy. Judgment no. 162/2014: Removal of the ban on heterologous donation.
4. Italian Ministry of Health. Ministerial Decree (D.M.) No. 272, 25 November 2024-National LEA.
5. De Rycke M, et al. (2022) Ethical and regulatory frameworks for embryo handling in Europe. Hum Reprod Update 28(4): 643-655.
6. Sesh Kamal Sunkara, Vivian Rittenberg, Nick Raine Fenning, Siladitya Bhattacharya, Javier Zamora, et al. (2011) Association between the number of eggs and live birth in IVF treatment: analysis of 400,135 cycles. Hum Reprod 26(12): 293-302.
7. Nikolaos P Polyzos, Panagiotis Drakopoulos, Jose Parra, Antonio Pellicer, Samuel Santos Ribeiro, et al. (2018) Cumulative live birth rates according to the number of oocytes retrieved after the first ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including~15,000 women. Fertil Steril. 110(6): 1109-1117.
8. Hipp HS, et al. (2023) Oocyte number and live birth rate in donor egg IVF cycles. Fertil Steril 120(4): 765-773.
9. Connolly MP, William Ledger, Maarten J Postma (2010) Economics of assisted reproduction: access to fertility treatments. Hum Fertil 13(4): 259-270.
10. Patricia Katz, Jonathan Showstack, James F Smith, Robert D Nachtigall, Susan G Millstein, et al. (2011) Costs of infertility treatment: results from an 18-month prospective cohort study. Fertil Steril 95(3): 879-885.
11. Myers ER, et al. (2021) Cost-effectiveness of mild ovarian stimulation protocols. Hum Reprod 36(7): 1921-1931.
12. Ferraretti AP, et al. (2014) Assisted reproduction in Italy: ten years of restrictive legislation and its impact. Reprod Biomed Online. 28(1):18-24.