

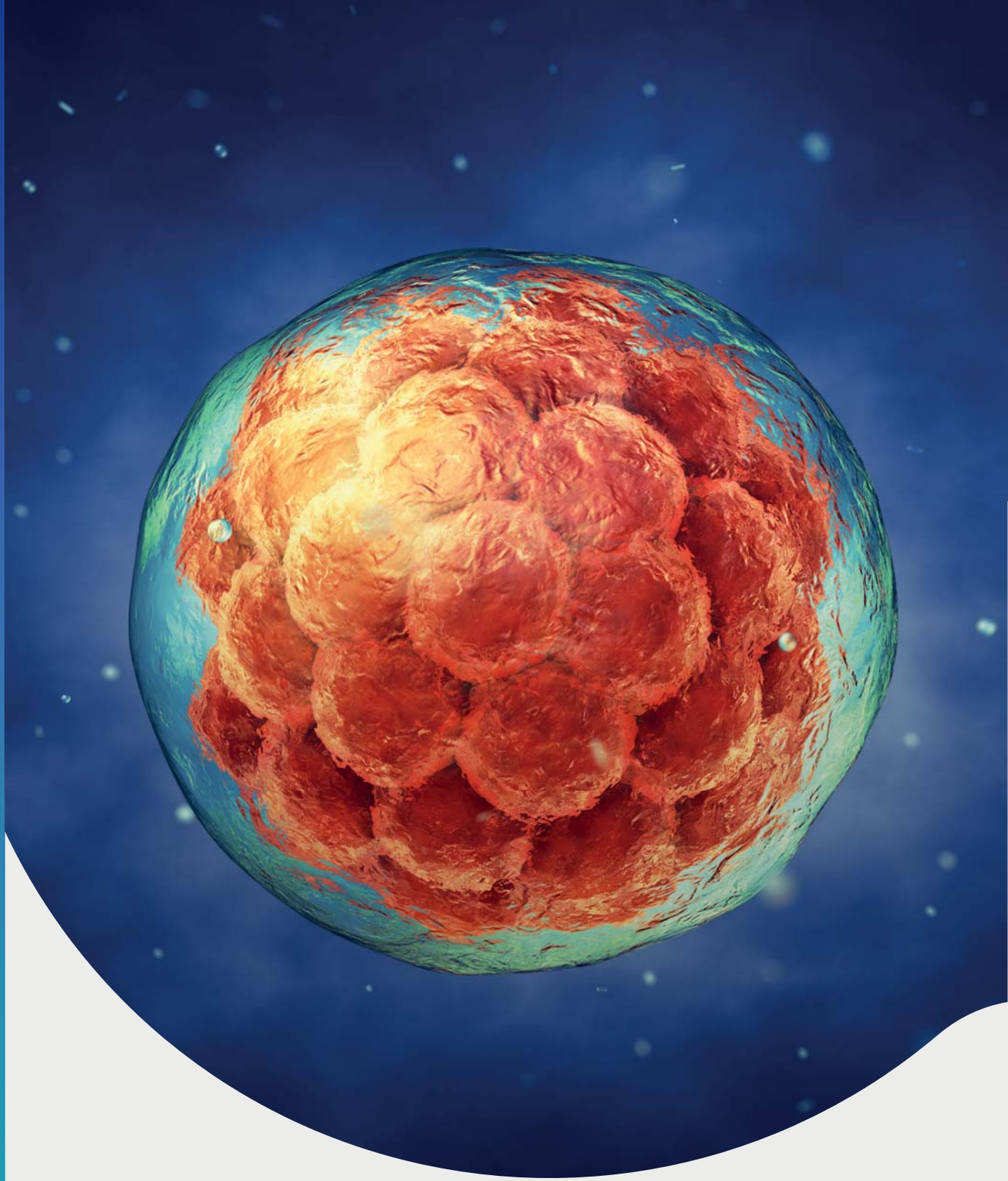


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# Number of embryos to transfer during IVF/ICSI

Guideline of European Society of Human Reproduction and Embryology

2023

The ESHRE guideline development group on the number of embryos to transfer during IVF/ICSI

## How to cite this guideline

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# Introduction

## Clinical need

More than four decades have passed since the birth of the first infant conceived through the use of assisted reproductive technology (ART). During these past four decades, more than ten million children have been born worldwide because of assisted conception. ART has increased the chance for people who have trouble conceiving to achieve a healthy pregnancy and birth. In the early years of *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI), clinicians may have transferred all available embryos into the uterus because of low implantation rates (IR) and suboptimal embryo culture and cryopreservation procedures. However, with medical and technical improvements in ART, the transfer of multiple embryos led to a greater number of high-order multiple pregnancies (HOM) compared to spontaneous pregnancies. Multiple pregnancies are associated with a wide range of maternal and neonatal complications ([Santana et al., 2018](#)).

Therefore, elective single embryo transfer (eSET) is considered the preferable route towards the key objective of ART and at present this is recommended by several international and national professional organisations ([ASRM, 2021](#), [De los Santos et al., 2016](#)). However, these recommendations are not followed in many countries, as can be observed in annual reviews ([Sunderam et al., 2022](#), [Wyns et al., 2020](#)).

Overall, there is a clear trend towards the transfer of fewer embryos in Europe, the USA, and Australia and New Zealand ([De Geyter et al., 2020](#)). In Europe, the proportion of transfers of two embryos is decreasing (from 56.7% in 2010 to 45.1% in 2018), and the transfer of three or more embryos is also decreasing (from 17.6% in 2010 to 4.2% in 2018), while the proportion of transfers of only one embryo at a time is on the rise (from 25.7% in 2010 to 50.7% in 2018) ([Kupka et al., 2014](#), [Wyns et al., 2020](#), [Wyns et al., 2021](#), [Wyns et al., 2022](#)). The transfer of fewer embryos at a time has led to a decrease in the twin pregnancy rate in Europe (from 25.7% in 2010 to 12.4% in 2018), but this rate is still higher than the 10% target rate recommended by ESHRE in 2000 ([The ESHRE Capri Workshop Group, 2000](#), [Kupka et al., 2014](#), [Wyns et al., 2020](#), [Wyns et al., 2021](#), [Wyns et al., 2022](#)).

Although the promotion of (e)SET by professional national and international societies (ESHRE, American Society for Reproductive Medicine (ASRM)) and scientific communities has had its effect on multiple pregnancy rates (MPRs) in certain countries, data show that there is still a considerable difference in the practice of (e)SET in Europe. This results in twin pregnancy rates varying from >25% in Serbia and Romania to 4% in Sweden and 5% in Iceland, where eSET is adopted in more than 80% of all cases (Calhaz-Jorge et al., 2020). Similarly, a questionnaire distributed by the guideline development group (GDG) before initiating the writing process of this guideline was answered by practitioners from 37 European countries and revealed significant differences in embryo transfer practices (unpublished data). The development of an evidence-based guideline supporting SET or double embryo transfer (DET) is therefore needed to harmonise the transfer policies and to promote (e)SET worldwide.

### Guideline development

This guideline was developed according to a well-documented methodology, universal to ESHRE guidelines and described in the manual for ESHRE guideline development (<https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Guideline-development-process>).

Details on the methodology of the current guideline are outlined in **annex 5**.

The GDG was composed of representatives of the Special Interest groups (SIG) of Safety and Quality in ART (SQART), Embryology, Implantation and Early Pregnancy, Psychology and counselling, Ethics and law, and Reproductive endocrinology, representatives of the European IVF Monitoring (EIM) Consortium and two patient representatives. The members of the GDG are listed in **annex 1**.

### Guideline scope

The purpose of this guideline is to provide guidance on embryo transfer policy. It entails a strategy for evaluating the number of embryos to transfer in groups of patients focusing on the cases where it is relevant to transfer one embryo and cases where it is relevant to transfer two embryos. This guideline is not intended for patients for whom multiple pregnancy is to be avoided at all cost due to underlying conditions (Vliska et al., 1999, Gerris, 2005). This guideline should not be used to justify DET in those patients.



## Target users of the guideline

The guideline is directed towards healthcare professionals who are involved in information provision and decision-making regarding who need embryo transfer. This includes, but is not limited to, reproductive medicine specialists, gynaecologists, obstetricians, neonatologists, and embryologists.

The document can also be used by policy makers and regulators as a source of independent information on the relevance of single and multiple embryo transfer which can be used to guide national recommendations and policies for reimbursement.

For the benefit of patient education and shared decision-making, a patient leaflet based on this guideline has been developed (see <https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Embryo-transfer>).

## Patient population

The current guideline focusses on patients undergoing embryo transfer in the context of a fertility treatment.

This guideline, in line with the research, terminology, and discussion in ART is focused on couples, women, and men. The guideline group recognises that there are single women, same sex couples or individuals who are transgender, who do not menstruate, who do not have a uterus or who do not identify with the terms used in the literature. For the purposes of this guideline, we have attempted to use the neutral terms “couples/individuals undergoing ART” or “patients” whenever possible. Use of any other terms is not intended to isolate, exclude, or diminish any individual’s experience nor to discriminate against any group.

## Terminology

This guideline uses terms and definitions as described in the international glossary on infertility and fertility care ([Zegers-Hochschild et al., 2017](#)). SET refers to single embryo transfer and DET refers to double embryo transfer. If embryologists can choose from more than one embryo, the transfer is considered elective. Thus, eSET is the transfer of one embryo in cases where there are more than one to choose from. In some scientific articles, especially those from the early 2000s, data on SET cycles were analysed but the texts may not have indicated whether this was the only embryo available or not. For these articles, the abbreviation of (e)SET was



used. Other types of SET exist: compulsory SET (cSET) applies to cases when only one embryo is available for transfer; and medical SET, which is SET applied to women in whom a multiple pregnancy represents an *a priori* increased medical risk compared to the overall population (Vilksa et al., 1999, Gerris, 2005) (**Box 1**). Spontaneous pregnancies are defined as gestations that did not originate from medical treatment. An alternative term that can also be used in texts intended for patients is non-assisted pregnancies/gestations. These terms were agreed upon with the representative from Fertility Europe. A list of abbreviations used in this document is included in **annex 4**.

## Outcomes

This guideline summarises existing knowledge about SET and DET in terms of medical, financial, and psychosocial consequences and evaluates social, legislative, and economic factors. Clinical factors such as previous unsuccessful ART treatments, duration of infertility, previous pregnancy, female age, ovarian response, and endometrial characteristics are also considered. Embryologic factors evaluated in the guideline include evaluation of fresh and cryopreserved embryos, time-lapse (TL) morphokinetics, and preimplantation genetic testing. The guideline also summarises the consequences and risks of other ET strategies such as the transfer of more than two embryos with or without foetal reduction. Considerations on treatments with donated oocytes/embryos and on gestational carriers are presented in separate chapters.

Main outcome measures extracted from these studies included live birth rates, per cycle and/or cumulative (LBR and/or cLBR), multiple pregnancy rate (MPR), prematurity or preterm birth rate (PBR), and maternal and child morbidity.

### *Box 1: Medical single embryo transfer.*

Medical SET is applied to women in whom a multiple pregnancy represents an *a priori* increased medical risk compared to the overall population (Vilksa et al., 1999, Gerris, 2005). This includes women with:

- Severe systemic disease (e.g. insulin-dependent diabetes).
- Congenital uterine anomalies (e.g. septate uterus).
- Bad obstetric history: e.g. previous premature birth < 32 gestational weeks.

## Patient perspectives, experiences, and preferences

A Europe-wide online patient survey on patient experiences and preferences regarding the number of embryos to transfer was developed by the GDG in collaboration with Fertility Europe. The aim was to investigate patient knowledge, preferences, and experiences related to the number of embryos to transfer during ART treatment (**annex 7**). The results of this survey were used to better define the patients' preferences and perspectives in terms of counselling.

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# List of all recommendations

Chapter	Recommendation	Strength of evidence*	Quality of evidence**	Justification
<b>Part A – Consequences and risks of multiple pregnancy</b>				
<b>Medical risks related to multiple pregnancy/birth</b>				
	Medical risks that should be considered before the transfer of more than one embryo are the higher rates of maternal, foetal, and neonatal complications.	Strong	⊕⊕⊕○	There is consistent evidence showing that multiple pregnancies are associated with higher maternal and neonatal risks compared to singleton pregnancies, irrespective of the mode of conception.
I	The GDG recommends that whenever the transfer of >1 embryo is considered, the patient should be provided with clear information about the higher risk of pregnancy loss, ectopic pregnancy, pre-eclampsia, gestational diabetes, antepartum and postpartum haemorrhage, caesarean section, stillbirth, preterm birth, low birth weight, neonatal intensive care admission and neonatal death associated with multiple pregnancies. The GDG also recommends that the patients sign an additional consent form if >1 embryo is transferred.	GPP		Informed consent prior to accepting the transfer of >1 embryo implies that adequate counselling has been provided regarding the risks associated with pregnancy complications.
<b>Financial issues of multiple pregnancy/birth</b>				
	It is recommended to consider the increased direct costs related to obstetric care of multiple pregnancies and paediatric care of twins and triplets.	Strong	⊕⊕⊕○	Moderate-quality evidence demonstrates higher overall direct costs in multiple pregnancies. There is low-quality evidence that overall indirect costs are higher in multiple pregnancies, regardless of the point of comparison (mother- vs. child-based comparison).
II	It is recommended to consider increased indirect costs with multiple pregnancies due to sick leave days, over-the-counter medication, loss of productivity because of an ill child.	Strong	⊕⊕○○	

	The GDG recommends that cost-related information should be provided and discussed with the patient(s) at the treatment planning stage.	GPP		The GDG recommends that the impact of costs with singleton and twin pregnancy is discussed with the patients prior to the decision on the number of embryos to transfer, at the time of planning the treatment.
<b>Psychosocial issues of multiple pregnancy/birth</b>				
III	Clinicians should consider the possible complications of multiple pregnancies with regards to mental health postpartum, emotional distress and possible marital problems, as well as the influence of personality characteristics, sociodemographic factors, and family functioning, on the mental health of parents and offspring regardless of the number of children born.	Strong	⊕⊕○○	Moderate-quality evidence shows higher likelihood of stress and depression in mothers of ART children and especially in mothers of ART multiples, compared to mothers of children from spontaneous pregnancies. Clinicians should be aware of these risks in order to help patients through a successful decision-making process. Psychosocial dimensions that should be considered for patients before taking the decision of the transfer of one or more embryos are sociodemographic factors, family functioning, marital relationship, and education.
	The GDG recommends that information on possible psychosocial complications should be provided to patients at the treatment planning stage.	GPP		
<b>Part B – Social, legislative, and economic factors</b>				
I	The GDG encourages legislative and health insurance policies that promote the practice of eSET.	GPP		
<b>Part C – Clinical criteria</b>				
<b>Previous unsuccessful ART treatments</b>				
I	The decision to perform DET instead of eSET should not be based on the number of previous unsuccessful ART treatments.	Strong	⊕○○○	Very low-quality evidence from a single retrospective study indicates similar clinical pregnancy rates but lower MPR if one embryo is transferred compared to two embryos. Furthermore, there is no scientific evidence indicating that repeated failed cycles can be compensated by increasing the number of embryos per transfer.
<b>Duration of infertility</b>				
II	The decision to perform DET instead of eSET should not be based on the duration of infertility.	Strong	⊕○○○	Low-quality evidence shows that this factor has no effect on LBR when analysing the results with respect to the number of embryos transferred in eSET and DET treatment cycles.

Previous pregnancy/live birth				
III	The decision to perform DET instead of eSET should not be based on previous pregnancies or live births from ART.	Strong	⊕○○○	Low-quality evidence demonstrates that this factor is not a significant variable for predicting LBR and MPR in SET and DET cycles.
Female age				
	The decision to perform DET instead of eSET should not be based on female age.	Strong	⊕⊕○○	High-quality evidence is more abundant for women aged <38 years and of lower quality for those aged 38 years or more due to fewer studies. Regardless of female age, cLBR is higher after eSET but rates of multiple pregnancy are higher after DET. Consequently, obstetric complications such as preterm birth and perinatal mortality are observed more often after DET. In some studies of women ≥38 years, cLBR and MPR can be similar to the ones after DET. Advanced maternal age is in itself associated with increased obstetric risks, which should be taken into account at the time of planning of ART treatment.
IV	Women aged less than 38 years should receive eSET.	Strong	⊕⊕⊕○	
	Women aged 38 years or more should receive eSET.	Strong	⊕○○○	
Ovarian response				
	For normal responders, eSET is recommended.	Strong	⊕○○○	Very low-quality evidence shows similar cLBR in normal responders with DET and eSET.
V	The GDG recommends eSET in patients with low or high ovarian response.	GPP		No evidence for high and low responders with regards to eSET vs. DET was identified. However, high responders are at high risk of OHSS and late onset OHSS is more frequent in multiple pregnancies. And low responders have only a few embryos, many of which cannot be classified as good-quality ones. Therefore, the GDG recommends that no more than one embryo be transferred in all high and low responders having a fresh ET.
Criteria related to the endometrium				
VI	The decision to perform DET instead of eSET in fresh embryo transfer cycles should not be based on endometrial characteristics.	Strong	⊕○○○	Promoting DET in the presence of abnormal endometrial characteristics, especially if potentially reversible, reduces the chance of at least one of the embryos available ultimately

	The decision to perform DET instead of eSET in a frozen embryo transfer cycles should not be based on endometrial characteristics.	Strong	⊕○○○	interacting with an endometrium which may be more favourable for conception.
<b>Treatments with donor oocytes and donated embryos</b>				
VII	Only eSET should be practiced for patients undergoing ART with donor oocytes.	Strong	⊕⊕⊕○	Multiple pregnancies may increase the already high pregnancy risks and complications in pregnancies achieved through donor oocytes/embryos, compared to pregnancies using autologous oocytes.
	Only eSET should be practiced for patients undergoing ART with donated embryos.	Strong	⊕○○○	
<b>Gestational carriers</b>				
VIII	Only eSET should be practised for gestational carriers.	Strong	⊕○○○	Increased MPR and PBR were observed in the group receiving DET. The data are comparable to high risks observed using donor oocytes. Transferring one embryo minimises those risks and should therefore be strongly recommended.
	The GDG recommends that both gestational carriers and intended parents be counselled that DET is associated with greater risk of pregnancy and perinatal complications in surrogate pregnancies.	GPP		
<b>Part D – Criteria related to the embryo</b>				
<b>Fresh embryo transfer</b>				
<b>Cleavage stage</b>				
I.1	In fresh cleavage-stage embryo transfer, the decision to perform DET instead of eSET should not be based on embryo criteria.	Strong	⊕⊕⊕○	The evidence assessed failed to show an increase of LBR following DET as compared to eSET when embryos with similar quality are transferred in a fresh cycle. Moreover, if embryo quality is not taken into account, transferring two cleavage-stage embryos in fresh cycles led to a higher LBR at the cost of a substantial increase in the risk of MPR.
<b>Blastocyst stage</b>				
I.2	In fresh blastocyst transfer cycles, the decision to perform DET instead of eSET should not be based on blastocyst morphology/quality.	Strong	⊕⊕⊕○	When balancing the benefit of higher LBR against the risks related to higher MPR and considering the higher risk of monozygotic twinning with blastocyst transfer, eSET is associated with higher benefit/risk ratio.

Frozen embryo transfer				
II	When reporting research on vitrified-warmed treatments, the GDG recommends including details on the minimal embryo criteria for vitrification and/or transfer as well as on the selection of devices or embryos for thawing and warming e.g., randomly picked or according to quality criteria (e.g., first embryos with the best quality were selected).	GPP		
	The GDG recommends cryopreserving one embryo per device in order to facilitate the practice of SET and for traceability purposes.	GPP		
Cryopreserved-warmed cleavage-stage				
II.1	In cryopreserved-warmed cleavage-stage embryo transfer cycles, the decision to perform DET instead of SET should not be based on embryo criteria.	Strong	⊕⊕⊕○	There is no reason related to embryo quality to perform DET instead of eSET when cryopreserved-warmed cleavage-stage embryos are transferred since the increased LBR with DET is associated with a substantial increase in MPR.
Vitrified-warmed blastocyst stage				
II.2	In vitrified-warmed blastocyst transfer cycles, SET should be applied regardless of the quality of the vitrified blastocyst.	Strong	⊕○○○	There seems to be no reason related to embryo morphology to perform DET instead of SET when vitrified-warmed blastocysts are transferred since the increased LBR is associated with a substantial increase in MPR.
Time-lapse morphokinetics				
III	TL imaging-derived parameters for embryo selection should not be considered a factor to perform DET instead of eSET.	Strong	⊕○○○	There is currently no evidence that supports DET instead of SET based on this parameter.
Preimplantation genetic testing				
IV	PGT-A outcomes should not be considered when deciding to perform DET instead of eSET.	Strong	⊕○○○	PGT is seen as an eSET strategy and low to moderate evidence was found showing that the use of eSET following PGT-A minimises the risk of multiple pregnancies without affecting LBR.



## Part E – Other strategies for embryo transfer

### Transfer of more than two embryos

I	Transfer of more than two embryos is not recommended.	Strong	⊕○○○	The transfer of more than two embryos carries an unacceptable increase in the risk of HOM and ectopic pregnancies.
<b>Foetal reduction</b>				
	In patients who conceived HOM following multiple embryo transfer, foetal reduction can be considered to reduce the risk of maternal complications.	Conditional	⊕○○○	The evidence for this recommendation to rely on embryo reduction in case of HOM pregnancy is very weak.
II	The transfer of two or more embryos with the intention of performing foetal reduction in case of multiple embryo implantation instead of (e)SET is not recommended.	Strong	⊕○○○	Low-quality evidence from one retrospective study showed that selective foetal reduction following DET increased maternal and neonatal complications compared to SET.
	The GDG recommends against the transfer of more than two embryos with foetal reduction after multiple embryo implantation considering the high risks of the procedure.	GPP		Foetal reduction is associated with ethical issues that can be avoided by transferring only one embryo.

## Part F – Patient Counselling on embryo transfer

### Patient counselling

I	<p>The GDG strongly recommends that healthcare professionals discuss with the patient a number of issues related to the number of embryos to transfer. Main topics include:</p> <ul style="list-style-type: none"> <li>- Medical, economic, social and psychological consequences of transferring &gt;1 embryo.</li> <li>- Patient wishes regarding family building.</li> <li>- Clinical, science-based recommendations for the specific patient case.</li> </ul> <p>Key elements to the discussion, and the decision-making process regarding the number of embryos to transfer are the following:</p> <ul style="list-style-type: none"> <li>- Patient involvement, which ensures a decision that reflects both healthcare professional' good clinical judgement and the patients' values and personal context.</li> <li>- Involvement of both members of the patient couple.</li> </ul>	GPP		
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\*We labelled the recommendations as either “strong” or “conditional” according to the GRADE approach, the strength of a recommendation reflects the extent to which a guideline panel is confident that desirable effects of SET outweigh undesirable effects, or vice versa, across the range of patients for whom the recommendation is intended. A conditional recommendation is formulated when the GDG is uncertain whether the balance is clear. Good practice points (GPP) are mainly based on the expertise and opinion of GDG members and are written by the GDG to support the recommendations.

\*\*The number of pluses reflect the overall quality of evidence across all the critical outcomes essential to the recommendation made: 3 pluses refer to moderate quality evidence from meta-analysis or RCTs; 2 pluses and 1 plus refer respectively to low quality evidence and very-low quality evidence from observational studies.

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# Pictorial summary

Figure 1 The benefits of transferring only one embryo at a time



# Part A. Consequences and risks of multiple pregnancy

## I. Medical risks related to multiple pregnancy/birth

*Key question. Which pregnancy-related risks should be considered before the transfer of more than one embryo?*

A Cochrane review, encompassing 17 randomised controlled trials (RCTs), demonstrated that cLBRs are similar when comparing repeated SET to one cycle of DET (risk ratio (RR) 0.95; 95% confidence interval (CI) 0.82 – 1.10, 4 studies; 985 participants; low-quality evidence) ([Kamath et al., 2020](#)). SET was associated with reduced MPR compared to a single DET cycle (Peto odds ratio 0.13; 95% CI 0.08-0.21, 4 studies, 985 participants). This means that for a clinic with a 42% chance of live birth following a single cycle of DET, the chance following repeated SET would be between 34% and 46%. The same study also revealed that in cases with 13% risk of multiple pregnancy following a single cycle of DET, the risk following repeated SET would be between 0% and 3%. MPR following DET is influenced by a number of factors that are further elaborated in parts C and D of this guideline.

### *Evidence*

A systematic review, including 60 observational studies comparing maternal and neonatal outcomes in singleton and twin pregnancies following ART treatments, indicated significantly higher maternal and perinatal health risks in twin pregnancies compared to singletons ([Eapen et al., 2020](#)). Maternal health risks encompassed antenatal hospitalisation (31.2% (901/2890) vs. 13.0% (1128/8707), respectively; odds ratio (OR) 2.6; 95%CI 1.9-3.5), caesarean section (76.6% (62558/81644) vs. 45.5% (90396/198809), respectively; OR 3.7; 95%CI 3.3-4.1), gestational diabetes (10.9% (7191/65991) vs. 8.9% (13917/156202), respectively; OR 1.2; 95%CI 1.1-1.3), preterm labour (69.1% (1432/2071) vs. 17.2% (602/3502) respectively; OR 6.3; 95%CI 3.6-11.0), pregnancy-induced hypertension (12.1% (7903/65134) vs. 5.6% (8671/156202), respectively; OR 2.0; 95%CI 1.9-2.3), pre-eclampsia (7.2% (173/2417) vs. 3.9% (167/4295), respectively; OR 1.9; 95%CI 1.4-2.6), placental abruption (1.1% (690/60736) vs. 0.8% (1243/148792), respectively; OR 1.3; 95%CI 1.2-1.5), placenta praevia (1.5% (904/60705) vs. 2.1% (3118/148734), respectively; OR 0.8; 95%CI 0.7-0.9) and postpartum haemorrhage (10.5% (464/4400) vs. 4.3% (483/11116), respectively; OR 2.2; 95%CI 1.2-4.1). Foetal and neonatal risks included congenital anomaly (2.5% (898/35722) vs. 2.3% (1080/47464),

respectively; OR 1.1; 95%CI 1.0-1.2), preterm birth rate (52.6% (149927/285078) vs. 21.4% (185082/863038), respectively; OR 8.3; 95%CI 7.8-8.9), early preterm birth rate <32 gestational weeks (31.8% (5816/182811) vs. 0.91% (5763/632722), respectively; OR 3.5; 95%CI 3.1-3.9), very preterm birth rate <28 gestational weeks (10.8% (24430/189177) vs. 2.2% (13701/631847), respectively; OR 5.5; 95%CI 5.2-5.9), low birth weight (52.3% (74836/143190) vs. 8.7% (28435/328034), respectively; OR 10.6; 95%CI 9.9-11.4), NICU admission rate (37.5% (25847/68854) vs. 9.0% (15580/172485), respectively; OR 6.5; 95%CI 5.8-7.3), perinatal mortality rate (2.9% (561/19368) vs. 1.2% (315/26548), respectively; OR 2.4; 95%CI 2.1-2.8), and stillbirth rate (5.4% (910/16745) vs. 2.4% (944/30390), respectively; OR 2.2; 95%CI 1.8-2.6).

A large retrospective cohort analysis assessing the risk of pre-eclampsia in 12,810 singleton and 8,378 twin pregnancies conceived through ART treatment demonstrated that within the singleton subgroup, the transfer of multiple embryos increased the risk of pre-eclampsia (adjusted RR 1.1; 95%CI 1.0-1.2) (Sites et al., 2020). Within the twin subgroup, the transfer of >2 embryos increased the risk of pre-eclampsia (adjusted RR 1.1; 95%CI 1.0-1.2).

Another retrospective cohort study involving 138,435 children conceived through ART treatment stratified the risk of major congenital anomalies, small for gestational age, small birth weight and premature birth based on the number of embryos transferred (Luke et al., 2021). Compared to singleton births following SET, the risks were increased in singleton births following DET or triple embryo transfer (TET) for major congenital anomalies (DET adjusted OR 1.1; 95%CI 1.0-1.3; TET or more adjusted OR 1.2; 95%CI 1.0-1.4), small for gestational age (DET adjusted OR 1.1; 95%CI 1.0-1.2; TET or more adjusted OR 1.2; 95%CI 1.1-1.3), low birth weight (DET adjusted OR 1.1; 95%CI 1.0-1.1; TET or more adjusted OR 1.2; 95%CI 1.1-1.3), and preterm birth (DET adjusted OR 1.06; 95%CI 1.0-1.1; TET or more adjusted OR 1.1; 95%CI 1.1-1.2).

Similarly, several retrospective studies comparing twins or HOM gestations to singletons, conceived either without medical assistance or by ART treatment, showed that multiple pregnancies were associated with higher maternal, foetal, and neonatal health risks (D'Souza et al., 1997, Gupta et al., 2020, Makhseed et al., 1998, Pinborg et al., 2004, van Heesch et al., 2014) (**tables 1 and 2**).

Table 1 Outcomes regarding maternal risks associated with ART singleton, twin, and HOM pregnancies.

Study	Maternal health risks in ART pregnancies														
	Threatened miscarriage rate (%)			Pre-eclampsia rate (%)			Gestational diabetes rate (%)			Antepartum haemorrhage rate (%)			Caesarean section rate (%)		
	1 foetus	2 foetuses	≥3 foetuses	1 foetus	2 foetuses	≥3 foetuses	1 foetus	2 foetuses	≥3 foetuses	1 foetus	2 foetuses	≥3 foetuses	1 foetus	2 foetuses	≥3 foetuses
D'Souza et al., 1997	-	-	-	-	-	-	-	-	-	-	-	-	26.7	-	55.5
Makhseed et al., 1998	6.9	6.4	40.9	18.9	16.1	33.3	-	-	-	6.9	6.9	33.3	6.8	26.7	55.5
Pinborg et al., 2004	-	-	-	6.2	13.4	-	4.1	5.6	-	-	-	-	26.2	58.1	-
van Heesch et al., 2014	-	-	-	-	-	-	-	-	-	-	-	-	22.5	-	41

Table 2 Outcomes regarding the neonatal risks associated with ART singletons, twins, and HOM children.

Study	Foetal and neonatal health risks in ART pregnancies and children														
	Intrauterine growth retardation rate (%)			Gestational age (weeks)			Birth weight (g)			NICU admission rate (%)			Neonatal death rate (%)		
	singletons	twins	HOM	singletons	twins	HOM	singletons	twins	HOM	singletons	twins	HOM	singletons	twins	HOM
D'Souza et al., 1997	-	-	-	38.8	-	35.2	3016	-	2078	-	26.2	58.1	-	-	-
Makhseed et al., 1998	6.5	25.8	48.1	39.4	38.8	35.2	3380	3016	2078	1.7	0	25.9	1.7	-	11.1
Pinborg et al. 2004	-	-	-	-	-	-	-	3387±638	2509±659	-	-	-	14 (18.2%)	18 (33.5%)	-
van Heesch et al. 2014	-	-	-	39.4	-	36.3	3362	-	2441	2.8	-	12.2	0.4	-	1
Gupta et al., 2020	-	-	-	37.2	35	-	2760	2030	-	-	-	-	-	-	-

NICU: neonatal intensive care unit.

Moreover, multiple pregnancies leading to a higher risk of premature delivery is subsequently associated with higher risk of neonatal complications such as developmental delays, visual and hearing impairment, cerebral palsy, infection, digestive and metabolic problems ([Wainstock et al., 2023](#)).

A potentially fatal complication of the transfer of multiple embryos is ectopic pregnancy, the rates of which increase along with the number of embryos transferred up to approximately 20-fold ([Anzhel et al., 2022](#), [Bu et al., 2016](#), [Cirillo et al., 2022](#), [Li et al., 2015](#), [Perkins et al., 2015](#), [Santos-Ribeiro et al., 2016](#)). When comparing outcomes of (e)SET and DET, the risk of an extrauterine pregnancy is elevated after the transfer of two embryos vs. one, regardless of development stage or cryopreservation status (OR ranging 1.3-1.4) ([Anzhel et al., 2022](#), [Santos-Ribeiro et al., 2016](#)). In one retrospective study, a factor associated with lower odds of ectopic pregnancy was the transfer of a top-quality embryo (OR 0.7; 95%CI 0.6-0.9) ([Anzhel et al., 2022](#)).

Pregnancies that begin as multiple gestations may undergo spontaneous reduction and continue as singleton gestations. A single-centre retrospective study compared the clinical outcome of spontaneous foetal reduction following DET (n=865) to the clinical outcomes of singleton pregnancies following SET (n=4667) ([Wang et al., 2022](#)). Singleton pregnancies after spontaneous foetal reduction had a reduced LBR (OR 0.7; 95%CI 0.5-0.98) and an increased risk of low-birth weight (OR 1.5; 95%CI 1.1-2.1).

Finally, even if a singleton pregnancy develops after DET, it is associated with an overall higher risk of neonatal death (OR 2.7; 95%CI 1.3-5.6) and a higher risk of low birth weight in DET (OR 1.6; 95%CI 1.2-2.3) compared with singleton pregnancies after SET, as shown by a Swedish study comprising 1 115 863 singleton births, of which 30 713 singletons were born after SET and 5123 after DET ([Rodriguez-Wallberg et al., 2023](#)).

*Recommendation*

<p><b>Medical risks that should be considered before the transfer of more than one embryo are the higher rates of maternal, foetal, and neonatal complications.</b></p>	<p><b>Strong</b>    ⊕⊕⊕○</p>
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The GDG recommends that whenever the transfer of >1 embryo is considered, the patient should be provided with clear information about the higher risk of pregnancy loss, ectopic pregnancy, pre-eclampsia, gestational diabetes, antepartum and postpartum haemorrhage, caesarean section, stillbirth, preterm birth, low birth weight, neonatal intensive care admission and neonatal death. The GDG also recommends that the patients sign an additional consent form if >1 embryo is transferred.

GPP

#### *Justification*

*There is consistent evidence demonstrating that multiple pregnancies are associated with higher maternal and neonatal risks compared to singleton pregnancies, irrespective of the mode of conception [see **annex 2** – summary of findings (sof) table 1]. Informed consent prior to accepting the transfer of >1 embryo implies that adequate counselling has been provided regarding the risks associated with multiple pregnancies (**annex 11**). The strength of evidence for the recommendation to counsel patients about the maternal and neonatal risks associated with multiple pregnancy following the transfer of >1 embryo relies on data from observational studies with a retrospective design. These risks are also well documented in spontaneous multiple pregnancies and conducting randomised controlled trials (RCT) on this topic in ART patients is not considered ethically acceptable. Therefore, the GDG concluded that the existing evidence from observational studies is sufficient to provide patients with clear information about the risks associated with multiple pregnancies.*

#### *Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## II. Financial issues of multiple pregnancy/birth.

ART treatments are costly and can account for as much as 25% of annual household expenditure in some countries ([Collins, 2002](#)). Consequently, the cost of ART is an important aspect of treatment. One reason for opting for DET is the belief that it is associated with lower expenses compared to SET ([Gleicher and Barad, 2006](#), [Ryan et al., 2004](#)). Different conclusions can be drawn depending on the scope of analysis. Various options include the following:

- Costs can be limited to the ART treatment only ([Fiddelers et al., 2006](#)), but can also include perinatal costs ([Gerris et al., 2004](#)).
- The analysis evaluates only direct medical costs ([Hernandez Torres et al., 2015](#)), or both direct and indirect costs ([Kjellberg et al., 2006](#)).
- The time period studied can end at positive pregnancy test, or at delivery, or weeks, months or years after the birth.
- Costs can be calculated per delivery ([Lemos et al., 2013](#)) or per child ([Sitler et al., 2019](#)).
- The study can include good prognosis and/or bad prognosis patients.
- The number of treatments being compared can involve scenarios such as two eSET cycles compared to one DET cycle ([Monteleone et al., 2018](#)), one eSET cycle compared to one DET cycle (e.g. [Fiddelers et al., 2006](#)), or all treatments performed during a specific time period ([Polinder et al., 2008](#), [Veleva et al., 2009](#)).
- Finally, determining the incremental cost that a patient or society is willing to pay for a higher chance of a live birth ([Fiddelers et al., 2006](#)) is based on personal value judgements and is, therefore, challenging to translate into guidelines for routine clinical practice.

Costs associated with ART treatments can be categorised into direct and indirect costs (**Box 2**).

### *Box 2 Direct and Indirect costs of ART treatment.*

#### **Direct costs include the following:**

- Costs of ART treatment: outpatient doctor visits, monitoring visits before, during or after hormonal stimulation (for blood samples and ultrasound examinations), medical procedures (oocyte aspiration, micromanipulation, embryo transfer, freezing, thawing) and hospital admissions ([De Sutter et al., 2002](#), [Gerris et al., 2004](#), [Lukassen et al., 2005](#), [Stillman et al., 2009](#), [Veleva et al., 2009](#), [Velez et al., 2014](#)).
- Obstetric costs, including outpatient consultations, sonography, chorionic villus sampling, amniocentesis, blood samples, medical procedures, surgery, delivery and hospital admissions, telemedicine ([Carpinello et al., 2016](#), [Gerris et al., 2004](#); [Koivurova et al., 2004](#), [Motohashi et al., 2004](#)).
- Paediatric costs: outpatient consultations, medical procedures, surgery and hospital admission, telemedicine ([Carpinello et al., 2016](#), [Gerris et al., 2004](#), [Koivurova et al., 2004](#), [Motohashi et al., 2004](#)).

#### **Indirect costs are related to the following:**

- Loss of productivity such sick leaves, maternal leave, parental leave ([Santana et al., 2018](#), [Fiddelers et al., 2006](#); [Kjellberg et al., 2006](#)).
- Out-of-pocket costs: travel, over-the-counter medication, informal care, housekeeping ([Fiddelers et al., 2006](#)).

*Key question. Which financial issues should be considered for couples/individuals planning a singleton or multiple pregnancy/birth?*

*Evidence*

There is considerable heterogeneity in studies comparing the cost-effectiveness of eSET and DET. This variability stems from differences in the clinical scope of analysis, the availability and type of reimbursement, and whether the reporting centre is also involved in the treatment of pregnancies, births, and children.

*Direct costs*

When analysing costs of ART treatment, the outcome from cost-effectiveness analysis seems to depend on the treatment policy examined. Early studies, characterised by small cohort sizes and considerable heterogeneity, found that direct costs of eSET were similar to those of DET (Fiddellers et al., 2006, Hernandez Torres et al., 2015, Kjellberg et al., 2006, Lukassen et al., 2005).

By contrast, population-based studies of direct ART costs found eSET to be the less expensive policy. The first of these examined the impact of introducing the eSET strategy in Northern Finland in the period 2000-2005 and compared ART treatment costs to the pre-eSET period of 1995-1999 and to live births at term (Veleva et al., 2009). This study, which included only charges for ART treatment and medication costs from a payer perspective until the pregnancy test, revealed that a term live birth in the eSET period was €19,889 less expensive than those in the preceding DET period. Similar cost reductions were observed in Quebec during the introduction of eSET policy in 2009-2011, where all government costs for ART treatment and related maternity and child costs per live birth until the end of the first year post-partum were included (Velez et al., 2014). Costs per baby conceived decreased by CAD\$6155 (€4572).

In fact, there is strong evidence that including obstetric and/or neonatal costs into the analysis demonstrates that DET is associated with higher costs, compared to eSET. The first study to include ART treatment, pregnancy and neonatal costs was performed in 2004 (Gerris et al., 2004). This intention-to-treat analysis of observational data showed a higher total cost after a DET cycle than after an eSET cycle for both the mother and the children: €4700±3239 (eSET) versus €8,613±10,004 (DET), entirely due to significantly higher neonatal costs after DET.

Studies focusing solely on obstetric and/or neonatal costs consistently indicated that singletons conceived after ART treatment are the less costly option. For instance, a Dutch analysis

investigating costs from pregnancy to six weeks after delivery observed that the medical cost per twin pregnancy was over 5-fold than the expenses incurred following a singleton pregnancy: €13,469 vs. €2,550 (Lukassen et al., 2004). Similarly, a study of total health care costs from conception until the end of the neonatal period in Finland found that ART singletons were associated with approximately a three-fold reduction in costs compared to ART twin siblings (one singleton: €5,780, one twin: €15,580) (Koivurova et al., 2004). The difference was even more substantial in a small observation analysis from Japan (Motohashi et al., 2004) in which obstetric, delivery and neonatal costs were 1,889,000 yen (€13,127) per one ART twin sibling and only 173,000 yen (€1203) per ART singleton. Delivery and neonatal costs per type of transfer (SET or DET) were investigated in the United States in a small series of patients (Carpinello et al., 2016). Costs for SET (all singletons) were \$71,860 (€67,234), while for the DET cohort consisting of 57% twins, costs were more than two-times higher at \$171,350 (€160,318).

Paediatric costs of ART children after the neonatal period were also lower in singletons, although differences with twins were less pronounced. An analysis of hospital care costs from the post-neonatal period up to the age of 7 years found that costs per child were about €206 for ART singletons and €302 for an ART twin sibling (Koivurova et al., 2007). However, if the birth and neonatal periods are included in the analysis, hospital costs from birth up to the age of 5 years were over 3.3-fold higher for a cohort consisting of 96% twins, compared with singletons (van Heesch et al., 2015). Finally, hospital costs from birth to the age of 5 years in Australia found that costs of a singleton in 2009-2010 US dollars was \$2,730 (€2,554) and costs of a twin sibling was \$8,993 (€8,414) (Chambers et al., 2014).

#### *Indirect costs*

In some countries, obstetric and paediatric care is provided by universal health insurance and not by patients themselves. Analyses of indirect costs highlight the significant impact of money lost through sick leaves and absence from work. Two studies, one from Sweden and one from the Netherlands, evaluated costs from ART treatment until the postpartum period. Thurin-Kjellberg and co-workers calculated the total mean cost for absence from work per pregnancy to be €1,602 for eSET and €2,359 for DET (Kjellberg et al., 2006). The eSET group also had a significantly fewer days of sick leave during pregnancy (14.1 vs. 23.0 days).

Indirect costs were highest during pregnancy in the study from the Netherlands that investigated costs from start of ART treatment until 42 weeks after embryo transfer (Fiddelers et al., 2006). Indirect costs, which included sick leave or maternity leave, leave of absence, loss of leisure time, out of pocket costs and informal care with eSET and DET, were €750 vs. €785 during ART, €1149 vs. €2516 during pregnancy, and €493 vs. €1105 during the delivery-postpartum period, respectively.

Finally, the effect of insurance status on patients' choice of eSET was investigated in the United States (Stillman et al., 2009). The authors found that use of eSET is associated with having ART insurance because patients are more likely to choose eSET when not under financial pressure to transfer multiple embryos.

#### Recommendation

It is recommended to consider the increased direct costs related to obstetric care of multiple pregnancies and paediatric care of twins and triplets.	Strong ⊕⊕⊕○
It is recommended to consider increased indirect costs with multiple pregnancies due to sick leave days, over-the-counter medication, loss of productivity because of an ill child.	Strong ⊕⊕○○
The GDG recommends that cost-related information be provided and discussed with the patient(s) at the treatment planning stage.	GPP

#### Justification

*Costs related to pregnancy after ART can be categorised into two main groups: direct costs, related to ART treatment, obstetric and paediatric care, and indirect costs, associated with loss of productivity such as sick leaves. While direct costs are seldom fully paid by ART patients, indirect costs are often borne by the patients, even in countries with paid sick leave policies.*

*Evidence from early studies is low because of study heterogeneity and small subject numbers. However, moderate-quality evidence from later analyses demonstrates higher direct costs in multiple pregnancies. Additionally, there is low-quality evidence that indirect costs are higher in multiple pregnancies, regardless of the point of comparison (mother- vs. child-based comparison).*

*It is important for insurers to recognise that the likelihood of higher direct and indirect costs is not limited to multiple pregnancies and twins/triplets; even singleton pregnancies and children born following ART treatment may incur higher costs.*

#### *Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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### III. Psychosocial issues of multiple pregnancy/birth

When compared to spontaneous pregnancies, becoming parents through medically assisted reproduction carries added stress before and during pregnancy related to the procedures, timing, fears, costs and outcomes (Eugster and Vingerhoets, 1999). Furthermore, care providers should be aware of the likelihood of stress and depression after delivery in mothers of multiples and patients undergoing ART (van den Akker et al., 2016). Throughout the years, many studies have emphasised the difficulties and risks of multiple pregnancies, not only in terms of physical consequences in mothers and offspring, but also in the mother's mental health and the development of the children. Parental stress is associated with poorer quality parenting and parent-child interactions (Deater-Deckard, 1998).

The impact on the psychosocial health of mothers, fathers and offspring of ART should be considered before the decision on the number of embryos to transfer.

***Key question. Which psychosocial issues should be considered for couples/individuals having a singleton or multiple pregnancy/birth?***

#### *Evidence*

A systematic review suggested that mental health outcomes in the postpartum and early childhood periods (e.g., depression, anxiety, parenting stress) are, in general, worse for parents of multiples versus parents of singletons, and may be worse in the case of higher-order multiples versus twins (Wenze et al., 2015). This review found no clear evidence for differences in mental health outcomes in the antenatal period between patients expecting singletons versus multiples, however, the maternal antenatal depressive symptoms may be higher among patients with multiples from spontaneous pregnancies versus parents of ART multiples. On the other hand, outcomes until the end of the first year postpartum may be worse for parents of multiples resulting from ART versus multiples from spontaneous pregnancies. One hypothesis is that in the group that conceived without medical assistance, a multiple pregnancy has a more negative psychological impact as it is more unexpected than in the ART group; the latter group is aware of the possibility of a multiple pregnancy when more than one embryo is transferred, which could be a part of the decision-making process.

Results of a meta-analysis showed that mothers of multiples after ART exhibit significantly more stress and depression compared with mothers of singletons after ART (van den Akker et al., 2016). Results indicate that the effects for depression were stronger at  $\leq 1$ -year post-

partum than at >1-year post-partum (but the difference is seen as late as 4-years post-partum), suggesting that patients need more support to cope with ART multiples following the first year post-delivery. The authors suggest that their data can be used to deter multiple ETs and encourage eSET, given the clear evidence for the effects in the first two years post-partum.

Birth circumstances (prematurity, multiple births, parity) and psychosocial factors (sociodemographic factors, family functioning, marital relationship, and education) interfere with mental maternal health (MMH) over the course of four years after delivery, as shown in a prospective observational study ([Porat-Zyman et al., 2018](#)). According to the results of this study, shortly after birth, poorer MMH was more frequent in mothers who gave birth prematurely or were characterised by insecure partnership (including anxious attachment with deep fear of abandonment, avoidant attachment with fear of intimacy, or a combination of both anxious and avoidant attachment), lower marital quality, younger age, or a higher level of education. After one month from birth, improvement in MMH was shown in mothers who had given birth prematurely or were younger, more highly educated, or multiparous, while the MMH of mothers with insecure attachment or lower marital quality did not improve over time. The MMH of older or less educated mothers deteriorated over time. Marital quality mitigated or exacerbated the effects of birth circumstances and insecure attachment style on MMH starting shortly after giving birth.

Another observational study of mothers of both full term (88 mothers of twins, 99 mothers of singletons) and preterm (59 mothers of twins, 36 mothers of singletons) babies, showed that marital quality provided the strongest explained variance for both well-being and distress without a significant difference between mothers of twins and singletons ([Noy et al., 2014](#)). This was followed by mother's somatic health and levels of insecure attachment. Maternal grandmother's support was also found to contribute to well-being.

Co-parenting also seems to be a significant coping strategy to reduce the level of parenting stress in singleton and twin mothers, irrespective of their personal and obstetric characteristics ([De Roose et al., 2018](#)).

A prospective longitudinal study showed significant individual variability in parenting stress across mothers of preterm infants at 4-, 24-, and 36-months post-term ([Spinelli et al., 2013](#)).

Having multiples seemed to have the strongest effect on the development of parenting stress at 4 months compared to having singletons. Furthermore, mothers of infants with more medical risks and shorter hospitalisation (with less nursing support), and mothers with lower education and more depressive symptoms, reported higher levels of parenting stress. Parenting stress decreased over time, with more profound effect in mothers of multiples and for mothers with lower education than in mothers of singletons or for mothers with higher educational levels. At the same time, an increase in parenting stress scores over 36 months was negatively associated with a maternal positive affective behaviour during interaction with the infant. These results suggest the importance of analysing individual differences in parenting stress as well as factors that could influence these differences.

In an observational study of more than 2500 parents, mothers of twins reported perceiving themselves as less effective in achieving parental goals and more hostile and reactive towards their infants as compared to mothers of singletons ([Boivin et al., 2005](#)).

Although there are not many studies focusing on fathers, an observational study including 57 families with eighty 6- to 12-year-old ART children (50 singletons and 30 twins) showed that while mothers exhibit similar interactional behaviours toward twins and singletons, fathers had fewer optimal behaviours toward twins compared to singletons and were less engaged in supportive communication with their twins ([Anderson et al., 2017](#)).

A small observational study showed that three-year-old twins or triplets conceived after ART do not seem to experience markedly raised levels of emotional or behavioural disturbances compared to ART singletons ([Golombok et al., 2007](#)). However, the study by Anderson and colleagues showed that older twins (6 to 12 years old) displayed less engaged behaviour (less supportive communication) with their mothers and fathers compared to singletons of similar age ([Anderson et al., 2017](#)). The authors concluded that these results suggest that there is reason to be concerned about fathers' interactions with middle-aged twins and twins' engagement in their relationships with both parents.

*Recommendation*

<p>Clinicians should consider the possible complications of multiple pregnancies with regards to mental health postpartum, emotional distress and possible marital problems, as well as the influence of</p>	<p>Strong ⊕⊕○○</p>
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personality characteristics, sociodemographic factors, and family functioning, on the mental health of parents and offspring regardless of the number of children born.

The GDG recommends that information on possible psychosocial complications should be provided to patients at the treatment planning stage.

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#### Justification

Moderate-quality evidence shows higher likelihood of stress and depression in mothers of ART children and especially in mothers of ART multiples, compared to mothers of children from spontaneous pregnancies. Clinicians should be aware of these risks in order to help patients through a successful decision-making process. Psychosocial dimensions that should be considered for patients before taking the decision of the transfer of one or more embryos are sociodemographic factors, family functioning, marital relationship, and education.

#### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## Part B: The impact of patient preferences, regulatory factors and reimbursement policies

While the guideline's main focus is on the medical aspects of eSET versus DET, it is important to acknowledge that regulatory and reimbursement factors can also have an effect on embryo transfer practices as they impact attitudes of patients, fertility clinics or both. In some cases, patients' personal preferences can also affect transfer decisions.

*Key question. Which personal, regulatory and reimbursement factors are expected to affect the decision for number of embryos to transfer? (Narrative)*

### *Patient preferences*

Several studies indicate that positive attitudes towards DET and twin pregnancies are widespread in the ART patient population, even when informed about the additional risks involved (Blennborn et al., 2005, Højgaard et al., 2007, Mendoza et al., 2018, Okohue et al., 2010, Pinborg et al., 2003, Ryan et al., 2004, Twisk et al., 2007). This contrasts with the general perspective of health care professionals, cataloguing a twin pregnancy as an adverse outcome of ART (The ESHRE Task Force on Ethics and Law, 2003, Hartshorne and Lilford, 2002). The survey on patient attitudes regarding embryo transfer undertaken by the GDG (**annex 7**) revealed that currently, 15% of infertility patients in Europe have a clear preference for twins. This modest percentage could reflect changes in patient perceptions that have occurred during the last decade in parallel with the increase in practise of eSET observed in Europe (Wyns et al., 2022). The GDG survey also revealed that the leading reason for a patient's preference for twins was the desire to have several children as soon as possible (49.5%), followed by a general fondness towards twins (41.7%).

The combination of a positive attitude towards twins and the eagerness to maximise the likelihood of a pregnancy in the fresh transfer cycle may explain why patient's preference for DET persists in a subset of patients, even if that may imply a higher risk of health problems and even disabilities in future children as a result of the decision to transfer more than one embryo. A study by Scotland and co-workers found that it is not uncommon for patients undergoing ART treatment to prefer a child with a disability over no child at all (Scotland et al., 2007) and

another study demonstrated that even though counselling and assertive communication techniques lead to more patients choosing eSET over DET, a significant proportion retains a favourable attitude towards twins, despite awareness of the health risks ([Van Peperstraten et al., 2010](#)).

It should be acknowledged that at present, most patients in Europe do not have a clear preference towards twins or singleton(s). According to the results of the current GDG survey ([annex 7](#)), one third of patients (33.3%) preferred singleton(s), while half of the patients (50.0%) had no preference towards twins or singleton(s). Furthermore, the leading reason for a patient's preference for singleton(s) was the desire to have an easier and less risky pregnancy (82.7%).

#### *Regulatory factors and reimbursement policies*

Legislation and regulations have a clear impact on embryo transfer decisions. Guidelines without legal imposition tend to have a much smaller impact ([Hornstein, 2016](#)). Examples of European countries that have linked public funding to a clinical policy favouring eSET over DET are Belgium, Czech Republic, Slovenia, Sweden, and The Netherlands. The reimbursements systems of Belgium and Slovenia support couples when only one embryo is transferred during the first two ART cycles in women up to 35 years of age. The Netherlands has a similar policy but the limit for reimbursement is set at 38 years of age. The Czech Republic reimburses an extra (4<sup>th</sup>) IVF cycle if the first two attempts were SET, while Sweden only allows DET exceptionally and does not allow TET ([Calhaz-Jorge et al., 2020](#)). The countries that have adopted SET as part of reimbursement criteria have seen rapid declines of multiple ETs and MPRs, without negative impact on the cLBR ([Bergh, 2007](#), [Bissonnette et al., 2011](#), [Ergun et al., 2013](#), [Peeraer et al., 2014](#), [Salame et al., 2011](#), [Saldeen and Sundstrom, 2005](#), [Umstad et al., 2013](#)). A regulation setting an upper limit to the clinic's overall MPR has had similar results ([Human Fertilisation & Embryology Authority, 2021](#)). Results regarding costs of ART treatment have shown a decrease in overall expenses after a conversion from DET to eSET ([Veleza et al., 2009](#), [Velez et al., 2014](#)).

Regulation can also have the opposite effect, for example when public funding is linked to minimal success rates of the clinic without taking the number of embryos transferred or MPRs into account, which is currently the case in Austria, Bulgaria, and Romania ([Calhaz-Jorge et al.,](#)



2020). In line with this, the latest EIM report revealed that in Austria, 26.9% of fresh transfers were DET and 0.2% were TET ([Wyns et al., 2022](#)). The corresponding figures for Romania were 52.1% DET and 9.1% TET. Bulgaria does not report numbers of embryos transferred, however published MPRs showed a decline from 32.7% ([Kozovski et al., 2007](#)) to 25.0-27.1% ([Stamenov et al., 2017](#)).

Legislative requirements for eSET might be perceived as interfering with patient rights and liberties. Previously, due to divergent opinions of health care professionals and patients, the mandate of (e)SET has been flagged by a number of authors as an insufficiently motivated infraction against patient autonomy ([Gleicher and Bard, 2013](#), [Gleicher et al., 2009](#), [Meldrum et al., 2018](#), [Tremellen et al., 2015](#)). This is particularly the case when patients estimate their financial burden increased through the eSET policy. Depending on their reimbursement scheme, patients' personal cost may be lower for DET than for eSET, even though the overall cost is higher. This is, for example, the case when ART treatment is not covered by health insurance, but perinatal and paediatric care is ([Sitler et al., 2019](#)). This observation should be considered by health insurance programmes, both public and private. Better reimbursement policy for assisted reproduction may motivate more patients to ultimately consent to SET rather than DET as illustrated by several studies ([Hamilton et al., 2018](#), [Jain et al., 2002](#), [Provost et al., 2016](#), [Styer et al., 2016](#)). When treatment of infertility is not reimbursed, patients will most likely take the cost of reaching their preferred family size into consideration when they have to decide about the number of embryos to be transferred. Patients may perceive DET as a cheaper and more cost-effective option, especially when the preferred family size consists of two or more children.

### Conclusion

Only 16% of ART patients in Europe currently seem to prefer twins over singletons. This preference can be partially explained by the desire to have several children as soon as possible. Legislations or reimbursement programmes that are based on eSET or on acceptable MPRs promote both minimal costs and risks. The results of such policies demonstrate low MBRs without jeopardising LBRs. Reimbursement schemes based on other criteria may result in high MBRs. When legislative or reimbursement factors create motives to prefer DET over SET despite the clinical contra-indications for DET, the preferred remedy is an adjustment of those factors. However, given that patients and physicians must often make decisions within these

suboptimal conditions, it appears advisable to retain the option of deviating from an eSET policy on an individual, case-by-case basis, after providing and documenting information regarding all the potential additional risks and burdens to the patient.

The GDG encourages legislative and health insurance policies that promote the practice of eSET.

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## Part C: Clinical criteria

### I. Previous unsuccessful ART treatments

The number of previously failed treatment trials is one of the factors that affects most the outcome of the next ART trial ([Templeton et al., 1996](#)). In a large population-based observational cohort study, LBR gradually decreased with each additional ART cycle, from 28.5% in the first cycle to 16.8% in the fifth cycle ([McLernon et al., 2016](#)). This has been confirmed in other retrospective studies that have been undertaken to support the decision about the number of embryos to be transferred. One such analysis showed that two previous unsuccessful IVF treatments were associated with lower chance for live birth when compared with no previous IVF (OR 0.5, 95%CI 0.2-0.9) ([Strandell et al., 2000](#)). In another model, each unsuccessful ART cycle decreased the odds of ongoing implantation (OR 0.6, 95%CI 0.4-0.98) ([Thurin et al., 2005](#)). However, when compared to other prognostic factors, such as female age and the number of available embryos, the number of failed treatment attempts has been considered a weak prognostic factor ([Roberts, 2010](#)). After unsuccessful treatments, care providers and patients alike might still consider transferring a higher number of embryos to ensure a live birth.

*Key question. Should the number of previous unsuccessful ART treatments be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, what is the cut off?*

#### *Evidence*

At present, there are no prospective studies analysing the impact of the number of previous unsuccessful ART treatments on LBR as final outcome with respect to DET vs. (e)SET.

One retrospective study reported results from FET treatments after eSET with no pregnancy, of which 40 were eSET and 102 DET ([Monteleone et al., 2016](#)). This study demonstrated that while clinical pregnancy rate (PR) was not significantly different after eSET and after DET (eSET: 42.5% vs. DET: 35.3%), the MPR was higher in DET cycles (eSET: 5.9% vs. DET: 22.2%).

After a review of the available literature, the GDG found no evidence on how many unsuccessful cycles could justify DET instead of (e)SET. No evidence was also found for treatments with donor oocytes or donated embryos.

## Recommendation

The decision to perform DET instead of eSET should not be based on the number of previous unsuccessful ART treatments.

Strong

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## Justification

Very low-quality evidence from a single retrospective study indicates similar clinical pregnancy rates but lower MPR if one embryo is transferred compared to two embryos. Furthermore, there is no scientific evidence indicating that repeated failed cycles can be compensated by increasing the number of embryos per transfer. Therefore, eSET was recommended for safety reasons.

## Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## II. Duration of infertility

Longer duration of infertility inversely correlates with the likelihood of spontaneous pregnancy (Hunault et al., 2004, Leridon and Spira, 1984). While the duration of infertility had no significant effect in the model proposed by Roberts and co-workers (Roberts et al., 2010), in the model proposed by McLernon and colleagues, duration of infertility negatively impacted the overall outcome of ART (McLernon et al., 2016).

**Key question. Should the duration of infertility be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, what is the cut off?**

### Evidence

Despite the importance of the duration of infertility for the prediction of the outcome of ART, no studies that specifically compared the outcome of eSET versus DET with respect to the duration of infertility were found. In two retrospective cohort studies comparing the outcome of eSET versus DET, the duration of infertility was one of the compared baseline characteristics of both cohorts. The results from these studies are briefly shown here to illustrate the possible lack of effect on ART outcomes of infertility duration.

In the first retrospective study of 404 treatment cycles, the mean duration of infertility was five years in SET patients (281 cycles) and six years in DET patients (123 cycles) (Yilmaz et al., 2013). No significant difference in LBR was observed between SET and DET (31.7% vs. 26%, respectively), while the number of multiple pregnancies was significantly higher in DET cycles (0 for SET vs. 12 for DET).

A similar conclusion was reached for patients with a mean duration of infertility of  $2.3 \pm 2.2$  years and  $2.2 \pm 1.2$  years in eSET (40 cycles) and DET (102 cycles), respectively (Monteleone et al., 2016). Clinical PR did not vary significantly (42.5% vs. 35.3% for eSET and DET, respectively), while the MPR was higher in DET (5.9% vs. 22.2% for eSET and DET, respectively). No data on LBR was reported.

No evidence was found regarding this question for cycles with donor oocytes or donated embryos.

### Recommendation

The decision to perform DET instead of eSET should not be based on the duration of infertility.	Strong ⊕○○○
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### Justification

Duration of infertility is an important item in the patient's history since it is one of the main criteria for the decision to initiate ART. However, low-quality evidence showed that this factor does not seem to be associated with LBR when analysing the results with respect to the number of embryos transferred in (e)SET and DET treatment cycles. eSET was recommended for safety reasons.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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### III. Previous pregnancy/live birth

Pregnancy from an ART cycle has been found to be a good prognostic indicator of pregnancy in a subsequent cycle, with an increased chance of both live birth and multiple birth ([Engmann et al., 2001](#), [Kupka et al., 2003](#), [Molloy et al., 1995](#), [Simon et al., 1993](#)). Besides a positive association with LBR, a lack of correlation with MPR in a subsequent cycle was also observed in a group of articles, some of which evaluated previous ART or a mix of spontaneous and ART pregnancies/live births ([Bhattacharya et al., 2013](#), [Lintsen et al., 2007](#), [McLernon et al., 2016](#), [Roberts et al., 2010](#), [Templeton and Morris, 1998](#)). A single retrospective study of 2107 DET cycles showed that having a previous pregnancy or a previous childbirth was not associated with a difference in the likelihood of LBR and MPR ([Strandell et al., 2000](#)).

None of the studies above specifically compared the outcomes of SET and DET cycles.

***Key question. Should a previous pregnancy/live birth from ART treatment be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART?***

#### *Evidence*

A prediction model that was validated on several data sets ([Luke et al., 2014](#)) found that prior full-term birth was associated with higher LBR in all models. A later study by the same team evaluated the impact of previous live births specifically on outcomes from eSET or DET ([Luke et al., 2015](#)). In this study, eSET over two cycles was compared with one DET to examine the effect of this embryo transfer strategy on cumulative cLBR and multiple birth rates. The study included data from a first fresh cycle including 33,065 SET cycles and 126,921 DET cycles and data from a second fresh or frozen-thawed SET cycle including 8682 fresh SET cycles and 6747 frozen-thawed SET cycles. Using a stepwise approach, the variable number of previous full-term births (0, 1,  $\geq 2$ ) included in the logistic regression model was ultimately eliminated given that it was a non-significant variable in predicting LBR and MPR.

No evidence was found regarding this question for cycles with donor oocytes or embryos.

#### *Recommendation*

The decision to perform DET instead of eSET should not be based on previous pregnancies or live births from ART.	Strong ⊕○○○
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### Justification

Although the number of previous pregnancies from the ART cycles is associated with the likelihood of a successful pregnancy from a subsequent treatment, low-quality evidence showed that this factor was likely not a significant variable for predicting LBR and MPR in SET and DET cycles.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## IV. Female age

Female age is an important factor predictive of success in ART. It is recognised that the number and viability of oocytes decrease with age, which causes a marked decline in fecundity several years earlier than the onset of menopause. This fertility decline is clinically relevant to patients from their mid-30's onwards ([Baird et al., 2005](#)).

In line with this observation, female age has historically been one of the criteria included in guidelines on eSET with autologous oocyte treatment. For example, in earlier guidelines, patients with a good prognosis up to 35 years old were recommended eSET while patients aged 37 years or older could be recommended DET ([Min et al., 2010](#), [Scotland et al., 2011](#)). A more recent guideline has included the stage of embryo development alongside with female age ([ASRM, 2021](#)). According to that guideline, patients younger than 35 years should receive SET regardless of embryo stage, and eSET should also be strongly considered for patients aged 35-37 years ([ASRM, 2021](#)). For older patients, the same guideline offers the transfer of one or several embryos as a possibility, depending on embryo development stage at the time of transfer.

*Key question. Should female age be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, what is the cut off?*

### *Evidence*

The specific impact of age has been difficult to disentangle from other factors due to the fact that well-designed studies investigating the outcome of SET vs. DET have mainly included young patients with good prognosis ([Ma et al., 2022](#)). Moreover, study design has been heterogeneous: many analyses have evaluated fresh cycles only, or predetermined combinations of fresh and FET cycles, which may not necessarily correspond to what is observed in real-life work. Scientific evidence on the effect of female age on eSET and DET outcomes has been summarised in **table 3**.

If (e)SET and DET are compared after a single cycle, LBR favours DET. The largest systematic review and meta-analysis included 85 studies (14 randomised controlled trials and 71 observational studies) comparing mostly eSET vs. DET in per-transfer analyses ([Ma et al., 2022](#)). LBR was higher after a DET in patients aged <35 years (OR 0.7; 95%CI 0.6-0.8;  $I^2=85\%$ ; 12 studies) and 35-40 years (OR 0.8; 95%CI 0.7-0.9;  $I^2=69\%$ ; 6 studies). However, in patients aged  $\geq 40$  years such differences were not observed (OR 0.9; 95%CI 0.5-1.4;  $I^2=69\%$ ; 4 studies). MPR

was lower in women aged <35 years receiving SET (OR 0.03; 95%CI 0.03-0.05;  $I^2= 0\%$ ; 11 studies) or those aged 35-40 years (OR 0.04; 95%CI 0.03-0.06;  $I^2= 0\%$ ; 5 studies), whereas the difference was not significant in women  $\geq 40$  years old (OR 0.3; 95%CI 0.06-2.0;  $I^2= 0\%$ ; 3 studies). Compared to DET, SET was associated with lower obstetric and neonatal risks including preterm birth (9.9% vs. 31%; OR 0.3; 95%CI 0.2-0.3;  $I^2=0\%$ ; 13 studies), antepartum haemorrhage, caesarean section, low birth weight, low Apgar score and neonatal intensive care unit (NICU) admission.

Outcomes differ if the analyses include several sequential embryo transfers per patient. In the latest Cochrane systematic review that included 17 RCTs and data on 2505 patients ([Kamath et al., 2020](#)), most studies had a maximum age threshold and the majority of the women included in the studies were under 36 years old, with an expected good prognosis. In that population, there were no differences in cLBR after two consecutive SETs versus one DET (risk ratio (RR) 0.95; 95%CI 0.8-1.1; 4 RCTs; low-quality evidence), whereas MPR after repeated SET was significantly lower, compared to that of DET (OR 0.1; 95%CI 0.08-0.2; 4 RCTs; moderate quality evidence).

A large retrospective cohort study reported on data of 49,333 patients who underwent an initial eSET (n=17,576) or initial DET (n=31,757) ([Mejia et al., 2021](#)). Overall, cLBR was higher after a fresh eSET followed by the second frozen-thawed eSET cycle, compared to a single DET (74.0% vs. 57.0%; adjusted OR 1.3; 95%CI 1.26-1.4). After stratification by age, eSET cycles were associated with an increased cLBR compared to initial DET cycles for women <38 years old (<35 years: adjusted OR 1.3, 95%CI 1.2-1.4 and 35-37 years: adjusted OR 1.3; 95%CI 1.2-1.4). eSET was also associated with reduced MBR compared to DET in all age categories <40 years old (8% vs. 34%; adjusted OR, 0.13; 95%CI 0.12-0.14). No differences were observed in women  $\geq 40$  years old. Preterm birth rate and perinatal mortality rates after eSET were reduced compared to DET (1.2% vs. 2.8% and 0.5% vs. 1.2%, respectively, for eSET vs. DET).

Outcomes of 1224 fresh embryo transfers with known embryo quality in women aged 36-39 years who had eSET or DET were compared in another retrospective cohort study ([Veleva et al., 2006](#)). While LBR did not differ significantly after eSET and DET (26.0% vs. 21.9%), cLBR was higher after eSET (41.8% vs. 26.7%, for eSET and DET) and MPR was higher after DET (1.7 vs. 16.6%, for eSET vs. DET).

A retrospective study compared the outcomes of 264 eSET cycles to those of 364 DET cycles in patients aged 40-44 years ([Niinimaki et al., 2013](#)). LBR per cycle was similar (13.6% vs. 11.0% for eSET vs. DET) but cLBR was higher after eSET (22.7% vs. 13.2% for eSET vs. DET). No twin pregnancies were observed after fresh eSET, but in the DET group, there were three sets of twins (7.5%). Another study focusing on outcomes of 411 patients aged 41-43 years who received blastocyst transfers reported higher LBR and MPR after fresh DET, compared to fresh eSET (19.3% vs. 26.5% and 0 vs. 17.5%, respectively, for SET vs. DET) ([Tannus et al., 2017](#)). However, when this retrospective analysis also included subsequent FET, the cLBR was similar after eSET and DET (28.0% vs. 31.1%, OR 1.7; 95%CI 0.9-3.4) while the cumulative MBR was lower in the eSET group (0 vs. 14.9%, for eSET vs. DET).

A large prospective study using the UK Human Fertilisation and Embryology Authority data investigated perinatal outcomes after ART depending on the number of embryos transferred in relation to maternal age ([Lawlor and Nelson, 2012](#)). In total, 124,148 ART cycles resulting in 33,514 births were included in the analysis. LBR per cycle was higher after DET than after SET in all age categories (OR 3.1; 95%CI 2.6-3.8 for <40 years; OR 2.3; 95%CI 2.2-2.5 for ≥40 years). MBR was higher after DET but the odds of multiple birth were lower in women ≥40 years old than in younger patients (OR 20.6; 95%CI 14.1-29.9 for <40 years; OR 4.3; 95%CI 1.6-11.9 for ≥40 years). The same pattern was observed for preterm birth (<37 weeks) (OR 2.3; 95%CI 1.9-2.7 vs. OR 1.3; 95%CI 0.7-2.2, for <40 years vs. ≥40 years, respectively) and severe preterm birth (<33 weeks) (OR 2.3; 95%CI 1.7-3.2 vs. OR 1.0; 95%CI 0.4-2.9, for <40 years vs. ≥40 years, respectively).

A retrospective study evaluated 1104 FET cycles of grade BB or lower quality blastocysts ([Arab et al., 2020](#)). In patients <39 years old (n=744 SET, n=74 DET), LBR was similar (20.2% vs. 12.2%, for SET vs. DET), whereas MPR was significantly higher after DET (1.6% vs. 6.7%, for SET vs. DET). In the group of patients ≥40 years old (n=63 SET, n=34 DET), no significant differences were found in LBR (6.3% vs. 0, for SET vs. DET) or MPR (0 vs. 0).

A retrospective study reporting eSET from 464 clinics in the United States during 2013 found no significant differences in LBR following eSET in patients <35 years vs. 35-37 years old ([Mancuso et al., 2016](#)). DET was associated with higher LBRs than eSET, particularly in patients aged 35-37 years and when more embryos were available from the cycle. MPR was lower for

eSET compared with DET (1.7% vs. 39.4%, for eSET vs. DET respectively for patient aged >35 years and 1.7% vs. 32.0% for SET vs. DET respectively, for patients aged 35-37 years).

Table 3 Effects of age categories on ART outcomes following eSET and DET.

Outcome	Study	Type of study	Age categories			
			<35y	35-37y	38-39y	≥40y
LBR	(Ma et al., 2022)	Systematic review	Higher in DET	Higher in DET	Higher in DET	No difference
	(Arab et al., 2020)	Observational study	No difference	No difference	No difference	No difference
	(Mancuso et al., 2016)	Observational study	No difference	Higher in DET		
	(Lawlor and Nelson, 2012)	Observational study	Higher in DET	Higher in DET	Higher in DET	Higher in DET
	(Veleva et al., 2006)	Observational study		No difference*	No difference	
	(Tannus et al., 2017)	Observational study				Higher in DET
	(Niinimaki et al., 2013)	Observational study				No difference
cLBR	(Kamath et al., 2020)	Systematic review	No difference**			
	(Mejia et al., 2021)	Observational study	Higher in eSET	Higher in eSET	No difference	No difference***
	(Veleva et al., 2006)	Observational study		Higher in eSET *	Higher in eSET	
	(Tannus et al., 2017)	Observational study				No difference
	(Niinimaki et al., 2013)	Observational study				Higher in eSET
MPR	(Ma et al., 2022)	Systematic review	Higher in DET	Higher in DET	Higher in DET	No difference
	(Kamath et al., 2020)	Systematic review	Higher in DET **			
	(Mejia et al., 2021)	Observational study	Higher in DET	Higher in DET	Higher in DET	No difference
	(Arab et al., 2020)	Observational study	Higher in DET	Higher in DET	Higher in DET *	No difference
	(Mancuso et al., 2016)	Observational study	Higher in DET	Higher in DET		
	(Lawlor and Nelson, 2012)	Observational study	Higher in DET	Higher in DET	Higher in DET	No difference
	(Veleva et al., 2006)	Observational study		Higher in DET *	Higher in DET	
	(Tannus et al., 2017)	Observational study				Higher in DET
	(Niinimaki et al., 2013)	Observational study				Higher in DET
Preterm birth	(Ma et al., 2022)	Systematic review	Higher in DET	Higher in DET	Higher in DET	Higher in DET
	(Mejia et al., 2021)	Observational study	Higher in DET	Higher in DET	Higher in DET	Higher in DET
	(Lawlor and Nelson, 2012)	Observational study	Higher in DET	Higher in DET	Higher in DET	No difference
Perinatal mortality	(Mejia et al., 2021)	Observational study	Higher in DET	Higher in DET	Higher in DET	Higher in DET
	(Lawlor and Nelson, 2012)	Observational study	Higher in DET	Higher in DET	Higher in DET	No difference

\* 36-37y; \*\* <36y; \*\*\* >40y.

### Recommendation

The decision to perform DET instead of eSET should not be based on female age.	Strong	⊕⊕○○
Women aged less than 38 years should receive eSET.	Strong	⊕⊕⊕○
Women aged 38 years or more should receive eSET.	Strong	⊕○○○

### Justification

It has been suggested that the efficacy of ART might increase with DET in older women ([Scotland et al., 2011](#)). Available evidence does not confirm this hypothesis even though current evidence from RCTs regarding women of 38 years of age or older is scarce [see [annex 2 – sof table 2&3](#)]. Moreover, several observational studies suggest that results in women aged 38 years or older are similar to observations in younger patients (see [table 3](#)): while LBR in the first transfer cycle can be higher after DET, cLBR is not higher after DET. Observational studies should be interpreted with caution, as we cannot exclude the possibility of an overrepresentation of poor prognosis patients in the SET or DET group, leading to bias. However, studies also point toward higher rates of multiple pregnancies and obstetric complications such as preterm birth and perinatal mortality after DET in all age groups, except for women aged  $\geq 40$  years, for which MPRs can be similar after DET and after eSET.

It is worth noting that conducting an RCT based on female age is difficult to envisage. According to calculations presented in one study ([Lawlor and Nelson, 2012](#)), the sample size required for an RCT to detect a difference in the numbers needed to treat with eSET versus DET of at least five for preterm birth and low birth weight, comparing women younger than 40 years with those aged 40 years or older, is 140,000 patients. This is a daunting task; moreover, at least two RCTs were stopped prematurely because of strong patient preferences for a specific number of embryos to transfer ([McLernon et al., 2010](#)).

Advanced maternal age is associated with increased obstetric risks including preterm birth, low birth weight, hypertensive disorders, stillbirth, and caesarean delivery ([Jacobsson et al., 2004](#), [Kenny et al., 2013](#), [Lean et al., 2017](#), [Reddy et al., 2006](#), [Yogev et al., 2010](#)). As these risks are even more aggravated in multiple pregnancies, avoidance of multiple pregnancies is even more important in this group than in younger groups of patients. These specific health risks should be evaluated and considered at the time of planning ART treatment. A discussion with the patient during the planning stage is also recommended, please see the chapter on patient counselling (Part F).

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## V. Ovarian response

The term ovarian response to gonadotropin stimulation for ART describes immediate outcomes from ovarian stimulation such as numbers of follicles and retrieved oocytes. Different factors have historically been used to define ovarian response to gonadotropin stimulation, such as female age ([Templeton et al., 1996](#)), number of oocytes retrieved ([Surrey and Schoolcraft, 2000](#), [Veleva et al., 2005](#)), antral follicle count ([Bancsi et al., 2002](#), [Broer et al., 2013a](#), [Broer et al., 2013b](#)), anti-Mullerian hormone levels ([Broer et al., 2013a](#), [Broer et al., 2013b](#), [Oehninger et al., 2015](#)), early follicular phase follicle stimulating hormone (FSH) levels ([Arce et al., 2013](#), [Soldevila et al., 2007](#)), E2 levels ([Broekmans et al., 2006](#)), mean daily gonadotropin dose ([Shaker et al., 1992](#)), and total gonadotropin dose ([Faber et al., 1998](#)).

Classifying the patients into separate groups according to ovarian response is important for predicting the success of treatment cycle. Low responders are expected to have low pregnancy and LBRs whereas high responders are at risk of developing the potentially life-threatening ovarian hyperstimulation syndrome (OHSS) ([The ESHRE Guideline Group on Ovarian Stimulation, 2020](#)).

Definitions for low, normal and high ovarian response used in the present guideline are the ones that were detailed in ESHRE Guideline on ovarian stimulation ([The ESHRE Guideline Group on Ovarian Stimulation, 2020](#)). A low ovarian response is observed when there are  $\leq 3$  follicles of  $\geq 11$  mm on the day of trigger and/or  $\leq 3$  oocytes retrieved with conventional stimulation. A high ovarian response is observed when, with conventional stimulation, there are  $> 18$  follicles on the day of trigger and/or  $> 18$  oocytes retrieved. Normal response is between low and high responses and is defined by 4-18 follicles and/or oocytes retrieved with conventional stimulation. Conventional stimulation is defined as ovarian stimulation with daily gonadotropin doses of 150-225 IU with long or antagonist protocols ([The ESHRE Guideline Group on Ovarian Stimulation, 2020](#)).

***Key question. Should ovarian response (i.e., low, normal or high) be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, what is the appropriate transfer strategy for low, normal or high responders?***

### *Evidence*

Only one RCT examined outcomes in eSET and DET patients ([Moustafa et al., 2008](#)) that can be classified as normal responders. In this RCT, 40 and 41 patients had eSET and DET, respectively.

SET and DET patients had similar numbers of oocytes retrieved (9.8±2.5 vs. 10.2±2.3; non-significant (NS)). cLBR was similar in the two groups (45.0% vs. 46.3%, for SET and DET, respectively). Cumulatively, there were no multiple pregnancies in the eSET group versus 8 (14.0%) in the DET group. The study did not, however, compare outcomes in low or high responder patients and therefore could not answer the question on whether ovarian response should be considered a factor in deciding to apply DET instead of SET.

The GDG found no scientific evidence for patients with low, or with high ovarian response.

#### Recommendation

For normal responders, eSET is recommended.	Strong ⊕○○○
The GDG recommends eSET in patients with low or high ovarian response.	GPP

#### Justification

*There is very low-quality evidence from only one quasi-randomised controlled trial showing cLBRs after eSET and DET in normal responders. No evidence for high and low responders with regards to eSET vs. DET was identified. However, high responders are at risk of OHSS (The ESHRE Guideline Group on Ovarian Stimulation, 2020). Careful pre-treatment evaluation of risk factors, selection of the most appropriate stimulation protocol together with strict monitoring are essential part of ART treatment for patients at risk of OHSS. While freeze-all is the current standard of care in most cases at risk of OHSS, some patients proceed to embryo transfer despite these risks. However, late-onset OHSS is more frequent in multiple pregnancies (De Leener et al., 2006) and therefore, in order to minimise this type of OHSS, the GDG recommends that no more than one embryo be transferred in all high responders having a fresh ET.*

*Low responders have only a few embryos, many of which cannot be classified as good-quality ones. The GDG recommendation for low responders is based on the evidence for cleavage-stage and blastocyst transfer with fresh and frozen-thawed embryos, that can be found in these respective chapters: D.I and D.II.*

#### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## VI. Criteria related to the endometrium

### VI.1. Criteria related to the endometrium in a fresh cycle

Controversy continues regarding whether endometrial assessment in ART is clinically beneficial or not ([Craciunas et al., 2019](#), [Shakerian et al., 2021](#)). Nonetheless, given the recent widespread interest in novel markers of endometrial receptivity and since at least endometrial thickness measurement is routinely performed in most treatment centres, the GDG decided to assess whether any endometrial characteristics (e.g., thickness, morphology, previous endometrial receptivity biomarker with an abnormal result, presence of intracavitary fluid/adhesions, amongst others) could be an important finding to opt for DET instead of eSET.

**Key question.** *In a fresh cycle, should endometrial criteria be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, what is the appropriate cut off?*

#### *Evidence*

A retrospective study included 2478 patients who performed a fresh embryo transfer and attempted to evaluate whether multiple clinical or laboratorial criteria could predict a reduced risk of twin live birth following DET at cleavage-stage ([Huang et al., 2020](#)). The overall LBR per transfer was 62.0%, while the twin LBR was 39.0%. Beyond female age, the number of top-quality embryos and the number of previous embryo transfers, endometrial thickness also seemed to be associated with twin LBRs. Specifically, a 1 mm increase in endometrial thickness was associated with an increased risk of twinning (OR 1.4; 95%CI 1.1-1.7). That said, twin rate remained very high (above 20.0%) even in cycles with an endometrial thickness below 7 mm.

The GDG found no evidence of specific endometrium criteria related to the decision to transfer one vs. two embryos in donor cycles.

#### *Recommendation*

<b>The decision to perform DET instead of eSET in fresh embryo transfer cycles should not be based on endometrial characteristics.</b>	<b>Strong</b> ⊕○○○
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#### *Justification*

*Only low-quality evidence retrieved from a single study potentially relating endometrial thickness to twin LBRs after fresh cleavage stage DET was found. No other evidence could be found in the blastocyst stage setting or regarding other endometrium criteria. The GDG considered that, in the absence of more evidence, the currently most acceptable approach following a diagnosis of an abnormal endometrial criteria should be further evaluation and not*

DET. Promoting DET in the presence of abnormal endometrial characteristics, especially if potentially reversible, may even be counter-intuitive, since it reduces the chance of at least one of the embryos available ultimately interacting with an endometrium which may be more favourable for conception (Liao et al., 2021).

*Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

## VI.2. Criteria related to the endometrium in a frozen-thawed embryo transfer cycle

**Key question.** *In FET, should endometrial characteristics be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART (with own gametes or with donated oocytes/embryos) (hormonal substitution vs. ovulatory cycle)? If yes, what is the appropriate cut off?*

*Evidence*

A retrospective study including 768 hormonal-substituted FET cycles sought out to evaluate whether endometrial thickness could predict pregnancy outcomes after FET with one or several day 2-3 embryos, comparing two subgroups of endometrial thickness (those with 7-8 mm versus cycles with 9-14 mm) (El-Toukhy et al., 2008). A sensitivity analysis was performed in patients who underwent SET (42 cycles versus 41 cycles, respectively) and, in those with an endometrial thickness of 7-8 mm, the CPRs seemed numerically reduced (9.5% versus 19.5%). However, the difference in this small sample was not statistically significant and, most importantly, MPRs were never reported throughout the study.

There is no evidence supporting the application of DET instead of SET using either endometrial thickness or other endometrium characteristics as factor in the decision-making process in FET.

*Recommendation*

<b>The decision to perform DET instead of eSET in frozen embryo transfer cycles should not be based on endometrial characteristics.</b>	<b>Strong</b> ⊕○○○
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*Justification*

No evidence could be found assessing both efficiency and safety when deciding between SET versus DET using either endometrial thickness or other endometrial characteristics. The justification of the recommendation in FET cycle is the same as for fresh embryo transfer cycle (refer to PICO 7- justification).

*Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## VII. Treatments with donor oocytes and donated embryos

*Key question. Should a different embryo transfer strategy be applied for patients undergoing ART with donor oocytes and donated embryos?*

Current evidence indicates that use of donor oocytes should be considered an independent risk factor for complications during pregnancy, even in recipients that are young and healthy, as indicated by a prospective controlled study ([Rodriguez-Wallberg et al., 2019](#)). Compared with pregnancies resulting from autologous ART, pregnancies resulting from donor oocyte cycles are characterised by higher risks of hypertensive disease, premature delivery, caesarean section and small for gestational age babies, ([Jeve et al., 2016](#)).

### *Evidence*

#### *ART with donor oocytes*

Pregnancy risks and complications have been found to be higher after the use of donor oocytes, compared to pregnancies using autologous oocytes. In a systematic review including 11 observational studies, the risk of hypertensive complications found was significantly higher (OR 3.9; 95%CI 3.2-4.8) in pregnancies with donor oocytes compared with homologous oocytes, and excess risk was found both in singleton pregnancies and twin pregnancies (OR 3.1; 95%CI 2.19-4.24 and OR 3.6; 95%CI 2.6-5.2, respectively) ([Jeve et al., 2016](#)). A subgroup analysis of two studies reporting on women older than 40 years, the risk for hypertensive disorders was significantly higher with donor oocytes pregnancy compared to autologous oocytes IVF pregnancy (23% (30/129) vs 10% (18/168); OR 2.33; 95%CI 1.21-4.49; two studies). The meta-regression for the covariate of age showed that the occurrence of hypertensive disorders in the studies was independent of age (95%CI 0.9-1.1). Secondary outcomes including small for gestational age (OR 1.8; 95%CI 1.3-2.6), caesarean section (OR 2.7; 95%CI 2.2-3.3) and pre-term delivery risk (OR 1.3; 95%CI 1.08-1.66) were also significantly higher in donor oocyte pregnancies.

A pilot RCT considered a total of 65 oocyte recipients with at least two good-quality embryos on day 3 ( $\geq 6$  blastomeres,  $< 10\%$  fragmentation) obtained from donor oocytes. Patients were randomly allocated to eSET (n=34) or DET (n=31) ([Clua et al., 2015](#)). LBR was similar in the two groups (44.1% vs. 54.8% for eSET vs. DET) but the twin pregnancy rate was much higher in DET group compared to eSET group (0% vs. 47.7%, for eSET vs. DET). This study was stopped prematurely due to risks associated with elevated twin pregnancy rate.

The retrospective observational study of Arab et al. included data collected between January 2008 and December 2019 on day 5/6 vitrified-warmed blastocyst transfers (n=153) in donor oocyte patients ([Arab et al., 2020](#)). Poor-quality blastocysts had grading  $\leq 3BB$ . The study reported no differences in LBR after poor-quality embryo SET (n=126) or DET (n=27) (11.7% vs. 22.2%, for SET vs. DET). No differences in MPR were reported between SET and DET (3.1% vs. 7.4%, for SET vs. DET).

Outcomes of 27,033 first treatments with fresh donor oocytes in patients aged 18-59 years were analysed in a retrospective study of ART cycles performed in the United States during 2004-2013 ([Mersereau et al., 2017](#)). For blastocyst transfers where there were  $\geq 1$  embryo available for cryopreservation, the LBR did not significantly differ following DET when compared to SET (56.1% vs. 66.6% for SET vs. DET). The MPR was lower with SET cycles compared to DET cycles (<2% vs. 49%, for SET vs. DET). After a cleavage-stage embryo transfer with  $\geq 1$  embryo available for cryopreservation, LBR was lower in SET compared to DET (38.5% vs. 53.1% for SET vs. DET). The MPR was higher in DET cycles (0% vs. 35.3% for SET vs. DET). There was little to no effect of maternal (recipient) age on LBR and MPR in these oocyte donor cycles. The retrospective study by Fishel and colleagues evaluated also donor oocyte cycles in women aged >38 years (n=2296), in which the transfer of one embryo selected by objective morphokinetic algorithms (TL embryos) was compared to the transfer of two embryos selected according to conventional embryology selection parameters and developed in standard incubator (standard embryos) ([Fishel et al., 2017](#)). No difference in LBR was observed after SET with a TL embryo and DET with a standard embryo (OR 0.98, 95%CI 0.6-1.8). Increased rates of multiple births by about 30 to 40% were observed after DET compared to SET regardless of age, incubator type, and embryo stage.

An investigation was performed by the Society for Assisted Reproductive Technology (SART) on U.S. treatments with donated oocytes from donors younger than 35 years and with recipients aged 41-42 years ([Acharya et al., 2016](#)). This analysis revealed that in cleavage-stage transfer cycles (n=2787), PR (55.0% vs. 41.4%) and LBR (45.8% vs. 33.3%) were higher after DET, compared with (e)SET. Twin pregnancy (33.0% vs. 1.1%) and HOM rates (0.7% vs. 0%) were also higher after DET. Concerning treatments with blastocysts (n=10,236), (e)SET was associated with lower PR (65.0% vs. 72.7%), LBR (53.5% vs. 63.1%), MBR (1.7% vs. 52.8%) and HOM rate (0 vs. 2.5%), compared with transfer of 2-6 blastocysts.



### ART with donated embryos

The current evidence on pregnancy outcome after embryo donation is scarce, as only few studies have been performed and the practice of embryo donation is relatively recent in many European countries ([Peigné et al., 2023](#)). A retrospective cohort study from six clinics in France compared the outcome of 73 singleton pregnancies with donated embryos and 136 singleton pregnancies after autologous FET. Most hypertensive disorders of pregnancy observed in the embryo donation group were severe, and they were more common after embryo donation than after autologous FETs (OR 2.1; 95%CI 1.1-4.0). Caesarean sections were also more frequent in the embryo donation group (47.3 vs. 29.2%, respectively).

### Recommendation

Only eSET should be practiced for patients undergoing ART with donor oocytes.	Strong	⊕⊕⊕○
Only eSET should be practiced for patients undergoing ART with donated embryos.	Strong	⊕○○○

### Justification

*Multiple pregnancies may increase the already high pregnancy risks and complications in pregnancies achieved through donor oocytes/embryos, compared to pregnancies using autologous oocytes. Furthermore, there is strong evidence to recommend that patients undergoing ART with donor oocytes or with donated embryos should receive eSET regardless of recipient age. In fresh cleavage-stage ET in donor oocyte cycles, decision for DET instead of eSET should not be based on embryo criteria. In vitrified-warmed blastocyst transfer donor cycles, SET should be applied regardless of the quality of the blastocyst. TL imaging-derived parameters for embryo selection should not be considered a factor to apply DET instead of eSET in donor cycles.*

*There is lack of published evidence regarding treatments with donated embryos. However, until such data is available, the GDG considered that the transfer of donated embryos should be conservatively deemed comparable to embryos created with donated oocytes only in terms of prognosis for the recipient. Therefore, suggesting SET for treatments involving donated embryos appeared to be the most rational choice.*

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## VIII. Gestational carriers

A population-based study found that in gestational carrier pregnancies, multifoetal gestation was associated with significantly increased odds of preterm birth, caesarean section, and neonatal morbidity but not with an increased risk of other obstetric morbidities, compared to singleton gestation ([Swanson et al., 2021](#)). Nearly two decades ago, the ESHRE task force on ethics and law strongly recommended that only one embryo should be transferred in order to prevent multiple pregnancies and to avoid unnecessary endangerment of the surrogate's and the future child's health ([Shenfield et al., 2005](#)).

### *Key question. Should a different embryo transfer strategy be applied for gestational carriers?*

#### *Evidence*

At present there are no randomised studies reporting on pregnancy outcomes following eSET and DET in gestational carriers. The first Australian nationwide cohort study reported on 360 treatments on gestational carriers in 2004-2011 ([Wang et al., 2016](#)). Of these, 68.9% received SET and 30.5% received DET. LBR was not different after SET or DET (19.0% vs. 19.1%, respectively) while MPR and the preterm birth rates were higher in DET cycles (0% vs. 22.7% and 12.8% vs. 30.8%, for SET vs. DET).

A retrospective cohort study reported outcomes of 583 FET cycles with vitrified high-grade blastocysts (grade BB or higher) to gestational carriers, with 427 SETs and 156 DETs ([Namath et al., 2021](#)). The overall LBR was lower in the SET group (36.8% vs. 51.3%,  $p < 0.001$ ). The MPR, the preterm birth rate and the very preterm birth rate were increased in the DET group (MPR: 1.9% vs. 20.0%, Preterm birth: 13.4% vs. 40.0%,  $< 0.001$  and very preterm birth: 0.6% vs. 6.3%, NS, for SET vs. DET).

#### *Recommendation*

Only eSET should be practised for gestational carriers.	Strong ⊕○○○
The GDG recommends that both gestational carriers and intended parents be counselled that DET is associated with greater risk of pregnancy and perinatal complications in surrogate pregnancies.	GPP

### *Justification*

*Increased MPR and preterm birth rate were observed in the group receiving DET. The data are comparable to high risks observed using donor oocytes. Transferring one embryo minimises those risks and should therefore be strongly recommended.*

### *Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## Part D: Criteria related to the embryo

In their search for the embryo with the highest implantation potential, embryologists can apply invasive and/or non-invasive assessment techniques. Of the latter group, morphological scoring at different stages of preimplantation development is the most practiced approach to evaluate the embryo's developmental quality ([Ebner et al., 2003](#)).

In 2011, an embryo grading system for cleavage-stage embryos and blastocysts was introduced in the Istanbul consensus ([Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011](#)). According to this grading system, a cleavage-stage embryo is categorised as good quality at day 2 (44±1 h post-insemination) by having four equally sized mononucleated blastomeres in a three-dimensional tetrahedral arrangement, with <10% fragmentation. Similarly, a good-quality day 3 embryo (68±1 h post-insemination) should have eight equally sized mononucleated blastomeres with <10% fragmentation.

In the case of blastocysts (116±2 h post-insemination), “an optimal embryo at this developmental stage will be fully expanded through to hatched blastocyst with an inner cell mass (ICM) that is prominent, easily discernible and consisting of many cells, with the cells compacted and tightly adhered together, and with a trophectoderm (TE) that comprises many cells forming a cohesive epithelium” ([Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group Embryology, 2011](#)). This grading system for blastocysts was adapted from the original Gardner scale. In this scale the embryo score results from the degree of expansion (scores 1-6) as well as the ICM (grades A, B and C) and TE (grades A, B and C) ([Gardner and Schoolcraft, 1999](#)).

A recent Cochrane review of 32 RCTs including 5821 couples/individuals showed that the LBR per fresh ET was higher in the blastocyst transfer group (OR 1.3; 95%CI 1.1-1.5; 15 studies, 2219 women, low-quality evidence) ([Glujovsky et al., 2022](#)). The subanalysis of studies comparing the transfer of equal number of embryos including SET, showed that there is no difference between the transfer of cleavage-stage embryos or blastocysts in terms of LBR (OR 1.2; 95%CI 0.99-1.5; 9 studies, 1736 women). It must be noted that the cLBR was not reported in most studies included in this meta-analysis and therefore the higher LBR after fresh blastocyst transfer possibly does not translate into a higher cLBR. Moreover, the authors

reported that blastocyst transfer probably increases the MPR when only high-quality studies were considered (OR 1.3; 95%CI 1.0-1.7, 14 studies, 3399 women).

While the aim of the GDG was to focus on studies using the Istanbul consensus criteria, not all studies included in this chapter applied these criteria to assess embryo morphology. Whenever another scoring system was used, the system or criteria used are indicated.

This guideline has taken into consideration the embryo criteria separately for cleavage-stage embryos and blastocysts, in both fresh and FET.

Another factor to consider is the risk of monozygotic twinning (MZT), which can result from SET, regardless of grading or whether it is transferred at the cleavage-stage or blastocyst stage ([Hviid et al., 2018](#)). MZT is a rare event, with an incidence of 1.6% after blastocyst transfer and 0.4% after cleavage-stage ET, according to a meta-analysis ([Chang et al., 2009](#)). One study has shown that the chance of MZT is not affected by the number of embryos transferred, zona manipulation, or whether the embryo has been cryopreserved ([Liu et al., 2018](#)). A separate meta-analysis supported the view that zona manipulation techniques do not increase the MZT rate ([Hviid et al., 2018](#)). Two meta-analyses have highlighted that a younger maternal age may increase the incidence of MZT ([Busnelli et al., 2019](#), [Hviid et al., 2018](#)).

## I. Fresh embryo transfer

### I.1. Cleavage-stage

*Key question. In fresh transfer, should embryo criteria be considered a factor in deciding to apply DET instead of (e)SET at cleavage-stage for couples/individuals undergoing ART? If yes, which criteria are appropriate?*

Evidence on cleavage-stage embryo fresh cycles is mostly based on SET and not on eSET because in most of the studies, the only available embryo was transferred.

#### *Evidence*

An RCT trial was performed with 144 patients having  $\geq 4$  good-quality embryos (<20% fragmentation and even sized blastomeres at day 2) during their first fresh cycle. Patients were randomly assigned to eSET (n=74) or DET (n=70) ([Martikainen et al., 2001](#)). LBR per cycle was lower in the eSET group (29.7% vs. 40.0%, for eSET vs. DET). The MPR and the preterm birth rate were higher in the DET group (MPR: 5.0% vs. 39.0%; Preterm birth rate: 5.0% vs. 21.0%,

for eSET vs. DET). The number of preterm deliveries (gestation age <37 weeks) was six (21.0%) after DET and one (5.0%) after eSET.

A second RCT included 661 patients <36 years old, undergoing their first or second ART cycle with at least two good-quality embryos (<20% fragmentation, 4-6 cells at day 2, 6-10 cells at day 3). Patients were randomised to eSET (n=330) or DET (n=331) ([Thurin et al., 2004](#)). In the fresh cycle, LBR was significantly lower in the eSET group compared to the DET group (27.6% vs. 42.9%, for eSET vs. DET). The MPR was significantly lower in the eSET group compared to the DET group (0.8% vs. 33.1%, for eSET vs. DET).

A prospective cohort study was performed involving 130 eSET couples and 130 DET couples, undergoing their first cycles and matched according to female age and the numbers of embryos available, where the patient was aged <38 years old and there were ≥2 good-quality embryos (6-8 regular-sized blastomeres, <20% fragmentation), ([Le Lannou et al., 2006](#)). No difference in LBR was detected between the eSET and DET groups (26.1% vs. 31.5%). The MPR was higher in the DET couples (0% vs. 37.0%, for eSET vs. DET).

A prospective non-randomised study showed that in 53 cycles where eSET was performed, the transfers of fresh top-quality embryos resulted in 22 live births (41.5%) while in 98 DET cycles, 41 live births (41.8%) were obtained ([Fauque et al., 2010](#)). The cLBR was not significantly higher in the eSET group compared to the DET group (54.7% vs. 49.0%, respectively). The twin pregnancy rate was higher in the DET compared to SET group (0% vs. 41.5%, for SET vs. DET, respectively). Moreover, while there were no cases with preterm birth and a low Apgar score (0-6 at 1 min) in the eSET group, such complications were observed in one-fifth of the twin deliveries in DET group (0% vs. 20.6%, for eSET vs. DET). Perinatal mortality rates did not differ between the two groups.

In a retrospective analysis, SET and DET were performed in 83 PCOS patients and 76 PCOS patients undergoing an *in vitro* maturation, respectively ([Hatirnaz et al., 2016](#)). The embryos were graded as follows: grade 1= embryo with blastomeres of equal size, no cytoplasmic fragments (2.4% vs. 0.7%; for SET vs. DET); grade 2= embryo with blastomeres of equal size, minor cytoplasmic fragments, or blebs (61.5% vs. 78.3% for SET vs. DET); grade 3= embryo with blastomeres of distinctly unequal size, none or few cytoplasmic fragments (36.1% vs. 21.1%;

for SET vs. DET). The SET and DET groups had similar embryo quality and similar LBR (34.9% vs. 34.2%; for SET vs. DET, OR 1.2; 95%CI 0.4-3.8) after adjusting for confounding factors as female age, infertility duration, number of prior ART cycles and number of Metaphase II oocytes. However, the twin pregnancy rates were significantly higher in the DET group (2.4% vs. 9.2%; for SET vs. DET). Perinatal death rate was the only complication reported and was not different between groups (5.4% vs. 5.9%; for SET vs. DET).

In a retrospective study, patients were divided into three groups: 324 patients had DET with two good-quality embryos (group A), 127 had DET with one poor-quality embryo (group B), and 887 had SET with a good-quality embryo (group C) (Aldemir et al., 2020). LBRs were similar in all groups (27.5, 26.8 and 24.5% in group A, B and C respectively). MPR and preterm birth rate were significantly higher in group A and group B (MPR: 22.8, 13.0 and 3.4% in group A, B and C respectively (Preterm birth rate: 7.0, 7.1 and 3.6% in group A, B and C respectively).

Table 4 Effects of the transfer of fresh cleavage-stage embryo(s) of good and/or poor quality on outcomes of ART in eSET and DET patients in RCTs and observational studies – autologous cycles.

Fresh cleavage-stage embryo transfer					
Study	Type of study	Outcome (%)	Type of transfer		
			GQE-DET	MQE-DET*	GQE-SET
(Martikainen et al., 2001)	RCT	LBR	40.0	-	29.7
		MPR	39.0	-	5.0 <sup>a</sup>
(Thurin et al., 2004)	RCT	LBR	42.9	-	27.6 <sup>a</sup>
		MPR	33.1	-	0.8 <sup>a</sup>
(Le Lannou et al., 2006)	Observational study	LBR	31.5	-	26.1
		MPR	37.0	-	0 <sup>a</sup>
(Fauque et al., 2010)	Observational study	LBR	49.0	-	54.7
		MPR	41.7	-	0 <sup>a</sup>
(Hatirnaz et al., 2016)	Observational study	LBR	34.9	-	34.2
		MPR	9.2	-	2.4 <sup>a</sup>
(Aldemir et al., 2020)	Observational study	LBR	27.5	26.8	24.5
		MPR	7.0	7.1	3.6 <sup>a,b</sup>

GQE: good-quality embryo(s) only; MQE: embryos of mixed quality, i.e. good- and poor-quality.  
a. There was a significant difference compared to GQE-DET; b. There was a significant difference compared to MQE-DET



## Recommendation

In fresh cleavage-stage embryo transfer cycles, the decision to perform DET instead of eSET should not be based on embryo criteria.

Strong

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## Justification

The evidence assessed failed to show an increase of LBR following DET as compared to SET when embryos with similar quality are transferred in a fresh cycle [see **annex 2** – *sof table 4*]. Moreover, if embryo quality is not taken into account, transferring two cleavage-stage embryos in fresh cycles led to a higher LBR at the cost of a substantial increase in the risk of MPR.

## Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## 1.2. Blastocyst stage

*Key question. In fresh embryo transfer at blastocyst stage, should embryo criteria be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, which criteria are appropriate?*

It has been hypothesised that each blastocyst developing in vitro has a minimal implantation potential, but that viability may vary from blastocyst to blastocyst (Gardner et al., 1998). The potential to predict live birth is strongly dependent on blastocyst morphology, e.g., on the grade of expansion as well as on the quality ICM and TE (Ahlström et al., 2011, Subira et al., 2016, Thompson et al., 2013).

Furthermore, blastocyst transfer has been associated with significantly higher MZT rates compared to cleavage-stage transfer (6.8% vs. 0%, respectively) (Wang et al., 2019). In this context, several reports of HOM pregnancies have been published after SET (Sutherland et al., 2019, Woolnough et al., 2021) and DET (Schlueter et al., 2018).

### *Evidence*

Several observational studies and one RCT have dealt with the comparison of single and double blastocyst transfer, both elective and compulsory, without specific morphology evaluation (Eum et al., 2016, Friedman et al., 2011, Gardner et al., 2004, Mullin et al., 2012, Tannus et al., 2017). In three of these studies (Eum et al., 2016, Friedman et al., 2011, Mullin et al., 2012) a difference in CPR or LBR between (e)SET and DET was not observed, while in the remaining two analyses, DET was associated with higher CPR and LBR (Gardner et al., 2004, Tannus et al., 2017). All studies reported significantly higher MPR in DET as compared to eSET.

Four studies evaluated the morphology of all transferred blastocysts among other parameters. One RCT included 100 patients who had at least two good-quality blastocysts (quality 3BB or better) on day 5 (Abuzeid et al., 2017). Patients were randomised to eSET (n= 50) or DET (n= 50). The trial reported significantly higher LBR in the DET arm as compared to the eSET arm (49.0% vs. 70.0%; for eSET vs. DET). However, the MPR was also higher in the DET group (0% vs. 35.0%; for eSET vs. DET).

A retrospective investigation sought to find out whether the transfer of a good-quality blastocyst together with a poor-quality blastocyst would result in an outcome that is intermediary between DET and SET of only good-quality blastocysts (Aldemir et al., 2020). A

good-quality blastocyst was defined by grade 3BB or higher. Patients were divided into three groups: 174 patients had DET with two good-quality blastocysts (group A), 52 had mixed DET with one good- and one poor-quality blastocyst (group B), and 734 had (e)SET with a good-quality blastocyst (group C). The study reported that the LBR was significantly higher in group A compared to group B (40.2% vs. 19.2%) but not to group C (31.9%). MPRs were significantly higher in both DET groups (A, B) compared to the eSET group C (32.7% and 28.6%, respectively vs. 2.6% in group C). Preterm birth rates were similar in all three groups.

The outcomes following mixed-quality DET was further investigated in the study of Hill and co-workers ([Hill et al., 2020](#)). They conducted a retrospective study aimed to evaluate if transferring a second poor-/fair-quality morula/blastocyst together with a good-quality blastocyst negatively affects live birth. The quality of the ICM and TE was used to distinguish between good (AA, AB), fair (BA, BB, BC) and poor (CC, CB) blastocyst quality. Where the second embryo was an early blastocyst or a morula, no such grading could be performed. In this 3-year study, 889 mixed-quality DETs were compared with 3,751 SET of good quality. The primary analysis on cases with no supernumerary embryos to vitrify revealed that the LBR was higher when adding a second lower-quality embryo for transfer (44.0% vs. 50.0%, for SET and mixed-quality DET, respectively; OR 1.28; 95%CI 1.28–1.9). The MPR was also higher, being 1.0% in SET compared to 16.0% in mixed-quality DET cycles. The authors further elaborated on the developmental stage of the additionally transferred lower-quality embryo in several sub-analyses. Depending on whether this extra embryo was a fair-/poor-quality blastocyst or an early blastocyst (all adjusted for supernumerary embryos and female age), the associated LBRs improved from 49.0% (eSET) to 61% (fair-/poor-quality blastocyst: OR, 1.7; 95%CI 1.2-2.1) and 57% (early blastocyst: OR 1.4; 95%CI 1.1-1.6), respectively. An additional transferred morula did not increase LBR and it remained at 50% (OR 1.0; 95%CI 0.8-1.3). The MPR, however, was significantly higher in all these three subgroups with rates of respectively 27%, 22%, and 12% for the mixed-quality DET subgroups, which is of particular interest in case that the second transferred embryo was a morula which is usually associated with a lower live-birth rate if not formed before day 5 ([Shebl et al., 2021](#)).

Further evidence that a low-quality blastocyst added to a high-quality blastocyst in DET increases MPR comes from a retrospective analysis of Theodorou and co-workers ([Theodorou et al., 2021](#)). In this historical cohort study blastocysts were graded using modified Gardner

criteria. In detail, grade B for TE and ICM was further subcategorised into a B+ and a B– score based on both cell number and package. According to this, blastocysts graded as AA, AB+, AB–, B+A, B–A, B+B+ were classified as high-quality blastocysts, whereas blastocysts graded B–B+, B+B– or lower were considered low-quality blastocysts. As compared to eSET of a high-quality blastocyst only, DET of two high-quality blastocysts resulted in significantly higher LBR (51.0% vs. 61.0%; for SET vs. DET; OR 1.8; 95%CI 1.4-2.2) and MPR (1.9% vs. 42.5%; OR 49.3; 95%CI 24.7-98.3). When comparing eSET with mixed-quality DET, the difference in LBR did not reach significance (51.0% vs. 47.0%; for eSET vs. DET; OR 0.9; 95%CI 0.7-1.1), while MPR remained high (1.9% vs. 28.7%; OR 20.9; 95%CI 10.2-42.9).

The GDG found no evidence of specific fresh blastocysts embryo criteria related to the decision to transfer one vs. two embryos in cycles with donor oocytes or donated embryos.

Table 5 Effects of the transfer of fresh blastocyst (s) of good and/or poor quality on outcomes of ART in eSET and DET patients in RCTs and observational studies – autologous cycles.

Fresh blastocyst transfer							
Study	Type of study	Outcome (%)	Type of transfer				
			GQE-DET	MQE-DET	PQE-DET	GQE-(e)SET	PQE-(e)SET
(Abuzeid et al., 2017)	RCT	LBR	70.0	-	-	49.0 <sup>a</sup>	-
		MPR	35.0	-	-	0 <sup>a</sup>	-
(Aldemir et al., 2020)	Observational study	LBR	40.2	19.2 <sup>a</sup>	-	31.9	-
		MPR	32.7	28.6	-	2.6 <sup>a</sup>	-
(Hill et al., 2020)	Observational study	LBR	-	50.0	-	-	44.0 <sup>b</sup>
		MPR	-	16.0	-	-	1.0 <sup>b</sup>
(Theodorou et al., 2021)	Observational study	LBR	61.0	47.0	-	51.0 <sup>a</sup>	-
		MPR	42.5	28.7	-	1.9 <sup>a,b</sup>	-

GQE: good-quality embryo(s) only; MQE: embryos of mixed quality, i.e. good- and poor-quality; PQE: poor-quality embryo(s) only.  
a. There was a significant difference compared to GQE-DET; b. There was a significant difference compared to MQE-DET.

### Recommendation

In fresh blastocyst transfer cycles, the decision to perform DET instead of eSET should not be based on blastocyst morphology/quality.	Strong	⊕⊕⊕○
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## Justification

Good-quality evidence transferring two blastocysts in a fresh cycle is associated with higher MPR compared to SET with a good-quality blastocyst. This observation is consistent throughout studies surveyed and remained valid also for cases with transfer of one good-quality and one poor-quality blastocyst. PR and LBR can be higher, similar or lower after the transfer of two blastocysts vs. one, depending on morphologic quality. When balancing the benefit of higher LBR against the risks related to multiple pregnancy, and considering the higher risk of MZT with blastocyst transfer ([Wang et al., 2019](#)), SET is associated with better benefit/risk ratio and is the preferred strategy. Furthermore, a nationwide population-based analysis of singleton births after SET or DET in Sweden demonstrated a potentially higher risk of neonatal death (OR 2.7; 95%CI 1.3-5.6) after DET ([Rodriguez-Wallberg et al., 2023](#)).

## Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## II. Frozen-thawed embryo transfer

Over the years, the number of FET cycles in ART has increased worldwide and has become an essential part of ART treatment, increasing the cumulative LBRs ([Wyns et al., 2021](#)).

Evidence in both the sections on cleavage-stage embryo and blastocyst cycles is mainly based on SET, rather than eSET, since in most of the studies an eSET was not performed; rather, the only available embryo was transferred. Studies focusing on FET cycles that were included in this guideline show great variations of embryo quality at freezing. Thawing/warming is a procedure that can damage embryos. It has been previously shown that only 30 to 48% of embryos survive the cryopreservation process ([Guerif et al., 2002](#)). Furthermore, the development of thawed/warmed embryos might also be impaired. Fully intact frozen-thawed embryos have a higher developmental and implantation potential than embryos damaged by the cryopreservation method ([Wong et al., 2014](#)).

There is large variability with regards to the cryopreservation and thawing/warming procedures, which should be considered when comparing the results of different studies. A recent Cochrane review of 32 RCTs including 5821 couples/individuals showed that the cumulative PR was higher in blastocysts transfer when using vitrification as a method of freezing (OR 2.4; 95%CI 1.2-5.1, n= 1 RCT, moderate-quality evidence), but not when using slow freezing (OR 0.7; 95%CI 0.5-0.99; 4 RCTs, low-quality evidence) ([Glujovsky et al., 2022](#)).

Regarding the cryopreservation method of the studies included in this chapter of the guideline, in studies with cleavage-stage embryos, both slow- freezing and vitrification were used, while vitrification was applied in all studies of blastocysts. It should be noted that even if similar procedures and/or commercial kits are used, cryopreservation and thawing/warming protocols can differ among studies. Some clinics cryopreserve two embryos/blastocysts per device while other cryopreserve single embryos/blastocysts per device. Additionally, artificial shrinkage, especially in the case of blastocysts and/or assisted hatching could have been performed in combination with vitrification. Articles furthermore do not explain whether selection for warming was applied: whether embryos were randomly selected or if the best available one(s) were chosen according to pre-cryopreservation quality. This, along with use of overnight culture after thawing or warming, is expected to have an impact on outcome parameters.



All of these relevant methodological details are present in the evidence table (**annex 9**) if they were mentioned by the authors.

Further studies on vitrified-warmed treatment cycles should provide details on these parameters, including the quality criteria for vitrification and/or transfer, in the methodology section.

<p>When reporting research on vitrified-warmed treatments, the GDG recommends to include details on the minimal embryo criteria for vitrification and/or transfer as well as on the selection of devices or embryos for thawing and warming e.g., randomly picked or according to quality criteria (e.g., first embryos with the best quality were selected).</p>	<p>GPP</p>
<p>The GDG recommends to cryopreserve one embryo per device in order to facilitate the practice of SET and for traceability purposes.</p>	<p>GPP</p>

## II.1. Cryopreserved-warmed cleavage-stage

*Key question: In FET, should embryo criteria be considered a factor in deciding to apply DET instead of SET at cleavage-stage for couples/individuals undergoing ART? If yes, which criteria are appropriate?*

### *Evidence*

Different study formats were used: one cryopreserved-warmed SET vs. one cryopreserved-warmed DET, and also fresh SET and cryopreserved-warmed SET vs. one cryopreserved-warmed DET.

In a RCT performed in the period from May 2000 to October 2003, 661 patients were randomised shortly before embryo transfer to eSET (n=330) or DET (n=331) (Thurin et al., 2004). Patients were eligible for randomization if they were <36 years old, were undergoing their first or second ART cycle and had at least 2 good-quality embryos (<20% fragmentation, 4-6 cells on day 2, 6-10 cells on day 3). If there was no live birth after the fresh eSET, this was followed by the transfer of one warmed embryo, which resulted in a significantly lower MPR (0.8% vs. 33.1%, for SET vs. DET) but not a lower cumulative LBR (38.8% vs. 42.9%, for SET vs. DET), compared to fresh DET.

In an RCT, patients  $\leq 30$  years old with at least 1 good-quality embryo on day of transfer (grade I-II) were randomised to eSET (n=40) or DET(n=41) ([Moustafa et al., 2008](#)). The duration of follow-up was 1 year to allow inclusion of the outcomes of transfers of cryopreserved-warmed embryos. No difference was reported between DET and eSET in terms of cLBR and gestational age at birth. MPR was higher in DET (0% vs 14.0%, for SET vs. DET).

In a prospective, non-randomised study, a paired, case-control analysis was performed involving 130 SET and 130 DET couples, matched for the female age and the numbers of embryos available ([Le Lannou et al., 2006](#)). Inclusion criteria were: age <38 years, first cycle,  $\geq 2$  good-quality embryos (6-8 regular-sized blastomeres, <20% fragmentation). The cLBR was similar between the two groups (43.0% vs. 45.0%, for SET vs. DET), with a high percentage of twins following DET (34.0% vs. 0%).

A retrospective study analysed 775 (e)SET and 872 DET cycles ([Hydén-Granskog et al., 2005](#)). The criteria for cryopreservation were  $\geq 2$  blastomeres on day 2,  $\geq 4$  blastomeres on day 3, <50% fragmentation. Both LBR and MPR were significantly higher in DET cycles compared to eSET cycles (LBR: 19.2% vs. 25.7%; MPR:0% vs. 21.9%, for eSET vs. DET).

Another retrospective study included 420 SET and 822 DET patients ([Salumets et al., 2006](#)). Only embryos with morphology grades of 1-3A were considered suitable for cryopreservation (Grade 1: no fragmentation and equal blastomeres; Grade 2: <20% fragmentation and equal blastomeres; Grade 3A: no fragmentation and unequal blastomeres or 20-35% fragmentation, irrespective of the equality of the blastomeres). The LBR was higher in DET group compared to SET (14.3% vs. 18.7%, for SET vs. DET). LBR after FET was associated with the morphological grade of embryos transferred (LBR: 18.1% vs. 14.9% for grade 1-2 and 3A embryos respectively; OR 1.6; 95%CI 1.0-2.4).

Outcomes of 221 patients divided in three groups: 105 patients in DET group, 60 patients in cSET group (patients with only one cryopreserved embryo) and 41 patients in eSET group were reported in a retrospective study ([López-Regalado et al., 2014](#)). The inclusion criteria were: age <38 years, BMI 19-29 kg/m<sup>2</sup>, FSH  $\leq 15$  mIU/ml, first or second cycle, no pregnancy in their fresh cycles and  $\geq 2$  cryopreserved embryos A/B quality (7-9 cells, <20% fragmentation) available.

There was no difference in cLBR after two eSET cycles and one DET (34.1% vs. 30.0%, for eSET-FET vs. DET). The MPR was higher in the DET group (0% vs. 32.0%, for eSET vs. DET).

A more recent retrospective cohort study including patients who underwent their first FET included 3601 patients, of whom 1936 had SET and 1665 had DET in a cryopreserved-warmed cycle ([Racca et al., 2020](#)). Criteria for cryopreservation of embryos were:  $\geq 6$  blastomeres and  $< 20\%$  fragmentation. The LBR was similar between SET and DET (13.1% vs. 14.8%, respectively) and the MPR was higher in DET than in SET (1.9% vs. 16.7%, for SET vs. DET).

A final retrospective study analysed outcomes of 24,613 cleavage-stage FET considering different combinations according to embryo quality ([Zhu et al., 2020](#)). The authors showed an increase in LBR transferring 1 poor-quality embryo plus 1 good-quality embryo (mixed-quality DET) compared to the transfer of one good-quality embryo alone (SET-GQE) (37.5% vs. 25.6%; adjusted OR 1.3; 95%CI 1.04-1.5). However, mixed-quality DET also had a higher multiple live births compared with SET-GQE. No difference was observed in terms of LBR when 2 poor-quality embryos were transferred, compared to SET with 1 good-quality embryo (32.9% vs. 25.6%; adjusted OR 1.1; 95%CI 0.8-1.6). When only poor-quality embryos were transferred, raw data showed an increase in LBR with the transfer of two such poor-quality embryos (32.89% vs. 12.16%, in DET vs. SET).

The GDG found no evidence of specific cryopreserved cleavage-stage embryo criteria related to the decision to transfer one vs. two embryos in cycles with donor oocytes or donated embryos.

Table 6 Effects of the transfer of cryopreserved-warmed cleavage-stage embryo(s) of good and/or poor quality on outcomes of ART in eSET and DET patients in RCTs and observational studies – autologous cycles.

Cryopreserved-warmed cleavage-stage embryo transfer							
Study	Type of study	Outcome (%)	Type of transfer				
			GQE-DET	MQE-DET	PQE-DET	GQE-(e)SET	PQE-(e)SET
(Thurin et al., 2004)	RCT	cLBR	42.9	-	-	38.8	-
		MPR	33.1	-	-	0.8 <sup>a</sup>	-
(Moustafa et al., 2008)	RCT	LBR	37.5	-	-	42.9	-
		MPR	14.0	-	-	0 <sup>a</sup>	-
(Le Lannou et al., 2006)	Observational study	LBR	45.0	-	-	43.0	-
		MPR	34.0	-	-	0 <sup>a</sup>	-
(Hydén-Granskog et al., 2005)	Observational study	LBR	25.7	-	-	19.2 <sup>a</sup>	-
		MPR	21.9	-	-	0 <sup>a</sup>	-
(Salumets et al., 2006)	Observational study	LBR	18.7	-	-	14.3 <sup>a</sup>	-
		MPR	-	-	-	-	-
(López Regalado et al., 2014)	Observational study	LBR	30.0	-	-	34.1	-
		MPR	32.0	-	-	0 <sup>a</sup>	-
(Racca et al., 2020)	Observational study	LBR	14.8	-	-	13.1	-
		MPR	16.7	-	-	1.9 <sup>a</sup>	-
(Zhu et al., 2020)	Observational study	LBR	45.7 <sup>b</sup>	37.3 <sup>b</sup>	32.9	25.6	12.2 <sup>b</sup>
		MLBR	14.2	8.7 <sup>b</sup>	6.1	0.31	0

GQE: good-quality embryo(s) only; MLBR: multiple live birth rate; MQE: embryos of mixed quality, i.e., good- and poor-quality; PQE: poor-quality embryo(s) only.  
a. There was a significant difference compared to GQE- DET; b. There was a significant difference compared to SET-GQE.

### Recommendation

<p>In cryopreserved-warmed cleavage-stage embryo transfer cycles, the decision to perform DET instead of SET should not be based on embryo criteria.</p>	<p>Strong ⊕⊕⊕○</p>
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### Justification

When considering the studies comparing single versus double transfer of cryopreserved-warmed cleavage-stage embryos, the results are variable. Some, but not all studies show higher LBR with DET but all studies demonstrate higher MPR with DET. Discrepancies between studies can possibly be attributed to differences in cryopreservation methodology and thawing/warming procedure and the different criteria for selecting embryos for cryopreservation. In conclusion, there is no reason related to embryo quality to perform DET instead of SET when cryopreserved-warmed cleavage-stage embryos are transferred since the increased LBR with DET is associated with a substantial increase in MPR.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## II.2. Vitrified-warmed blastocyst stage

*Key question. In FET, should embryo criteria be considered a factor in deciding to apply DET instead of SET at blastocyst stage for couples/individuals undergoing ART? If yes, which criteria are appropriate?*

### *Evidence*

A retrospective propensity score matching (PSM) control study included data, collected in 2014 from 643 patients aged between 35 years and 39 years and compared outcomes of day 5/6 vitrified-warmed embryo transfer cycles ([Park et al., 2019](#)). The study reported a lower LBR in SET with one good-quality blastocyst ( $\geq 3BB$ ) compared to DET with two good-quality blastocysts (n= 102 cycles after PSM) (32.4% vs. 54.9% for SET and DET; OR 0.4; 95%CI 0.2-0.7). The MPR was significantly higher in the DET group (4.8% vs. 33.3%, for SET and DET; OR 0.1; 95%CI 0.02-0.5). Differences in the rates of preterm birth (defined as gestational age at birth before 37 weeks) did not reach the level of statistical significance (9.1% vs. 23.2% for SET and DET; OR 0.3; 95%CI 0.1-1.3), nor did the proportion of anomalies in the children born (2.9% vs. 1.3% for SET and DET). When SET of a good-quality blastocyst ( $\geq 3BB$ ) was compared to DET of a good-quality and a poor-quality blastocyst ( $< 3BB$ ) (mixed-quality DET) (n=93) no significant differences were observed in LBR (33.3% vs. 38.7%, for SET vs. DET; OR 0.8; 95%CI 0.4-1.4) but MPR was significantly lower in the SET group (2.5% vs. 21.4%, for SET vs. DET; OR 0.1; 95%CI 0.01-0.8). In the same comparison, the study reported there were no significant differences in either preterm birth rates (12.9% vs. 19.4%, for SET vs. DET; OR 0.6; 95%CI 0.2-2.3) or in anomalies in the children born (3.1% vs. 0%, for SET vs. DET).

These results are similar to the observations of a retrospective PSM control study SET and mixed-quality DET ([Wang et al., 2020](#)). Data on 520 matched day 5/6 vitrified-warmed embryo transfer cycles were collected during 2012-2019. Mixed-quality DET comprised the transfer of a good-quality blastocyst ( $\geq 3BB$ ) with a poor-quality one ( $\geq 3$  comprising of C TE or C ICM grade). A significantly higher LBR was reported after mixed-quality DET compared to SET of a good-quality blastocyst (47.9% vs. 41.0%, for DET vs. SET; OR 1.3; 95%CI 1.0-1.7). The MPR was significantly higher in the DET group (30.5% vs. 2.4% for DET vs. SET; OR 17.5; 95%CI 7.5-40.8).

Another observational study included data from 1009 vitrified-warmed blastocyst transfers (38.0% only day 5, 57.8% only day 6, 2.1% day 7, 1.3% mixed day 5/6 and 0.9%-day 6/7) ([Dobson et al., 2018](#)). The authors reported no differences in LBR between SET of a good-quality blastocyst versus ( $\geq 3BB$ ) (n=161) and a mixed-quality DET (a good-quality and a poor-

quality blastocyst with  $\geq 3$  containing C grade in ICM or TE; n=358) (32.7% vs. 24.2%, for SET vs. DET; OR 0.8; 95%CI 0.5-1.2), but a significantly higher MPR with DET (7.1% vs. 2.6% for DET vs. SET; OR 2.4; 95%CI 1.2-4.9).

The retrospective study of Van Landuyt et al. included 759 day 5/6 vitrified-warmed blastocyst transfer cycles performed between 2008 and 2010 (Van Landuyt et al., 2011). Early and expanded blastocysts were vitrified with A/B ICM/TE on day 5 and as fully expanded A/B blastocysts on day 6. A significantly lower MPR in cycles with SET compared to DET (1.3% vs. 21.9%, for SET vs. DET) was reported.

A retrospective study of Liu et al. analysing data from 2012-2013 of 741 day 5/6 vitrified-warmed embryo transfer cycles reported lower MPR after SET of one good-quality blastocyst ( $\geq 4BB$ ) than after DET with good-quality blastocysts (2.4% vs. 48.6%, for SET vs. DET) (Liu et al., 2014).

In a larger retrospective study day 5/6 vitrified-warmed blastocysts showed a higher LBR after DET of two good-quality blastocysts ( $\geq 3BB$ ) (n=519) when compared to after SET of one good-quality blastocyst (n=759) (43.0% vs. 60.3%, for SET vs. DET, adjusted OR 1.8; 95%CI 1.2-2.6) (Zhu et al., 2020). The MPR was higher after DET with two good-quality blastocysts (0.8% vs. 26.2% for SET vs. DET). The LBR was similar after mixed-quality DET of one good-quality and one poor-quality blastocyst ( $< 3BB$ ) (n= 266) (43.0% vs 53.8%, for SET vs. DET, adjusted OR 1.4; 95%CI 0.9-2.2) or DET with two poor-quality blastocysts (n= 160) (43.0% vs 46.3%, for SET vs. DET, adjusted OR 1.09, 95%CI 0.7-1.8) compared to SET of a good-quality blastocyst. MPR was lower for SET of one good-quality blastocyst compared to mixed-quality DET and DET of two poor-quality blastocysts (0.8% vs. 17.3% and 14.4% for SET vs. mixed-quality DET and DET with poor-quality blastocysts, respectively).

The retrospective observational study of Arab and colleagues analysed 1104 day 5/6 vitrified-warmed blastocyst transfers with poor quality ( $\leq 3BB$ ) performed in 2008-2019 to 856 patients (Arab et al., 2020). The study reported no differences in LBR between poor-quality SET (n=744) compared to poor-quality DET (n=74) 20.2% vs. 12.2%, for SET vs. DET, NS) in patients aged  $< 40$  years using own oocytes as well as in poor-quality DET in patients aged  $\geq 40$  years using their own oocytes after (n=63 and n=74, for SET and DET) (6.3% vs. 0%, for SET vs. DET). The

MPR was significantly lower in SET in patients aged <40 years using own oocytes (1.6% vs. 6.8% for SET and DET). No differences in MPR were reported between SET and DET in the patients aged ≥40 years using own oocytes (0% for SET and DET).

First blastocyst FET in 3362 patients after a complete freeze-all IVF/ICSI cycle performed in 2016-2018 were analysed in a retrospective study ([Chen et al., 2020](#)). SET of good-quality blastocyst (≤4BB) (n=1425) in patients aged <35 years was compared to three types of DET: DET of two good-quality blastocysts (≤4BB) (n=844) (54.2% vs. 64.6%, for SET vs. DET); to mixed-quality DET (with a poor-quality blastocyst being blast 3 or 4CC) (n=206) (54.2% vs. 64.1%, respectively); and to DET of two poor-quality blastocyst (54.2% vs. 48.6%, respectively) (n=183). The LBR of a poor-quality blastocyst SET (n=120) was lower than the one after DET with poor-quality blastocysts (36.7% vs. 48.6% SET vs. DET). The MPR in this age cohort was lower after a good-quality blastocyst SET compared to good-quality blastocysts DET (3.5% vs. 62.4%, for SET vs. DET); to mixed-quality DET (3.5% vs. 49.7%, respectively); and to DET of two poor-quality blastocysts (3.5% vs. 50.0%, respectively). The MBR of poor-quality SET was lower, compared to the one observed after poor-quality DET (0% vs. 50.0%; for SET vs. DET).

In the same study and using the same embryo quality criteria in treatments of patients aged >35 years, the authors observed lower LBR after good-quality SET (n=144), compared to LBR good-quality DET (n=269) (42.4% vs. 59.5%, for SET vs. DET). No differences in LBR between SET and mixed-quality DET (n=107) (42.4% vs. 48.6%, for SET vs. DET) and poor-quality DET (30.8%) (n=39) or poor-quality SET (n=25) (24.1%) were observed. MPR in patients aged >35 years was lower after good-quality SET compared to good-quality DET (6.3% vs. 49.2%, for SET vs. DET); to mixed-quality DET (6.3% vs. 42.6%, respectively) and to poor-quality DET (6.3% vs. 31.3%, respectively). The MBR after poor-quality SET (10.0%) was not different to the one observed in the other groups.



Table 7 Effects of the transfer of vitrified-warmed blastocyst(s) of good and/or poor quality on outcomes of ART in eSET and DET patients in observational studies – autologous oocyte cycles.

Vitrified-warmed blastocyst embryo transfer							
Study	Type of study	Outcome (%)	Type of transfer				
			GQE-DET	MQE-DET	PQE-DET	GQE-(e)SET	PQE-(e)SET
(Park et al., 2019)	Observational study	LBR	54.9	38.7	-	32.4 and 33.3	-
		MPR	33.3	21.4	-	4.8 and 2.5 <sup>b</sup>	-
(Wang et al., 2020)	Observational study	LBR	-	47.9	-	41.0 <sup>a</sup>	-
		MPR	-	30.5	-	2.4 <sup>b</sup>	-
(Dobson et al., 2018)	Observational study	LBR	-	24.2	-	32.7	-
		MPR	-	7.1	-	2.6 <sup>b</sup>	-
(Van Landuyt et al., 2011)	Observational study	LBR	-	-	-	-	-
		MPR	21.9	-	-	1.3 <sup>a</sup>	-
(Liu et al., 2014)	Observational study	LBR	-	-	-	-	-
		MPR	48.6	-	-	2.4 <sup>a</sup>	-
(Zhu et al., 2020)	Observational study	LBR	60.3	53.8	46.3	43.0 <sup>a</sup>	29.56
		MLBR	26.2	17.3	14.4	0.8 <sup>a,b,c</sup>	0.83
(Arab et al., 2020)	Observational study	LBR	-	-	12.16 <sup>*</sup> 0 <sup>**</sup>	-	20.21 <sup>*,c</sup> 6.3 <sup>**</sup>
		MPR	-	-	20.21 <sup>*</sup> 0 <sup>**</sup>	-	12.16 <sup>*,c</sup> 0 <sup>**</sup>
(Chen et al. 2020)	Observational study	LBR	64.6 <sup>†</sup>	64.1 <sup>†</sup>	48.7 <sup>†</sup>	54.3 <sup>†,a,b</sup>	48.6 <sup>†,c</sup>
			59.5 <sup>‡</sup>	48.6 <sup>‡</sup>	30.8 <sup>‡</sup>	42.4 <sup>‡,a</sup>	24.0 <sup>‡</sup>
		MPR	62.4 <sup>†</sup>	49.7 <sup>†</sup>	50.0 <sup>†</sup>	3.5 <sup>†,a,b,c</sup>	0.0 <sup>†,c</sup>
			49.2 <sup>‡</sup>	42.6 <sup>‡</sup>	31.3 <sup>‡</sup>	6.3 <sup>‡,a,b,c</sup>	10.0 <sup>‡</sup>

GQE: good-quality embryo(s) only; MLBR: multiple live birth rate; MQE: embryos of mixed quality, i.e. good- and poor-quality; PQE: poor-quality embryo(s) only.  
<sup>\*</sup> patients <40, using their own oocytes; <sup>\*\*</sup> patients ≥40y, using their own oocytes; <sup>†</sup>: patients <35y; <sup>‡</sup> patients ≥35y.  
a. There was a significant difference compared to GQE-DET, b. There was a significant difference compared to MQE-DET, c. There was a significant difference compared to PQE-DET

### Recommendation

<p>In vitrified-warmed blastocyst transfer cycles, SET should be applied regardless of the quality of the vitrified blastocyst.</p>	<p>Strong ⊕○○○</p>
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### Justification

When vitrified-warmed blastocysts are to be transferred, embryo morphology is not a reason to perform DET instead of SET, since the increased LBR is associated with a substantial increase in MPR.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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### III. Time-lapse morphokinetics

The incorporation of time-lapse (TL) technology in the monitoring and culture of embryos has been advantageous for laboratory processes and for improving knowledge on embryo development. It is no longer necessary to interrupt the culture conditions to observe embryos and, furthermore, morphokinetics and observation of abnormal cleavage have become a new embryo selection tool. The current section evaluates TL morphokinetics and observation of abnormal cleavage as a tool to support a decision for DET. In TL practice, embryo selection based on morphokinetic criteria varies between laboratories, rendering result assessment difficult ([Apter et al., 2020](#)). This can be explained by different culture conditions and different intrinsic population characteristics in each clinic and laboratory. Low quality evidence from a systematic review of RCTs including 1637 patients showed that TL selection and culture improve LBR and reduce early pregnancy loss ([Pribenszky et al., 2017](#)). However, a recent three-armed, multicentre, double-blind, RCT including 1731 patients showed that TL based embryo selection and uninterrupted culture conditions in a TL incubator do not improve clinical outcomes compared with routine methods ([Kieslinger et al., 2023](#)).

***Key question: Can TL morphokinetics be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART? If yes, which criteria and what is the appropriate cut off?***

#### *Evidence*

One retrospective study compared outcomes after the transfer of one embryo selected by objective morphokinetic algorithms (TL embryos) to the transfer of two embryos selected according to conventional embryology selection parameters and developed in standard incubator (standard embryos) (973 and 6948 deliveries, respectively) ([Fishel et al., 2017](#)). The LBR was lower after a single TL blastocyst transfer than after the transfer of two standard blastocysts in patients aged <38 years (OR 0.9; 95%CI 0.7-1.0). A similar difference was also observed in patients aged >38 years (OR 0.6; 95%CI 0.5-0.8). Increased rates of multiple births by about 30 to 40% were observed after the transfer of two embryos compared to SET regardless of age, incubator type, and embryo stage.

#### *Recommendation*

TL imaging-derived parameters for embryo selection should not be considered a factor to apply DET instead of eSET.	Strong	⊕○○○
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### *Justification*

*When choosing embryos for transfer or cryopreservation, laboratories with TL technologies may be able to implement their selection or ranking strategy more confidently (Apter et al., 2020). However, there is currently no evidence that supports DET instead of SET based on this parameter.*

### *Further information*

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## IV. Preimplantation genetic testing

The use of PGT, and particularly PGT-A, has increased over the years, despite the lack of consistent evidence of benefit ([Theobald et al., 2020](#), [Wyns et al., 2021](#)). One frequent reason mentioned by the proponents is that it may optimise the adherence to SET ([Forman et al., 2013](#), [Grifo et al., 2013](#), [Scott et al., 2013](#)). According to the ESHRE PGT consortium data collection in 2016-2017, the majority of transfers (87%) after PGT-A involved a single embryo ([van Montfoort et al., 2021](#)). The ASRM guideline recommended the transfer of one euploid embryo, regardless of the patient's age ([ASRM, 2021](#)).

As such, the GDG investigated whether there are any studies supporting a role for PGT-A in deciding to opt for DET instead of SET.

**Key question. Can the outcome of PGT-A testing of blastocysts be considered a factor in deciding to apply DET instead of (e)SET for couples/individuals undergoing ART?**

### Evidence

No evidence was found relating a specific PGT outcome (e.g., low mosaic, segmental), to justify DET versus eSET in treatments with the patients' own oocytes or with donor oocytes or embryos.

### Recommendation

<b>PGT-A outcomes should not be considered when deciding to apply DET instead of eSET.</b>
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**Strong**

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### Justification

To our knowledge, no studies have evaluated the impact of PGT testing while comparing outcomes of SET and DET. However, PGT is seen as an eSET strategy and low- to moderate-quality evidence was found showing that the use of eSET following PGT-A minimises the risk of multiple pregnancies without affecting LBR ([Forman et al., 2013](#), [Grifo et al., 2013](#), [Scott et al., 2013](#)). The GDG is of the opinion that in order to further the safety of ART treatment, this recommendation should be a strong one.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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# Part E: Other strategies for embryo transfer

## I. Transfer of more than two embryos

Whilst the aim of assisted conception is a healthy live singleton birth ([Min et al., 2004](#)), there are instances where patients or infertility specialists may request or recommend the transfer of more than two embryos, even though they are aware of the risks that accompany HOM.

The transfer of >2 embryos has been included in several historical or recent guidelines, mostly as part of ART treatment in patients of poorer-prognosis as the overall rate of multiple gestations is low in this age group ([ASRM, 2021](#), [Min et al., 2010](#)). Even though the number of embryos transferred in Europe is steadily declining, the transfer of >2 embryos is still practiced in several countries ([De Geyter et al., 2022](#)). Data from the European IVF monitoring data consortium showed that in 2016, TET was performed in 6.2% of the cycles and four or more embryos were transferred in 0.4% of the cycles ([Wyns et al., 2020](#)). These rates are declining, with 4.5% and 0.3% in 2017 and 3.9% and 0.3% in 2018, respectively ([Wyns et al., 2021](#), [Wyns et al., 2022](#)).

***Key question. In any patient undergoing ART, should the transfer of more than two embryos be applied considering the risks of the higher order pregnancies?***

### *Evidence*

Many studies have shown that transfer of >2 embryos results in higher PR/LBR and higher frequency of MPR and HOM when good-quality embryos are transferred ([Elizur et al., 2005](#), [Heijnen et al., 2006](#), [Ng et al., 2001](#), [Ruhmann et al., 2017](#)).

It is important to note that evidence on clinical outcome after the transfer of >2 embryos is largely based on articles published in the early 2000s, reflecting practices of that period. These analyses evaluated only a limited number of patient and embryo characteristics, making it difficult to draw definitive conclusions. However, all studies showed that transfer of >2 embryos resulted in higher frequencies of MPR and HOM. Furthermore, PR and LBR can be higher following transfer of >2 embryos, depending on study design.

A retrospective study of 1448 patients having their first ART cycle assessed the influence of the presence of day 2 good-quality supernumerary embryos on clinical outcome and the risk of multiple conception ([Salha et al., 2000](#)). When patients <35 years old had good-quality supernumerary embryos, TET of three good-quality embryos resulted in higher twin (12.5% vs. 11.9%) and triplet birth rates (2.1% vs. 0%) than DET of two good-quality embryos, without significantly improving the LBR (38.9% vs. 35.7%). When no good-quality spare embryo was present, patients who had TET rather than DET had a significantly higher LBR (32.7% vs. 19.4%) and singleton birth rate per cycle (20.8% vs. 14.4%), without significantly influencing the multiple birth rate. In patients >35 years old, TET instead of DET in the presence or absence of good-quality supernumerary embryos led to a significant improvement in clinical outcome, without being associated with a concurrent increase in the multiple birth rate. However, it should be noted that this study only assessed day 2 ETs. Furthermore, the sample subsets, e.g., >35 years old with good-quality supernumerary embryos, was extremely variable (DET n=10 vs. TET n=85).

Another retrospective study comparing the outcome of fresh transfer cycles of two (n=388) vs. three embryos (n=347) showed higher MPR after TET as compared to DET (15.5% twins in DET vs. 24.4% twins and 7.0% triplets in TET, OR for multiple pregnancy 2.1; 95%CI 1.1-4.2) ([Ng et al., 2001](#)). The IR was higher in the TET group (14.6% vs 18.6%, for DET vs. TET) but no significant differences in PR were observed between the two groups (21.6% vs. 24.8%, for DET vs. TET). No data on LBR were reported.

An analysis of factors predicting LBR following ART evaluated treatment data of 1928 patients who underwent 5310 consecutive ART cycles ([Elizur et al., 2005](#)). DET and TET were associated with better LBR compared to SET (adjusted OR 1.97, 95%CI 1.2-3.2 and adjusted OR 2.7; 95%CI 1.7-4.4, respectively). LBR was similar after TET and DET, but MPR was significantly higher in the TET group compared to DET (27.7% twins and 5.3% triplets after TET vs. 21.5% twins and 0% triplets after DET).

A small two-centre RCT reported the outcomes of cycles in patients ≥38 years old who either had DET over a maximum of four cycles (DET group, n=23) or TET over a maximum of three cycles (TET group, n=22) ([Heijnen et al., 2006](#)). The cumulative LBR was 47.3% after four cycles of DET (although 12 patients discontinued the study) and 40.5% after three cycles of TET. There



were no multiple pregnancies in the DET group whilst in the TET group, there were three triplet pregnancies (HOM 30.0%, 95%CI 7.0-65.0).

A more recent retrospective analysis studied consecutive fresh day 5 ETs (n=784) from 2007 to 2015. Three groups were compared: DET where only two embryos had reached a transferable stage (n=219); elective DET where two embryos were selected from several that had reached transferable stage (n=357); and TET where only three developing embryos were available (n=208) ([Ruhlmann et al., 2017](#)). Clinical PR was 42.9% vs. 61.1% vs. 58.2%, and MPR was 11.7% vs. 31.2% vs. 37.2%, respectively. HOM rate (at least three foetuses) was however lower in the two DET groups than in the TET group (1.1% vs. 0.9% vs. 14.1%, respectively).

A retrospective analysis of fresh day 3 ET cycles (n = 863) looked at whether transferring >5 day 3 embryos increased PR in patients aged over 40 years ([Combelles et al., 2005](#)). The study concluded that in these patients, 5 embryos was the optimum number to transfer, and transferring >5 embryos did not confer any additional benefit to clinical outcome compared to transferring ≤5 embryos. LBR was significantly higher with ≥5 embryos transferred compared to <5 embryos (22.3%, 22.6% and 4.3% when 5 embryos, 5 embryos and <5 embryos were transferred, respectively). However, the study reported high twin rates of 23.8%, 36.7% and 13.3% where >5 embryos, 5 embryos and <5 embryos were transferred, respectively.

Two time periods with different number of embryos transferred were compared in another retrospective study of ART cycles ([Setti et al., 2005](#)). In the first period, TET was performed in patients <36 years old (n=262) and transfer of four embryos in patients ≥36 years old (n=157); whilst in the second period, DET took place in patients <36 years old (n=332) and TET in patients ≥36 years old (n=277). The reduced number of embryos transferred in the second period did not significantly reduce the multiple pregnancies rates.

Two more studies support the restriction on transferring more than two embryos in FET cycles. Firstly, a retrospective study compared outcomes of FET cycles of DET or TET in patients aged <40 years ([Berin et al., 2010](#)). In patients aged <35 years (n=145), DET vs. TET resulted in similar PR and LBR, but the MPR was significantly higher in the TET group (41.0% for TET vs. 9.4% for DET) regardless of whether they conceived in their fresh cycle or not. In patients aged 35 to 39 years (n=93), there were no differences in the PR, MPR or LBR between the two groups and

the result of a prior fresh cycle had no effect on the subsequent FET. This study concluded that transferring extra embryos in a thawing cycle when a patient had a previous unsuccessful fresh cycle was not warranted.

A separate retrospective study analysed FET cycles (n=980) of cleavage-stage embryos, comparing DET (785 cycles) vs. TET (195 cycles) with similar embryo quality at cryopreservation. Higher MPR was observed in the TET group, with similar CPRs, IRs and LBRs in patients ≤39 years old ([Sun et al., 2012](#)). Among patients ≥40 years old (n=35), there were no differences in the CPR, IR, MPR or LBR between the two groups. Studies against the restriction of the transfer of more than two embryos in FET cycles included one retrospective study evaluating factors associated with transfer of day 7 blastocyst with delayed expansion in FET ([Richter et al., 2016](#)). This study concluded that whilst increasing number of embryos per transfer resulted in a significant increase in the live birth per ET, there was a significant decrease in the number of children born per transferred embryo. There was also a lower birth rate following day 7 transfer of blastocysts with delayed expansion (10% per transfer).

Transfer of more than two embryos is associated with an increased risk of ectopic pregnancy, a potentially life-threatening complication. The risk of ectopic pregnancy is increased following ART (0.26-1.5 per 1000 ART pregnancies) ([Clayton et al., 2007](#)) and several studies have shown that it increases in correlation with the number of transferred embryos ([Anzhel et al., 2022](#), [Bu et al., 2016](#), [Cirillo et al., 2022](#), [Li et al., 2015](#)). A multivariate analysis estimated that the odds of ectopic pregnancy increase 20-fold, especially when transferring more than two embryos at a time ([Perkins et al., 2015](#), [Pi et al., 2020](#)).

#### Recommendation

Transfer of more than two embryos is not recommended.	Strong ⊕○○○
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#### Justification

*The transfer of three embryos in fresh cycles carries an unacceptable increase in the risk of HOM and ectopic pregnancies. Furthermore, HOM births can also result from MZT and caution is recommended when deciding on transferring more than two embryos. The possible benefit from a potentially higher PR with the transfer of >2 embryos should be balanced against the high obstetrical and neonatal risks for multifoetal pregnancies. Clinics should evaluate their*

results continually and consider decreasing the number of embryos transferred to minimise undesirable outcomes associated with HOM.

#### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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## II: Foetal reduction

Foetal reduction aims to decrease the order of a multiple pregnancy to twin or singleton by selective foeticide. It is fraught with emotional, ethical, and practical difficulties and this reproductive choice is fiercely debated. Pregnancy loss and very early premature delivery are the two main risks of foetal reduction, and the rate of poor outcomes is correlated with the starting number of foetuses ([Evans et al., 2003](#)). In addition, risks associated with foetal reduction include vaginal bleeding, (sub)chorionic haematoma, pain, intrauterine infection, premature rupture of membranes, psychological issues such as anxiety, guilt and regret, and ethical issues such as instances when the sex of the foetuses is known ([Multifetal Gestations: Twin, Triplet, and Higher-Order Multifetal Pregnancies: ACOG Practice Bulletin, Number 231, 2021, Beriwal et al., 2020, Evans et al., 2014](#)).

In 1999, ESHRE workshop recommended to use foetal reduction as a last resort to reduce the risks associated with HOM ([Multiple gestation pregnancy, The ESHRE Capri Workshop Group, 2000](#)), but not as a planned strategy to address the expected higher rate of multiple pregnancy following the transfer of >1 embryos.

***Key question. In any patient undergoing ART, should the transfer of more than two embryos with embryo reduction after implantation be applied considering the risks of the procedure?***

*Evidence*

*Comparison of reduced multiple pregnancies with non-reduced multiple pregnancies*

***Reduction to twins***

Two meta-analyses of observational studies reported on the effectiveness and safety of multifoetal reduction to twins in women who conceived HOM without medical assistance, by ovulation induction or by assisted reproduction ([Anthoulakis et al., 2017, Zipori et al., 2017](#)). A third meta-analysis, a Cochrane review aiming to include only randomised controlled trials did not find any studies to include ([Dodd et al., 2015](#)).

The first meta-analysis of 24 observational studies involving 3209 women who conceived either without medical assistance, by ovulation induction or by IVF/ICSI, concluded that embryo reduction at 8 to 15 weeks of trichorionic triamniotic triplet pregnancies to twins is associated with a better pregnancy outcome compared with that of non-reduced triplets ([Zipori et al., 2017](#)). Particularly, there was a reduction in the rate of preterm delivery before 36 weeks (OR 0.1; 95%CI 0.1-0.4) and before 34 weeks (OR 0.2; 95%CI 0.1 – 0.3), gestational diabetes (OR

0.4; 95%CI 0.2-0.7), hypertensive disorders of pregnancy (OR 0.5; 95%CI 0.3-0.7), neonatal death (OR 0.32; 95%CI 0.12-0.84) and need for caesarean section (OR 0.2; 95%CI 0.1-0.3). The LBR and pregnancy loss (<24 weeks) rates were comparable between the two groups (OR 0.9; 95%CI 0.5-1.4 and OR 0.9; 95%CI 0.6-1.5, respectively).

The second meta-analysis of eight observational studies involving 1416 women who conceived either without medical assistance, by ovulation induction or by IVF/ICSI, concluded that embryo reduction to twins at 8 to 14 weeks in trichorionic triamniotic triplet pregnancies (TCT) reduces the risk of preterm birth (<34 weeks) without significantly increasing the risk of pregnancy loss (<24 weeks) compared to non-reduced TCT (Preterm birth rate: 17.3% (106/612) vs. 50.2% (233/464), for TCT vs. reduced-TCT; RR 0.4; 95%CI 0.3–0.5) ([Anthoulakis et al., 2017](#)). However, the difference of preterm birth rates was not significant in the case of a dichorionic triamniotic triplet reduction.

A prospective cohort study also compared results from reduced pregnancies after ovulation induction or IVF/ICSI to outcomes of spontaneous twin pregnancies. The outcomes of 10 quadruple (group 1) and 30 triplet pregnancies reduced to twins (group 2) were compared with the outcomes of 30 non-reduced twins (group 3) and the results showed that the mean gestational age at delivery and mean birth weights were significantly lower in group 1 (33.2; 35.9; 36.9 weeks and 1843, 2209, 2361 g, respectively) ([Groutz et al., 1996](#)). Group 1 was also characterised by the highest pregnancy complication rates compared to group 2 and 3, especially premature contractions (50%, 27% and 13%, respectively) and pregnancy-induced hypertension (40%, 23% and 7%, respectively).

### ***Reduction to singletons***

A systematic review of six studies involving 7398 women conceived either without medical assistance, by ovulation induction or by IVF/ICSI showed that the perinatal outcomes of dichorionic diamniotic twins reduced to singletons before 15 weeks of gestation were improved, compared to the clinical outcomes of non-reduced twins ([Jin et al., 2020](#)). The reduction was associated with a lower risk of preterm birth (RR 0.3; 95%CI 0.2-0.4; n=7297 women; 5 studies) without an increased risk of miscarriage (RR 1.6; 95%CI 0.9-2.8; n=7355 women; 5 studies).

In a retrospective cohort study, a total of 850 pregnancies involving 732 trichorionic triplets and 118 dichorionic triplets were subdivided into three subgroups: expectant treatment (subgroup 1), reduction to twins (subgroup 2) and reduction to singletons (subgroup 3) ([Liu et al., 2019](#)). The study found that, in the trichorionic triplet group, both subgroups with embryo reduction had better outcomes compared to expectant treatment, especially those reduced to singletons. These subgroups showed significantly higher LBR (85% (34/40), 95% (577/610) and 95% (21/22) for subgroup 1, 2 and 3, respectively), take-home baby rates (85% (34/40); 94% (575/610) and 95% (21/22) for subgroup 1, 2 and 3, respectively) and lower miscarriage rates (15% (6/40); 5%; (30/610) and 5% (1/22) for subgroup 1, 2 and 3, respectively), with significant difference in perinatal mortality (1% (1/96; 1% (15/1131) and 0% (0/21) for group 1, 2 and 3, respectively). In the dichorionic triplet group, similar patterns of pregnancy outcomes were observed, with significant improvement in gestational age, LBR, birth weight, and take-home baby rate in the group reduced to singletons.

#### *Comparison of reduced multiple pregnancies to singletons with primary singletons*

When comparing outcomes of triplet or twin pregnancies reduced to singletons to outcomes of primary singleton gestations, it was observed that the overall risk of pregnancy complications was higher in the reduced multiple pregnancy group ([Kristensen et al., 2022](#), [van de Mheen et al., 2015](#), [Yimin et al., 2022](#)). However, it should be noted that the initial number of embryos transferred (whether from SET, DET, or higher numbers) was not indicated in those studies.

A retrospective study of patients undergoing IVF or ICSI found that triplets reduced to singletons had higher preterm delivery rates (15.8% vs. 7.3%), low birth weight rates (12.3% vs. 4.32%), very low birth weight rates (2.3% vs. 0.4%), and small for gestational age rates (14.6% vs. 6.6%) compared to primary singletons, with a comparable pregnancy loss rate (5.3% vs. 5.4%) ([Yimin et al., 2022](#)).

The clinical outcomes of dichorionic twin pregnancies undergoing foetal reduction between 11-23 weeks (n=172) were compared to those of non-reduced twins (n=9563) and primary singleton pregnancies (n=16465) in another retrospective cohort study ([Kristensen et al., 2022](#)). Women conceived either without medical assistance, by ovulation induction or by IVF/ICSI. It was observed that while the likelihood of at least one live-born child did not

significantly differ between the groups, primary singletons had the best outcomes with higher birth weights, longer gestational ages, and fewer adverse pregnancy outcomes, including miscarriage (<24 weeks) and stillbirth. It is worth noting that the outcomes of twins reduced to singletons before 14 weeks were better than the outcome of twins reduced to singletons from 14 weeks.

A third retrospective cohort study compared the pregnancy clinical outcomes following ART of 118 patients with a twin pregnancy reduced to singletons with the outcomes of 611 women with a primary singleton pregnancy ([van de Mheen et al., 2015](#)). In the reduction group, the miscarriage rate (>24 weeks) was significantly higher compared with primary singleton group (14.4 vs. 0.7%, respectively; RR 5.3; 95%CI 3.9-7.2). Preterm birth rate was higher in the foetal reduction group (18.6% vs. 1%; RR 5.7; 95%CI 4.4-7.5). The median gestational age was 38.9 weeks for reduced pregnancies and 40.1 weeks for primary singletons.

***Key question: Should multiple ET with embryo reduction versus (e)SET be used for any couple undergoing ART?***

A single-centre retrospective study compared the clinical outcome of selective foetal reduction following DET (n=390) to the clinical outcomes of pregnancies following SET (n=4667) ([Wang et al., 2022](#)). After adjusting for age, infertility duration, types of infertility, states of embryos, body mass index, and other factors affecting SET or DET decisions (scar uterus, Mullerian anomalies, cervical troubles and uterine fibroids), multivariate regression analysis revealed that selective foetal reduction increased the risk of miscarriage (OR 2.4; 95%CI 1.4-3.9) and preterm birth rate (OR 1.5; 95%CI 1.1-2.1) and reduced the gestational age ( $\beta$  coefficient -0.3; 95%CI -0.5 to -0.1). Selective foetal reduction was also associated with reduced LBR (OR 0.5; 95%CI 0.3-0.8), reduced newborn birth weight ( $\beta$  coefficient -177.4; 95%CI -235.1 to -119.7) as well as an increased risk of low-birth weight newborns (OR 2.2; 95%CI 1.5-3.3).

***Recommendation***

<b>In patients who conceived HOM following multiple embryo transfer, foetal reduction can be considered to reduce the risk of maternal complications.</b>	<b>Conditional ⊕⊕○○</b>
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The transfer of two or more embryos with the intention of performing foetal reduction in case of multiple embryo implantation instead of (e)SET is not recommended.	Strong ⊕○○○
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The GDG recommends against the transfer of more than two embryos with foetal reduction after multiple embryo implantation considering the high risks of the procedure.	GPP
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### Justification

HOM gestations are associated with an increased risk of hypertensive disorders of pregnancy, gestational diabetes, need for caesarean section, and neonatal complications such as preterm birth, low birth weight, admission to neonatal intensive care and neonatal mortality. Selective embryo reduction of a HOM pregnancy may be considered with the aim to reduce the associated increased risks. RCTs on this topic are not available for obvious ethical reasons. Because of this, the strength of evidence supporting the recommendation of using embryo reduction in case of HOM relies on data from observational studies, most of which are retrospective, and is therefore very weak [see **annex 2** – sof table 5&6].

It is important to mention that study populations, initial and final number of fetuses in utero, gestational age at reduction, and foetal reduction indications were different in the included studies and were not necessarily considered as confounding factors when analysing the results. Thus, the GDG considers that the ideal solution to reduce the risks associated with the foetal reduction procedure is to apply (e)SET. Low-quality evidence from one retrospective study showed that selective foetal reduction following DET increased maternal and neonatal complications compared to SET.

It is noteworthy that the results of the survey conducted by the GDG (please see **annex 7**) showed that many patients (49.6% of the participants) have heard about foetal reduction, even though this has been recommended to only a minority of cases (3.7%). Most patients (40.7%) found that the idea of foetal reduction is not acceptable for them.

### Further information

Details of the literature study and evidence tables are available in **annex 8** and **annex 9**.

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# Part F: Patient counselling on embryo transfer

## Patient counselling

Two definitions can be used to describe patient counselling in ART: a narrower one in which support, advice and guidance are provided in order to help the patient with their infertility journey ([Gameiro et al., 2015](#)), and a broader one that includes advice and guidance about all aspects of the medical treatment, including obstetric care. Here, we have adopted the broader definition for counselling.

*Key question. Which issues are crucial for decision-making regarding the number of embryos to transfer and how should they be discussed with the patients? (NARRATIVE)*

*ART is a family building procedure*

The main goal of ART is to overcome obstacles in family building. The family building element has often been neglected in ART research. Research on patient preferences regarding the number of embryos to transfer has studied various medical treatment options, for example, the desirability of one DET vs. one SET ([Blennborn et al., 2005](#)) 59% of patients chose DET), of a pair of twins vs. one singleton ([Højgaard et al., 2007](#), [Mendoza et al., 2018](#)) 59% and 58% wanted twins, respectively), of one DET vs. one SET with various PRs for the two sets of transfers ([Twisk et al., 2007](#)), and whether ART mothers of 3-4-year-old twins and singletons in retrospect would have preferred twins or singletons ([Pinborg et al., 2003](#)). However, the essential question regarding the number of children that ART patients wish for their family has remained open, although the argument has been made that the wish for twins and HOM can be explained with the wish for >1 child that the patients want to have as soon as possible ([Gleicher and Barad, 2006](#), [Gleicher and Bard, 2013](#)). While fondness of twins has a perceived significant prevalence in modern-day society, the GDG could not identify a scientific article about this matter in the general population.

In order to better understand patients' wishes on family building and their understanding of ET-related issues, the GDG conducted an online survey in 30 different languages during 2021. The survey design and results are detailed in **annex 7**. Questions were organised into four sections:

demographics and background information, wishes and beliefs on family building and pregnancy, patient knowledge about the embryo transfer and patient experience with the procedure. Details of replies to questions from the first two sections are discussed here.

The results of our survey showed that most patients (85.6%) wish to have more than one child. Half of the patients (50.0%) did not have a preference toward singletons or twins, and among those who indicated a preference, singleton(s) were more preferred than twins (67.9% vs. 32.1%). It should be noted that while in earlier years a preference for twins was observed in about 58-59% of European patients ([Blennborn et al., 2005](#), [Højgaard et al., 2007](#)) ([Mendoza et al., 2018](#)), in the GDG's survey in 2021, preference for twins was considerably lower at 15.7% (of all patients). The main reason indicated for preferring singleton(s) was an easier and less risky singleton pregnancy (82.7%). By contrast, preference for twins could not be explained by a single factor. The desire to have several children as soon as possible (49.5%) was followed by a general fondness towards twins (41.7%) and a wish to avoid multiple hormonal stimulations of the ovaries (31.5%).

#### *Patient counselling as shared decision-making*

Shared decision-making is currently recognised as the ideal treatment decision-making process, and involves counselling and information sharing between healthcare professionals and patients that help to reach an agreement on treatment modalities ([Sandman and Munthe, 2010](#)). In this process, healthcare professionals should provide accurate and transparent information regarding the likelihood of treatment success with the aim of helping patients to build their own perceptions and decisions ([Blennborn et al., 2007](#)) by adopting a receptive attitude towards the interests and needs expressed by their patients ([Charles et al., 1997](#)). Shared decision-making not only supports a good balance between the patient's best interest and the clinician's respect for patient autonomy but also results in better patient adherence and compliance to treatment ([Brody et al., 1989](#)).

In the context of embryo transfer, there are varying degrees of shared decision-making. For example, for a young, good prognosis patient having her first embryo transfer, the treating physician will likely leave very little to no room for the patient's request for a multiple embryo transfer. For poor prognosis patients with repeated implantation failures or insufficient funds for several transfer cycles, the decision-making process will require a more thorough exploration of

the desires of the patient, as both SET and DET might be clinically safe to perform. However, even in the first scenario, there needs to be an exploration of patient values and desires so that the care provider can understand why the patient may be reluctant to accept eSET and can address their concerns, even if the provider considers eSET to be the only acceptable transfer option in their specific situation.

#### *Shared decision-making: information provision*

Scientific evidence demonstrates the benefits of providing patients with information regarding ART treatment success and health risks with the transfer of >1 embryo and multiple pregnancies. Providing information on the risks associated with twin pregnancies increased the desirability of eSET and decreased the desirability of DET ([Newton et al., 2007](#)).

Information that the patient should receive regarding the number of embryos to transfer includes the same categories a clinician needs in order to formulate a recommendation: information regarding the factors that predict the chance of pregnancy, respectively live birth with eSET and DET and as well as information about the medical, economic, social and psychological consequences of the birth of ART singletons and twins. These issues are already covered in the preceding chapters.

An evidence-based decision aid to promote shared decision-making on the number of embryos transferred (eSET vs. DET) was developed ([Van Peperstraten et al., 2010a](#)) and tested in an RCT ([van Peperstraten et al., 2010b](#)) during the years when eSET was not yet widespread practice in The Netherlands. The decision aid provided information about the chances of pregnancy with eSET and DET and the factors that predict chance of pregnancy, about the differences in complication rates of ART singletons and ART twins, and about the available options of the couple. This information was used for agreeing between eSET and DET. In couples who used the decision aid in addition to standard counselling (n=152), the proportion of those who wanted to decide for themselves on the number of embryos to be transferred increased, while this percentage remained the same in the control group (n=156). Levels of experienced knowledge and actual knowledge were also higher in the intervention group compared with the control group. There was a trend towards a more frequent choice of eSET among these couples (43% vs. 32%, NS). A more

recent study from the same team showed that using the decision aid and taking into account patient preferences decreased variation in hospitals practice of SET and DET during the RCT trial ([Brabers et al., 2016](#)).

Information provision regarding eSET vs. DET was investigated in 55 recipients of donated oocytes ([Clua et al., 2020](#)). Counselling included information about outcomes with SET and DET and about the obstetric and perinatal risks in multiple gestation both in the clinic and according to literature review. This counselling changed the preferences from DET to eSET in 41% of cases while among patients who preferred eSET, none changed their preferences. Following counselling, the patients attached less importance to the probability of pregnancy and more importance to maternal and perinatal risks.

#### *Shared decision-making: patient attitudes*

Becoming aware of patient attitudes and knowledge concerning the number of embryos to transfer, singleton, and twin issues are a key element of the shared decision-making process.

Research on ART patient counselling has revealed differences between men and women. In heterosexual couples, it is important to consider the needs of both the female and the male when providing information ([Blennborn et al., 2007](#), [Blennborn et al., 2005](#)). Men should be more involved and need more information in the decision to transfer one embryo only, while women are more aware of the risks but the decision to accept SET was more difficult for them.

**The GDG strongly recommends that healthcare professionals discuss with the patients a number of issues related to the number of embryos to transfer. Main topics include:**

- Medical, economic, social and psychological consequences of transferring >1 embryo.
- Patient wishes regarding family building.
- Clinical, science-based recommendations for the specific patient case.

**Key elements to the discussion, and the decision-making process regarding the number of embryos to transfer are the following:**

- Patient involvement, which ensures a decision that reflects both healthcare professionals' good clinical judgement and the patients' values and personal context.
- Involvement of both members of the patient couple.

GPP

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# Conclusions

As there is no evidence showing that cLBR in eSET is inferior to that in DET, and as published data clearly demonstrate that the MBR after DET significantly exceeds that after (e)SET, the GDG recommends eSET as the standard procedure whenever more than one embryo is available.

The following conditions warrant strictly the use of eSET:

- Donor oocyte and donated embryo recipients.
- Gestational carriers.
- Patients at risk of OHSS for whom a fresh embryo transfer is planned.
- Fresh cycle blastocyst transfers.

Neither the stage of embryo development nor its morphology at transfer, nor the ovarian response to ovarian hyperstimulation justifies DET instead of eSET. Issues related to the number of embryos transferred, including success rates and short- and long-term health, financial, and well-being aspects of life with singletons or twins, should be thoroughly discussed with patients. Patients must be specifically informed about the risks of a multiple pregnancy and the potential consequences of that in case of transferring two embryos instead of one. If more than one embryo is to be transferred, an additional written informed consent should be signed (**annex 11**).



# Annexes

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## Annex 1. Guideline Development Group

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None declared.

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None declared.

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None declared.

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None declared.

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None declared.

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None declared.

*Methodological support*

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None declared.

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None declared.

## Annex 2: Summary of findings tables

### Summary of findings table 1:

#### Medical risks associated with multiple pregnancies (twins or HOM) compared to singletons

**Patient or population:** Women undergoing ART

**Intervention:** Twins [intervention]

**Comparison:** singletons [comparison]

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with SET [comparison]	Risk with transfer of more than one embryo [intervention]				
Caesarean delivery (CS) (Eapen et al., 2020)	455 per 1,000	<b>755 per 1,000</b> (733 to 774)	<b>OR 3.7</b> (3.3 to 4.1)	280453 (23 observational studies)	⊕⊕⊕○ Moderate	
Gestational diabetes (GD) (Eapen et al., 2020)	89 per 1,000	<b>105 per 1,000</b> (97 to 113)	<b>OR 1.2</b> (1.1 to 1.3)	222193 (11 observational studies)	⊕⊕⊕○ <sup>a</sup> High	
Preterm labour (Eapen et al., 2020)	172 per 1,000	<b>567 per 1,000</b> (428 to 695)	<b>OR 6.3</b> (3.6 to 11.0)	5573 (6 observational studies)	⊕⊕○○ Low	
Pregnancy-induced hypertension (Eapen et al., 2020)	56 per 1,000	<b>105 per 1,000</b> (100 to 119)	<b>OR 2.0</b> (1.9 to 2.3)	221336 (11 observational studies)	⊕⊕⊕○ Moderate	
Pre-eclampsia (Eapen et al., 2020)	39 per 1,000	<b>71 per 1,000</b> (54 to 95)	<b>OR 1.9</b> (1.4 to 2.6)	6712 (7 observational studies)	⊕⊕⊕○ Moderate	
Preterm birth (Eapen et al., 2020)	214 per 1,000	<b>694 per 1,000</b> (680 to 708)	<b>OR 8.3</b> (7.8 to 8.9)	1148116 (43 observational studies)	⊕⊕○○ Low	
NICU admission (Eapen et al., 2020)	90 per 1,000	<b>392 per 1,000</b> (365 to 420)	<b>OR 6.5</b> (5.8 to 7.3)	241339 (11 observational studies)	⊕⊕○○ Low	
Perinatal mortality (Eapen et al., 2020)	12 per 1,000	<b>28 per 1,000</b> (25 to 33)	<b>OR 2.4</b> (2.1 to 2.8)	45916 (10 observational studies)	⊕⊕○○ Low	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; OR: odds ratio

<sup>a</sup> downgraded because the evidence is based on observational studies

## Summary of findings 2:

### DET compared to SET for women ≤37y

**Patient or population:** Women ≤37y undergoing ART

**Intervention:** DET

**Comparison:** SET

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with SET	Risk with DET				
Cumulative live birth rate (cLBR) <i>(Kamath et al. 2020)</i>	393 per 1,000	<b>413 per 1,000</b> (354 to 472)	<b>RR 1.05</b> (0.90 to 1.20)	985 (4 RCTs)	⊕⊕○○ Low <sup>a</sup>	
Cumulative multiple pregnancy rate (cMPR) <i>(Kamath et al. 2020)</i>	2 per 1,000	<b>16 per 1,000</b> (10 to 25)	<b>OR 7.70</b> (4.76 to 12.50)	985 (4 RCTs)	⊕⊕⊕○ Moderate <sup>b</sup>	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; OR: odds ratio; RR: risk ratio

#### GRADE Working Group grades of evidence

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

<sup>a</sup> Very serious risk of bias: high risk or unclear risk for allocation concealment, high risk of bias of performance, bias due to lack of blinding.

<sup>b</sup> Serious risk of bias: high risk or unclear risk of bias for allocation concealment.

### Summary of findings 3:

#### DET compared to SET for women 35-40y and women >40y

**Patient or population:** women 35-40y and women >40y undergoing ART

**Intervention:** DET

**Comparison:** SET

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with SET	Risk with DET				
Live birth rate 35-40y (LBR) <i>(Ma et al. 2022)</i>	269 per 1,000	<b>315 per 1,000</b> (280 to 355)	<b>OR 1.25</b> (1.06 to 1.50)	31294 (6 observational studies)	⊕⊕○○ Low	
Multiple birth rate 35-40y (MPR) <i>(Ma et al. 2022)</i>	7 per 1,000	<b>153 per 1,000</b> (107 to 194)	<b>OR 25.00</b> (16.67 to 33.33)	18867 (5 observational studies)	⊕⊕○○ Low	
Live birth rate women >40y (LBR) <i>(Ma et al. 2022)</i>	87 per 1,000	<b>98 per 1,000</b> (63 to 149)	<b>OR 1.15</b> (0.71 to 1.85)	5979 (4 observational studies)	⊕⊕○○ Low	
Multiple pregnancy rate >40y (MPR) <i>(Ma et al. 2022)</i>	0 per 1,000	<b>0 per 1,000</b> (0 to 0)	<b>OR 2.94</b> (0.49 to 16.67)	875 (3 observational studies)	⊕⊕○○ Low <sup>a</sup>	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; OR: odds ratio; RR: risk ratio

#### GRADE Working Group grades of evidence

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

## Summary of findings 4:

### DET compared to SET in fresh cleavage stage embryo transfer

**Patient or population:** fresh cleavage stage embryo transfer

**Intervention:** DET

**Comparison:** SET

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with SET[comparison]	Risk with DET[intervention]				
Live birth rate (LBR) <i>(Thurin et al., 2004)</i> <i>(Martikainen et al., 2001)</i>	405 per 1,000	<b>557 per 1,000</b> (493 to 621)	<b>OR 1.85</b> (1.43 to 2.41)	805 (2 RCTs)	⊕⊕⊕○ Moderate	
Multiple pregnancy rate (MPR) <i>(Thurin et al., 2004)</i> <i>(Martikainen et al., 2001)</i>	18 per 1,000	<b>45 per 1,000</b> (11 to 178)	<b>OR 2.640</b> (0.632 to 11.978)	283 (2 RCTs)	⊕⊕⊕○ Moderate	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; OR: odds ratio

#### GRADE Working Group grades of evidence

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.



## Summary of findings 5:

### Reduced triplets compared to non-reduced triplets for women with triplets

**Patient or population:** Women with a triplet pregnancy

**Intervention:** reduced triplets (abdominal KCl)

**Comparison:** non-reduced triplets

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with non- reduced triplets	Risk with reduced triplets				
Preterm delivery rate <32 weeks <i>(Zipori et al. 2017)</i>	281 per 1,000	<b>102 per 1,000</b> (72 to 138)	<b>OR 0.29</b> (0.20 to 0.41)	1144 (10 observational studies)	⊕○○○○ Very low <sup>a,b</sup>	
Preterm delivery rate <28 weeks <i>(Zipori et al. 2017)</i>	90 per 1,000	<b>33 per 1,000</b> (17 to 63)	<b>OR 0.35</b> (0.18 to 0.68)	951 (7 observational studies)	⊕○○○○ Very low <sup>a,b</sup>	
Pregnancy loss <24 weeks <i>(Zipori et al. 2017)</i>	82 per 1,000	<b>73 per 1,000</b> (45 to 116)	<b>OR 0.89</b> (0.53 to 1.48)	2184 (15 observational studies)	⊕○○○○ Very low <sup>a,b</sup>	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; OR: odds ratio

#### GRADE Working Group grades of evidence

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

a. Selection bias

b. Population Heterogeneity

## Summary of findings 6:

### Reduced twins to singletons compared to twins for women with a twin pregnancy

**Patient or population:** women with a twin pregnancy

**Intervention:** Reduced Twins to singletons

**Comparison:** Expectant management of twins

Outcomes	Anticipated absolute effects* (95%CI)		Relative effect (95%CI)	No of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with Twins	Risk with Reduced Twins to singletons				
Preterm delivery rate	400 per 1,000	<b>12 per 1,000</b> (88 to 160)	<b>RR 0.03</b> (0.22 to 0.40)	7297 (5 observational studies)	⊕○○○ Very low <sup>a,b</sup>	
Pregnancy loss	19 per 1,000	<b>30 per 1,000</b> (17 to 52)	<b>RR 1.57</b> (0.90 to 2.75)	7355 (5 observational studies)	⊕○○○ Very low <sup>a,b</sup>	

\*The risk in the intervention group (and its 95%CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95%CI).

CI: confidence interval; RR: risk ratio

#### GRADE Working Group grades of evidence

**High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.

**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

a. Selection bias

b. Population Heterogeneity

### Annex 3: Research recommendations

Having performed an extensive review of most scientific articles analysing data on embryo transfer up to September 2022, and having carefully considered available evidence also from the patient survey on the number of embryos to transfer, the GDG identified several topics with inconsistent, insufficient or non-existing evidence.

For the benefit of infertility patients who are treated with ART, the GDG recommends that future research, where possible in well-designed RCTs, should focus on addressing the following research gaps:

- Specific combinations of infertility diagnoses, female age, number of previous treatments, embryo quality that warrant the transfer of two instead of one embryo.
- Endometrial factors that are highly predictive of successful, or of unsuccessful implantation.
- Clinical outcomes in treatments with donated embryos.
- Patient attitudes towards embryo transfer- and pregnancy-related issues specifically in patients with no preference towards twins or singletons for live birth.
- Long-term follow-up and use of international registries to monitor outcomes.

In addition, when reporting research on vitrified-warmed treatments, the GDG recommends to include details on the minimal embryo criteria for vitrification and/or transfer, as well as on the selection of devices or embryos for thawing and warming. This may include information on whether embryos were randomly selected or chosen based on quality criteria (e.g., selecting the first embryos with the best quality).

## Annex 4: Abbreviations

ART	Assisted reproductive technology
ASRM	American Society for Reproductive Medicine
CI	Confidence interval
cLBR	Cumulative live birth rate
cSET	Compulsory single embryo transfer
DET	Double embryo transfer
EIM	European IVF monitoring
eSET	Elective single embryo transfer
(e)SET	(elective) Single embryo transfer
FET	Frozen embryo transfer
FSH	Follicle stimulating hormone
g	Gram
GDG	Guideline development group
GPP	Good practice point
HOM	Higher-order multiple
ICM	Inner cell mass
ICSI	Intracytoplasmic Sperm Injection
IR	Implantation rate
IVF	In vitro fertilisation
LBR	Live birth rate
mm	Millimetre
MMH	Mental maternal health
MPR	Multiple pregnancy rate
MZT	Monozygotic twinning
NICU	Neonatal intensive care unit
NS	Non-significant
OHSS	Ovarian hyperstimulation syndrome
OR	Odds ratio
PR	Pregnancy rate
PSM	Propensity score matching
RCT	Randomised controlled trial
RR	Risk ratio
SART	Society for Assisted Reproductive Technology
SET	Single embryo transfer
SIG	Special interest group
sof	Summary of findings table
SQART	Safety and quality in ART
TE	Trophectoderm
TET	Triple embryo transfer
TL	Time-lapse
y	Years old

## Annex 5: Methodology

### *Guideline development*

European Society of Human Reproduction and Embryology (ESHRE) guidelines are developed based on the Manual for ESHRE guideline development (N. Vermeulen, N. Le Clef, S. Mcheik, A. D'Angelo, K. Tilleman, Z. Veleva, W.L.D.M. Nelen, Manual for ESHRE guideline development, version 2020), which can be consulted at the ESHRE website (<https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Guideline-development-process> ). The principal aim of this manual is to provide stepwise advice on ESHRE guideline development for members of ESHRE guideline development groups. The manual describes a 12-step procedure for writing clinical management guidelines by the guideline development group, supported by the ESHRE methodological expert:

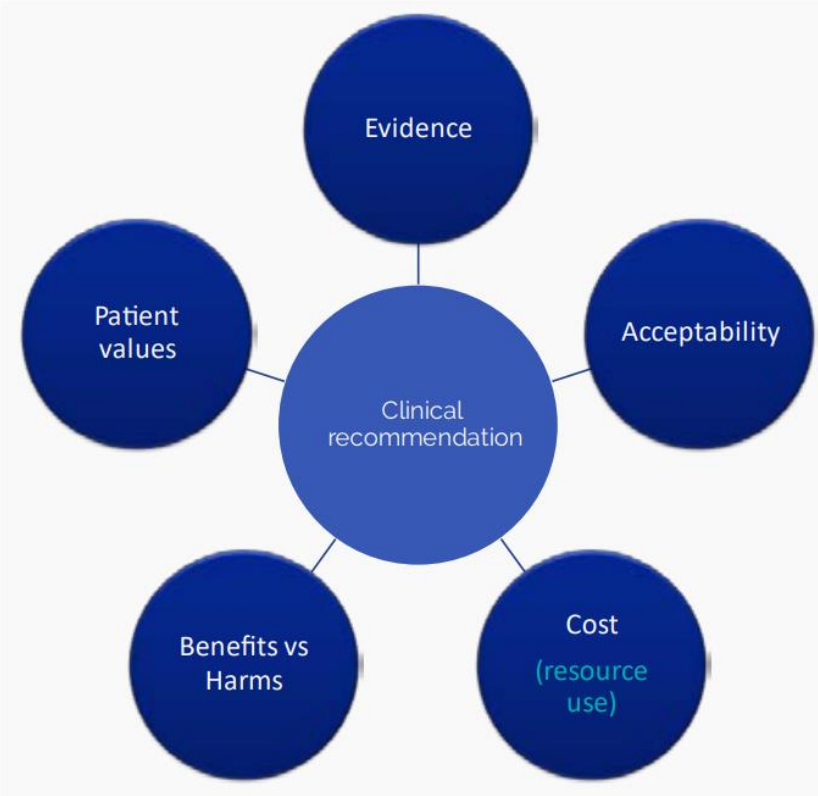


The current guideline was developed and funded by ESHRE, which covered expenses associated with the guideline meetings (travel, hotel and catering expenses) associated with the literature searches (library costs, costs associated with the retrieval of papers) and with the implementation of the guideline (printing, publication costs). Except for reimbursement of their travel expenses, GDG members did not receive any payment for their participation in the guideline development process.

The scope of the guideline and first version of the key questions were drafted by the coordinator and the immediate past co-ordinator of the ESHRE SIG SQART. A call was launched for experts in the field interested in joining the guideline development group. All applications were reviewed, and experts were selected based on expertise and geographical location. We strived towards a balance in gender and location within Europe. A meeting of the guideline development group was organised to discuss the key questions and redefine them through the PICO process (patients – interventions – comparison – outcome). This resulted in a final list of 22 key questions. Based on the defined key words, literature searches were performed by the

methodological expert (Dr. S. Mcheik). Key words were sorted to importance and used for searches in PUBMED/MEDLINE and the Cochrane library. We searched the databases from inception up to 22 September 2022. Relevant studies published after this date were added manually.

Literature searches were performed as an iterative process. In a first step, systematic reviews and meta-analyses were collected. If no results were found, the search was extended to randomised controlled trials, and further to cohort studies and case reports, following the hierarchy of the levels of evidence. References were selected or excluded by the methodological expert and expert GDG member based on title and abstract and knowledge of the existing literature. If necessary, additional searches were performed in order to get the final list of papers. The quality of the selected papers was assessed by means of the quality assessment checklist, defined in the ESHRE guideline manual. Furthermore, the evidence was collected and summarised in an evidence table according to GIN format (<http://www.g-i-n.net/activities/etwg>). The quality assessment and evidence tables were constructed by the expert GDG members.



Summary of findings tables are usually prepared according to the GRADE approach for all intervention studies with at least 2 studies per outcome. Where available, summary of findings tables (**annex 2**) were based on existing up-to-date well-executed systematic reviews, if necessary supplemented with additional recent RCTs. When there was no recent

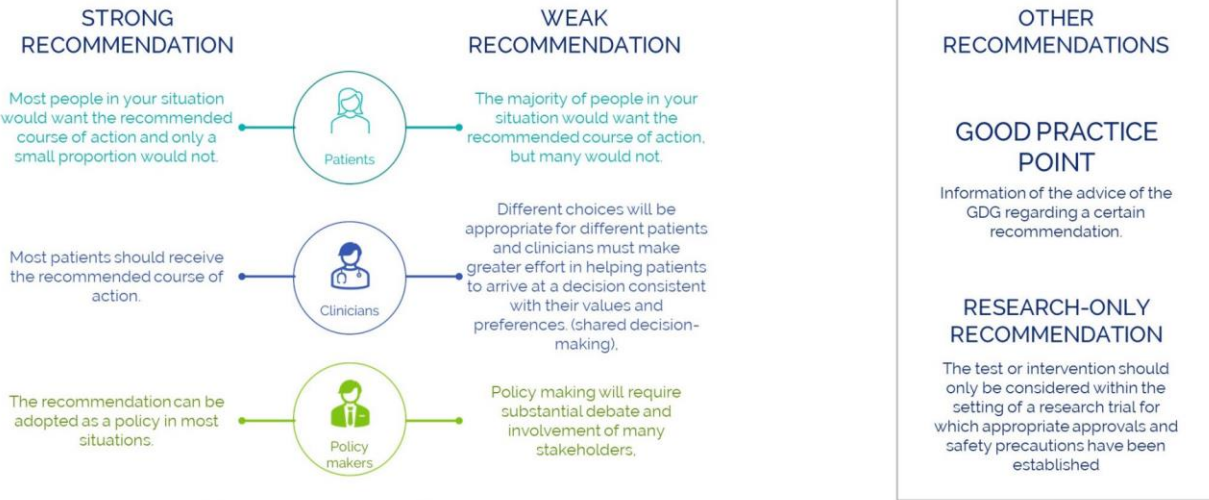
valid systematic review available, we systematically searched for relevant studies, as described

above, with focus on prospective (randomised) studies. Cumulative live birth rate, live birth rate and multiple pregnancy rate were considered the critical outcomes.

GDG meetings were organised to discuss the draft recommendations and the supporting evidence and to reach consensus on the final formulation of the recommendations. In a final step, all evidence and recommendations were combined in the ESHRE guideline: “Number of embryos to transfer during IVF/ICSI”.

*Implications of recommendations*

We labelled the recommendations as either “strong” or “weak/conditional” according to the GRADE approach, with appropriate wording for each option. Suggested interpretation of strong and weak/conditional recommendations by patients, clinicians and health care policy makers is as follows:



For each recommendation it is mentioned whether it is strong or conditional and what the quality of the supporting evidence was. In the justification section, more data are provided on the considerations taken into account when formulating the recommendations: balance between desirable and undesirable effects, certainty of the evidence of effects, certainty in how people value the outcome, acceptability and feasibility of the intervention. Impact on health equity and resource impact were only discussed where relevant.

Good practice points or GPPs are mainly based on the expertise and opinion of guideline group members. GPPs can be used to emphasize the importance of patient participation in decision-making about specific procedure, provide advice on the management of specific surgical

procedures for which there is an evidence-based recommendation, or advise caution where there is perceived risk of harm but no available direct evidence such as harms.

#### *Strategy for review of the Guideline draft*

After finalisation of the guideline draft, the review process was initiated. The draft guideline was published on the ESHRE website, accompanied by the reviewers' comments form and a short explanation of the review process. The guideline was open for review between 15 May and 23 June 2023.

To notify interested clinicians, we sent out an invitation to review the guideline by email to all members of the ESHRE SIG SQART, Embryology, Reproductive endocrinology, ethics and laws, psychology and patient counselling. An announcement was also posted on ESHRE website and social media pages. Selected reviewers were personally invited by email.

All reviewers that submitted comments are listed in **annex 6**. The review report, including further information on the review and a list of all comments per reviewer with the response formulated by the GDG is published on the ESHRE website.

#### *Guideline Implementation strategy*

The standard dissemination procedure for all ESHRE guidelines comprises publishing and announcement. Each guideline is published on the ESHRE Website and in Human Reproduction. The announcement procedure includes a newsflash on the ESHRE website homepage. All participants in the annual ESHRE meeting and all related national societies and patient organisations are informed about the guideline release. The latter are asked to encourage local implementation by, for instance, translations or condensed versions, but they are also offered a website link to the original document. Patient versions of the guideline will be developed by a subgroup of the GDG together with patient the representatives. The patient version is a translation of the recommendations in everyday language, with emphasis on questions important to patients. It aims to help patients understand the guideline's recommendations and facilitates clinical decision-making. To further enhance implementation of the guideline, the members of the GDG, as experts in the field, will be asked to make suggestions for tailor-made implementation interventions (e.g., option grids, flow-charts, additional recommendations, addition of graphic/visual material to the guideline).



### *Schedule for updating the guideline*

The current guideline will be considered for revision in 2027 (four years after publication). An intermediate search for new evidence will be performed two years after publication, which will inform the GDG of the necessity of an update. Every care is taken to ensure that this publication is correct in every detail at the time of publication. However, in the event of errors or omissions, corrections will be published in the web version of this document, which is the definitive version at all times. This version can be found at <https://www.eshre.eu/Guidelines-and-Legal>.

For more details on the methodology of ESHRE guidelines, visit [www.eshre.eu/Guidelines-and-Legal](http://www.eshre.eu/Guidelines-and-Legal).

## Annex 6: Stakeholder consultation (See also separate document)

The guideline draft was published for review for 6 weeks, between May 15 and June 23, 2023. All reviewers, their comments and the reply of the GDG are summarised in a review report, which is published on the ESHRE website as supporting documentation to the guideline. The list of representatives of professional organisation, and of individual experts that provided comments to the guideline is presented below:

<b>Representative</b>	<b>Participation on behalf of</b>
<b>Fazilah Abdul Hamid</b>	Advanced Reproductive Centre, Malaysian National University Hospital -Malaysia
<b>Vyacheslav Lokshin</b>	Kazakhstan Association of the reproductive medicine and Persona ART clinic - Kazakhstan
<b>Ernesto Viega Alvarez</b>	Commission ART of the Sociedad Española De Química Clínica (SEO) - Spain

<b>Reviewer</b>	<b>Country</b>
<b>Iana Malasevskaia</b>	Yemen
<b>Liisa Kuusipalo</b>	Finland
<b>Maha Malkawi</b>	UK
<b>Marco Sbracia</b>	Italy
<b>Demian Glujovsky</b>	Argentina
<b>Arianna D'Angelo</b>	UK/UAE
<b>Carlos Calhaz-Jorge</b>	Portugal
<b>Liliana Ramos</b>	The Netherlands
<b>Charles Coddington</b>	USA
<b>Ursula Eichenlaub Ritter</b>	Germany
<b>Jeanette Bogstad</b>	Denmark
<b>Anja Pinborg</b>	Denmark
<b>Janne Bentzen</b>	Denmark
<b>Keerti Singh</b>	Barbados
<b>Christiana Antoniadou Stylianou</b>	United Arab Emirates
<b>Lianne Marie Aquilina</b>	UK

- Annex 7: Survey Results (separate document)
- Annex 8: Literature study report (separate document)
- Annex 9: Evidence tables (separate document)
- Annex 10: Patient scenarios (separate document)
- Annex 11: Informed consent form (separate document)