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ASSISTED REPRODUCTION TECHNOLOGIES

Towards a more sustainable balance between optimal live birth rate and supernumerary embryos in ART treatments

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Abstract

Purpose To assess the relation between number of inseminated oocytes and cumulative live birth rate (CLBR) in order to provide guidance for limiting the number of surplus blastocysts.

Methods The study was a retrospective, single-center cohort analysis of 1223 ART complete cycles. Cycles were stratified according to female age (≤ 34, 35–38, and 39–42 years) and number of inseminated oocytes (1–5, 6–10, and > 10). Inclusion criteria were indication for IVF/ICSI with own spermatozoa and blastocyst culture up to day 6 of all embryos.

Results In patients younger than 35 years, insemination of more than ten oocytes produced an increase in overall blastocyst number, CLBR (40.3%, 54.3%, and 75.8%, respectively, for each oocyte group) and surplus embryo rate (12.9%, 27.8%, and 49.7% of cases for each group). Instead, in the middle age group, the use of more than ten oocytes was solely associated with an increase in the rate of surplus embryos (1.25%, 21.33%, and 28.68% of cases after stratification for oocyte number). In older patients, neither CLBR (9.1%, 23.9%, and 24.7%, respectively) nor rate of surplus embryos (2.0%, 7.1%, and 13.4% of cases for each group) were higher in cycles with more than ten inseminated oocytes.

Conclusion In women up to 38 years, sustainable CLBR are achieved while limiting the number of inseminated oocytes and the resulting blastocysts remaining unused. Based on this notion, novel treatment strategies could pursue high outcome rates, while alleviating the problems derived from surplus stored embryos.

Keywords Supernumerary embryos · Live birth · IVF · Fertilization · Cryopreservation · Maternal age

Introduction

For several decades, the question of how to express the clinical performance of assisted reproduction technology (ART) treatments has stirred an intense debate [1, 2]. Although different opinions persist, innovation in cryopreservation technology—namely, outstanding improvement in vitrification protocols—has marked a paradigm shift. Nowadays, embryos can be cultured to the blastocyst stage. Blastocysts can be then cryopreserved by vitrification, virtually without loss of material and developmental competence [3, 4]. This has led to the adoption of the currently widespread practice of sequential transfer of single fresh and vitrified blastocysts. Thereby, as a result of increased availability of stored embryos generated in a single stimulation cycle, across different countries the cumulative rate of live births per stimulation cycle has steadily increased. In parallel, the incidence of multiple births has been largely mitigated. The cumulative live birth rate (CLBR) per cycle has therefore become the most widely accepted criterion to assess clinical success in ART [5, 6].

While these developments mark an indisputable progress, a major problem remains unsolved. To achieve sustainable CLBR, multiple—often numerous—blastocysts are routinely produced in each cycle. As a result, over the decades, the number of surplus cryopreserved embryos has incessantly increased [7, 8]. In fact, especially when a live birth is achieved early in the course of a treatment, the remaining cryopreserved embryos are left unused. This is generating mounting concerns associated with the logistics, economics, and ethics of cryopreserved embryos. The problem is

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particularly acute in European countries in which embryo disposal is rigorously restricted. In extreme cases, not even embryos recognized as non-viable by preimplantation genetic screening for aneuploidy (PGT-A) can be disposed of or donated for research [9]. Regardless, the question of unused surplus embryos has acquired a growing and global dimension that calls for a long-term solution.

Future treatment strategies should be informed by the urgent need to constrain the number of cryopreserved embryos, while safeguarding clinical success. We postulated that the analysis of diverse factors influencing the number of embryos produced in each stimulation cycles could assist the development of novel practices consistent with this purpose. Here, we report on a study assessing the impact of the number inseminated oocytes in women of different maternal ages on CLBR and the number of transferred and surplus blastocysts.

Materials and methods

Study design, size, and duration

The study was a retrospective, single-center cohort analysis of 1223 ART complete cycles carried out between January 2010 and April 2022. All women were included only once in the study data set. As required by national law, the study (ID: 6306—Overall-outcomes-2023) was submitted for approval to the pertinent Ethical Committee (Comitato Etico Area Vasta Emilia Centro, CE-AVEC). Cycles were stratified according to female age: ≤ 34 years (Group 1); 35–38 years (Group 2); and 39–42 years (Group 3) and number of inseminated oocytes: 1 to 5; 6 to 10; and more than 10 (Table 1).

Participants/materials, setting, and methods

Inclusion criteria were indication for IVF/ICSI with own spermatozoa and blastocyst culture up to day 5/6 of the entire cohort of embryos. Oocyte donation, polycystic ovary syndrome, and preimplantation genetic testing (PGT) cycles were excluded from this analysis.

To assess the cumulative clinical outcome (CLBR), we focused on cycles considered completed, i.e., those of patients who had all their blastocysts transferred and/or had achieved a live birth. This defined the denominator of CLBR, while the nominator was the number of patients achieving a live birth. In these cycles, number of clinically usable blastocysts, number of blastocysts cumulatively transferred, surplus blastocysts, and CLBR were assessed. Primary endpoint was the number of surplus blastocysts, and secondary outcome was cumulative live birth rate.

Clinical and laboratory protocols

Ovarian stimulation and trigger of ovulation strategies were chosen according to patients' characteristics and based on gynecologists' judgement. Multifollicular growth was achieved with administration of rFSH or HMG. Oocytes were retrieved trans-vaginally 36 h after triggering with either hCG or GnRH analogues [10].

Fertilization was achieved by either standard IVF or ICSI. In ICSI cycles, 3 h after oocyte retrieval, cumulus cells were removed and nuclear maturation stage was assessed [11]. Only MII oocytes were inseminated by ICSI.

In standard IVF cases, approximately 4 h after oocyte pick up, oocytes were placed in a dish exposed to a 100,000–200,000/mL suspension of motile sperm

At fertilization check (16–18 h after insemination), oocytes displaying two pronuclei and two polar bodies were considered normally fertilized and further cultured. Embryos were cultured for 5–6 days and, where appropriate, transferred and/or cryopreserved by vitrification at the blastocyst stage.

Blastocysts were evaluated according to the degree of expansion and quality of the inner cell mass and trophectoderm cell, as previously described [4]. During the study period, changes to protocols staff and general setups were marginal, as shown by stability over time of laboratory and clinical key performance indicators.

For fresh cycles, luteal support was initiated after oocyte retrieval.

Blastocyst were cryopreserved using a Kitazato protocol (BioPharma Co., Japan) with a closed system device (HSV straw, Cryo Bio System, France) as previously described [4].

In supplemented FET cycles, estrogen (Climara, Bayer) and vaginal progesterone (Crinone, Merck Serono) were administered in a sequential regimen aimed at mimicking the endocrine exposure of the endometrium in a normal ovarian cycle [12].

Live birth was defined as the birth of at least one newborn after 24 weeks' gestation that exhibited signs of viability.

Statistics

Quantitative variables were compared with Student's *t* test for independent samples; chi-square analysis was performed for the comparison of categorical data. Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$. Data analysis was performed using GraphPad Prism (Version 9.5.1).

Univariable and multivariable logistic regressions were performed to evaluate associations with surplus

Table 1 Patient and treatment of study population stratified by female age

	Group 1 ≤ 34 years	Group 2 35–38 years	Group 3 39–42 years	Total	%	*p* value
Cycles characteristics						
Number of cycles (%)	445 (36%)	427 (35%)	351 (29%)	1223		
Female age ($m \pm$ ds)	31.49 ± 2.4	36.59 ± 1.1	40.46 ± 1.1	35.85 ± 4.0		$p > 0.001$
Male age ($m \pm$ ds)	36.0 ± 5.2	39.38 ± 4.4	42.78 ± 5.2	39.12 ± 5.7		$p > 0.001$
BMI	21.75 ± 3.3	21.45 ± 3.2	21.6 ± 3.4	21.6 ± 3.3		NS
Number of prev. cycles						
1–5 oocyte retrieved ($m \pm$ ds)	0.7 ± 1.2	1.2 ± 1.6	1.4 ± 1.8			
6–10 oocyte retrieved ($m \pm$ ds)	0.6 ± 1.1	0.8 ±1.2	1.1 ± 1.6			
≥ 11 oocyte retrieved ($m \pm$ ds)	0.5 ± 0.9	0.9 ± 1.2	0.8 ± 1.0			
TOT (number of cycles)	0.6 ± 1.1	0.9± 1.3	1.1 ± 1.5	0.8 ± 1.3		$p > 0.001$
Cause of infertility						
Female factor	125	135	108	368	30.1%	
Male factor	172	119	79	370	30.3%	
Female + male factor	60	51	61	172	14.1%	
Unexplained	88	122	103	313	25.6%	
Insemination method						
Coventional IVF	176	190	166	532	43.5%	
ICSI	269	237	185	691	56.5%	
Sperm characteristics						
Volume (ml) ($m \pm$ ds)	2.63 ± 1.3	3.02 ± 6.1	2.47 ± 1.1	2.72 ± 3.7		NS
N/ml ($m \pm$ ds)	26.20 ± 32.8	30.62 ± 37.1	31.27 ± 32.6	29.24 ± 34.4		NS
Motility ($m \pm$ ds)	38.08 ± 15.0	39.50 ± 22.1	40.41 ± 12.9	39.26 ± 14.1		$p > 0.005$
Morphology (normal forms) ($m \pm$ ds)	15.73 ± 9.0	16.90 ± 9.0	17.28 ± 8.6	16.60 ± 8.9		NS
Blastocyst rate						
1–5 oocyte retrieved (%)	51.79%	39.26%	43.12%			
6–10 oocyte retrieved (%)	47.22%	46.76%	37.94%			
≥ 11 oocyte retrieved (%)	45.86%	40.21%	31.43%			
TOT (%)	46.87%	43.10%	35.78%	42.64%		
Total blastocyst						
1–5 oocyte retrieved ($m \pm$ ds)	1.6 ± 0.8	1.39 ± 0.6	1.2 ± 0.6			
6–10 oocyte retrieved ($m \pm$ ds)	2.0 ± 1.1	2.0 ± 1.0	1.7 ± 1.9			
≥ 11 oocyte retrieved ($m \pm$ ds)	2.1 ± 1.3	2.4 ± 1.5	1.9 ± 1.1			
TOT (number of cycles)	425	396	313	1134		$p > 0.001$
Pregnant with surplus cryopreserved blastocyst						
1–5 oocyte retrieved	8/62 (12.9%)	1/80 (1.3%)	2/99 (2.0%)			
6–10 oocyte retrieved	64/230 (27.8%)	45/211 (21.3%)	11/155 (7.1%)			
≥ 11 oocyte retrieved	76/153 (49.7%)	39/136 (28.7%)	13/97 (13.4%)			
TOT	148/445 (33.3%)	85/427 (19.9%)	26/351 (7.41%)	259/1223 (21.2%)		
Pregnant with NO surplus cryopreserved blastocyst						
1–5 oocyte retrieved	12/62 (19.4%)	11/80 (13.8%)	5/99 (5.1%)			
6–10 oocyte retrieved	48/230 (20.9%)	44/211 (20.9%)	22/155 (14.2%)			
≥ 11 oocyte retrieved	19/153 (12.4%)	21/136 (15.4%)	10/97 (10.3%)			
TOT	79/445 (17.8%)	76/427 (17.8%)	37/351 (10.5%)	192/1223 (15.7%)		

blastocysts. The model included patient and cycle characteristics. We tested all 2-way interactions between pairs of predictors included in our multivariable analyses and used a Bonferroni-correction (for multiple testing) *p* value threshold of 0.05 to define statistical evidence of an interaction. The predictive value of the resulting model was assessed by calculating the area under the curve of the receiver operator characteristics (AUROC). To evaluate

the level of agreement between the estimated and the observed probabilities (calibration), the Hosmer–Lemeshow test was used. Univariable and multivariable logistic regressions were performed using IBM SPSS Statistics 28.

Results

Patient characteristics are reported in Table 1. The three age groups are distinct subpopulation, and therefore, the associated outcomes were not mutually compared statistically. In the younger age group (≤ 34 years), the total number of blastocysts was positively associated with number of inseminated oocytes. In the same group, the number of blastocysts transferred in the 1–5 oocyte subpopulation was significantly lower compared with cycles in which 6–10 oocytes or ≥ 11 oocytes were used. However, the difference between 1–5 oocytes and 6–10 oocytes was not significant (Fig. 1a).

The number of surplus blastocysts also significantly increased with increasing number of inseminated oocytes, across the three age groups (Fig. 1g).

The same trends were observed in group 2 (35 to 38 years) across all the inseminated oocytes categories (Fig. 1b, h).

In older patients, the same trends were observed with regard to the total number of transferred blastocysts. However, surplus blastocyst number was significantly different between 1–5 oocytes and 6–10 oocytes ($p < 0.05$), but not between 6–10 and > 11 oocytes (Fig. 1i).

In younger patients, CLBR was positively associated with the number of inseminated oocytes (Fig. 1d). In the middle age group, CLBR was significantly different between 1–5 and 6–10 oocytes, but not between 6–10 and > 11 oocytes (Fig. 1e). The same CLBR trend for was observed in older patients (Fig. 1f).

Data were further analysed in the selected population of patients achieving a live birth. In the younger group, the use of more than ten oocytes was associated to a more than three-time increase in the average number of supernumerary embryos (Fig. 2a). Although less prominent, this trend was also observed in the middle-aged group

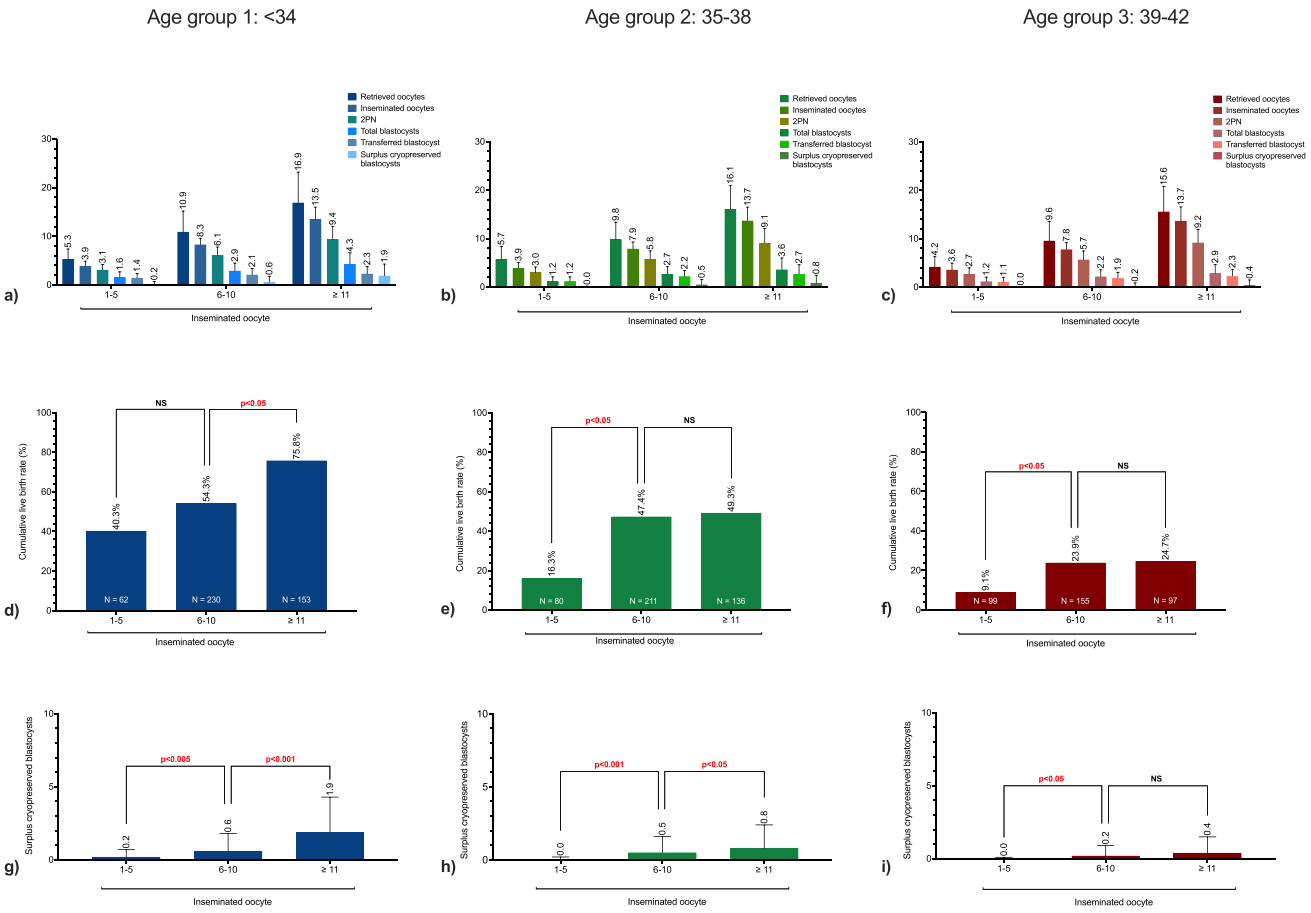

Fig. 1 Total blastocyst number (**a**–**c**), cumulative live birth rate (**d**–**f**), and cryopreserved blastocysts remaining unused (**g**–**i**) in ART cycles stratified by female age and number of inseminated oocytes

(Fig. 2b), while no significant differences were observed in the cycles of older age women (Fig. 2c).

We also assessed the percentage of patients with embryos still in storage. Fig. 3 shows that 49.7% of younger patients with more than 11 inseminated oocytes and achieving a live birth have surplus blastocysts. In the middle age group this rate was 28.7%. Figure 4 shows the distribution of number of cycles in relation to the number (1, 2, and ≥ 3) of blastocysts.

Finally, to further control for possible patient-specific confounding factors, maternal age, inseminated oocytes, and number of previous treatments were evaluated in a multivariate logistic regression analysis. From this assessment, all the variables emerged as a factor independently associated with the surplus blastocyst; in particular, an important association was found between the number of oocytes inseminated and surplus of blastocysts. The probability of surplus blastocysts was nine times greater when inseminating more than 10 oocytes, compared to less than 6 (Table 2).

Discussion

In this study, we retrospectively examined the association between number of inseminated oocytes, CLBR and surplus cryopreserved embryos. The aim was to define a suitable number of oocytes to use for treatment, in the attempt to

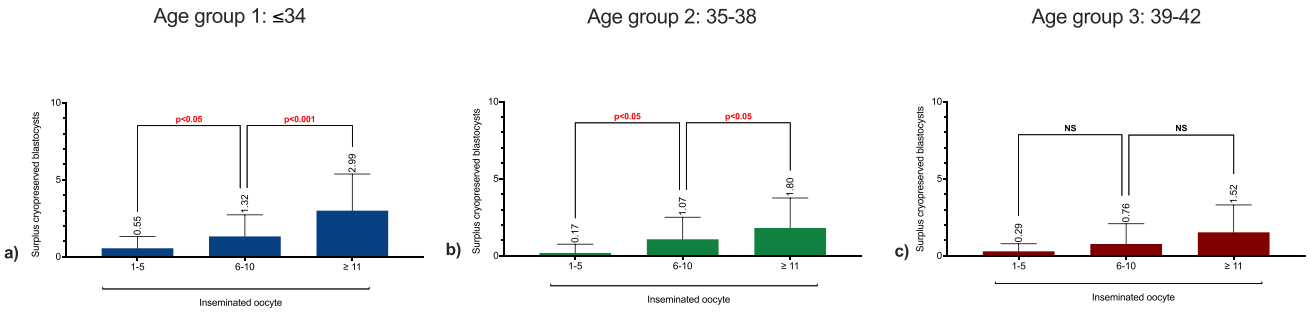

Fig. 2 Cryopreserved blastocysts remaining unused in ART cycles where a live birth was achieved, stratified by female age (**a**–**c**) and number of inseminated oocytes

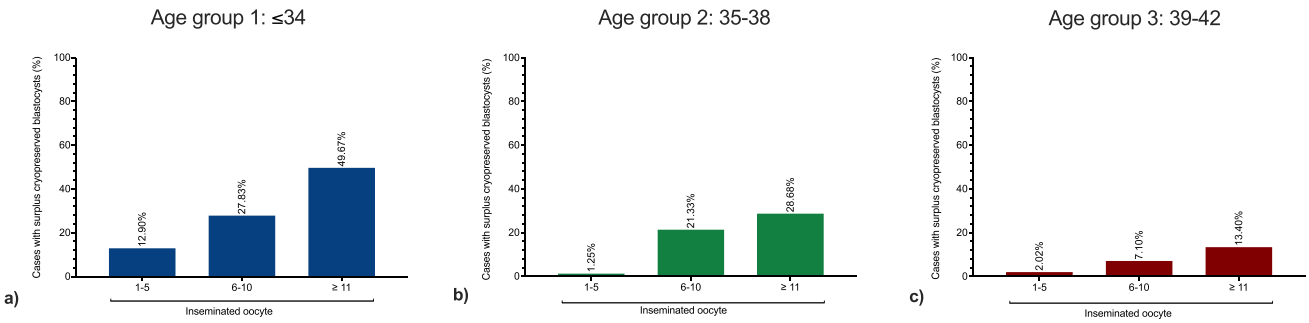

Fig. 3 Percentage of patients with embryos still in storage

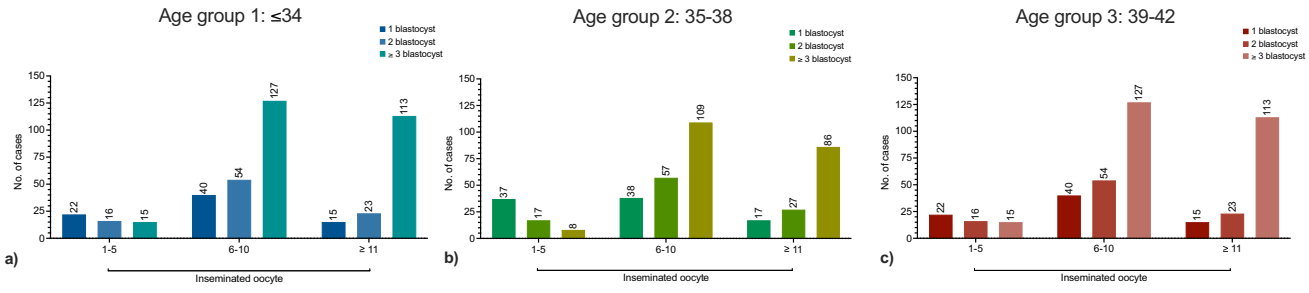

Fig. 4 Number of cycles in the three age groups with embryos still in storage reported according to the number of supernumerary blastocysts (1, 2, ≥3).

Table 2 Multivariate logistic regression analysis. Associations of potential predictors for surplus blastocyst

Characteristic	Categories	Univariable odds ratio of live birth (95% CI)	Multivariable[a] odds ratio of live birth (95% CI)	*p* value[b]
Maternal age (years)	≤ 34	1	1	< 0.001
	35–38	0.499 (0.366–0.679)	0.537 (0.389–0.741)	
	39–42	0.161 (0.103–0.251)	0.19 (0.12–0.3)	
Inseminated oocytes	1–5	1	1	< 0.001
	6–10	5.271 (2.787–9.969)	4.419 (2.316–8.432)	
	≥ 11	10.374 (5.465–19.692)	9.138 (4.766–17.52)	
Previous treatment	Yes	1	1	< 0.001
	No	0.536 (0.401–0.717)	0.643 (0.472–0.876)	

define a better balance between clinical outcome and production of embryos destined to remain unused. Cycles were stratified according to the number of inseminated oocytes and maternal age.

The study data show that, in patients younger than 35, inseminating more than ten oocytes produces an increase in both CLBR and surplus embryo rate. Instead, in the middle age group, the use of more than ten oocytes is solely associated with an increase in the rate of surplus embryos. In older patients, neither CLBR nor rate of surplus embryos are higher in cycles with more than ten inseminated oocytes. We therefore conclude that in younger and middle-aged patients inseminating up to ten fresh oocytes may be a possible treatment option, associated with the cryopreservation of surplus oocytes, which would then contribute to the cumulative outcome Nevertheless, we recognize that patient-specific characteristics may suggest the use of a higher number of fresh oocytes. For example, young and middle-aged couples at their first ART treatment and presenting with a relatively mild male factor might opt for having a maximum of ten oocyte inseminated, while surplus oocytes might be vitrified. In contrast, unfavourable clinical history or severe male factor would suggest the use of a larger number or the entire cohort of collected oocytes. Specifically, couples with a previous record of failed attempts, low fertilization rate, poor embryo quality would need the insemination or more than the eggs or even the entire cohort of collected material. Indeed, low fertilization rate is per se prognostic of lower CLBR [13], while poor embryo quality reduces the overall amount of embryo suitable for transfer with obvious implications for cumulative success. Poor oocyte morphological quality would also suggest the use of more than ten oocytes, irrespective of maternal age. Evidence on morphologically abnormal oocyte is controversial. However, most studies converge on the notion that specific oocyte abnormalities affect quality and quality of blastocyst yield, thereby affecting CLBR [14]. Better identification of such different cases could be achieved by future, larger and more in-depth analyses of patient characteristics. Further benefit might derive from the investigation of oocyte and sperm factors influencing fertilization rate and, in final analysis, yield of clinically usable blastocysts. Whatever the choice, patients would require exhaustive counselling in order to take the most appropriate decision fitting their specific needs. The relative efficiency of oocyte and embryo cryopreservation should be also considered. Both these modalities of preservation have crucially benefited from the higher performance and consistency of vitrification protocols. Nevertheless, vitrified oocytes do not survive warming with a 100% recovery rate. They also display a mild decrease in blastocyst development in comparison with fresh controls. These factors should be discussed with patients when considering the option to cryopreserve surplus oocytes: in fact, such a choice would imply a small, but measurable, reduction in CLBR. Furthermore, in light of these limitations, oocyte cryopreservation seems to be a realistic option only in cases where at least 5–6 surplus oocytes are available, in addition to those used fresh. Financial aspects should also be addressed. Of course, similarly to embryo cryopreservation, oocyte vitrification involves significant costs for patients. However, this drawback would be counterbalanced by the advantage of not facing ethical and possible legal dilemmas associated with protracted, and sometimes indefinite, embryo storage.

This analysis has the merit of assessing a poorly explored, but very important, aspect of ART treatment. However, we recognized that some factors, for example the study retrospective design and lack of external validation, limit the strength of our findings. Among such factors, absence of data on anti-Mullerian hormone (AMH) values of treated patients is a significant study limitation, In addition, not all couples were enrolled at their first treatment cycle and previous outcomes were not reported.

The rate of surplus embryos is a significant treatment outcome. It globally impacts on a number of economical, logistic, and ethical issues relevant to embryo cryopreservation, especially in countries where the law imposes the storage of such material for an unlimited period, even after definitive termination of a treatment. In Europe, no broad consensus exists on the question of supernumerary embryos remaining unused. For this reason, the European Court of Human Rights has granted a wide margin of decision to Member States [15,

16] on how to balance the interests of all relevant stakeholders. In Italy, ART is regulated by law no. 40/2004. This law is inspired by the concept of embryo protection, which results in unlimited storage of embryos remaining unused after the completion of a treatment. Due to overall increase in ART efficiency, especially derived from crucial progress in embryo cryopreservation [17], the number of surplus cryopreserved embryos has incessantly increased. In some countries, surplus material may also derive from embryos identified as aneuploid in PGT-A cycles, which likewise cannot be discarded by law. The problem is therefore destined to escalate over time. Previous studies investigated the association between the number of eggs used for treatment and live birth [18–20]; they indicated a non-linear relationship between the two variables and suggested the use of definite numbers of oocytes to the aim of maximizing the live birth rate. In particular, a recent study [20] aimed at establishing how many oocytes or embryos are needed to optimize the live birth rate and CLBR, in cycles of women with normal ovarian reserve. The results suggest that the CLBR is optimized with the use of twelve oocytes or the production of nine embryos. Notably, this conclusion is consistent with our findings, at least for young and middle-aged women. A recent SART retrospective study [21] including 402,411 cycles found that a higher number of collected oocytes increases the CLBR; this occurs without substantially impacting the live birth rate associated with the first embryo transfer or impairing the mean conversion rates of oocytes/2PNs and oocytes/blastocysts. Also, another study [22] tested and confirmed the hypothesis that the number of supernumerary blastocysts available for cryopreservation is a positive marker for implantation and live birth. Numerous other studies focused on ovarian stimulation and the optimal number of retrieved oocytes; however, none of them assessed the number of surplus embryos once a live birth is obtained.

Novel treatment strategies should aim at achieving high outcome rates, while alleviating the problems derived from unused stored embryos. In this perspective, the insemination of a limited, but appropriate, number of oocytes may be an option in good prognosis patients. In addition, non-inseminated oocytes could be cryopreserved and further contribute to increase the overall number of births.

Conclusions

We observed that in young and middle aged women, sustainable CLBR are achieved while limiting the number of inseminated oocytes. Based on this notion, novel treatment strategies could pursue high outcome rates, alleviating the problems derived from non-used stored embryos and relaunching cryopreservation as option for harnessing the potential of surplus oocytes. In final analysis, we propose that the option of limiting to ten the number of oocytes used fresh could be adopted for couples involving young and middle-aged women, at their first ART treatment, presenting with a relatively mild male factor and having at least 5–6 oocytes suitable for cryopreservation.

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Declarations

Conflict of interest The authors declare no competing interests

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