FVV in ObGyn, 2012, 4 (1): 51-58

Original paper

Does transferring three or more embryos make sense for a well-defined population of infertility patients undergoing IVF/ICSI?

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Abstract

Recently, there has been a marked increase in the use of Single Embryo Transfer (SET) subsequent to In Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI), with the aim of reducing the risk of multiple gestations. However, critics have stated that by reducing the number of embryos transferred, a group of women with an *a priori* reduced chance of pregnancy are at particular greater risk of undertreatment. This group includes women who are of a certain age (≥ 40 years) or have already received a number of – failed –IVF attempts. We wanted to study whether the practice of three or more embryos being transferred would be of added value to these patients and whether the strategy of Heavy Load Transfer (HLT) is likely to boost the pregnancy rates to an acceptable level. We performed both a literature study and a retrospective cohort analysis of 7,850 IVF/ICSI cycles of early cleavage stage embryo transfer.

Notwithstanding the limitations associated with this approach, we contend that HLT in the group of patients with poor prognosis should be recommended. This article outlines a suggested protocol within the legal framework relevant to Belgium.

Key words: **advanced maternal age, subfertility, Heavy Load Transfer, embryo transfer, multiple gestations, never pregnant prone.**

Introduction

There is a general consensus in the medical community that the number of embryos transferred during ART treatment should be limited in young women, so as to reduce the intrinsic risk of multiple pregnancies (Ombelet et al., 2005). Elective Single Embryo Transfer (eSET) is defined as the transfer of one good-quality embryo when there are at least two good-quality embryos available (Norian et al., 2009). Adopting a careful strategy of patient selection for single embryo transfer on the one hand, and the availability of at least one good-quality embryo to transfer on the other, are effective in reducing overall multiple pregnancy rates without a significant decrease in the percentage of live births (Criniti et al., 2005; Gerris et al., 1999). eSET is increasingly common in clinical practice and could provide a cheaper and potentially safer solution to women with a good prognosis (Gerris et al., 2009). ESHRE reports increases in SET implementation and subsequent steady decrease in multiple pregnancies (EHRE, 2010).

Belgium was one of the first countries to implement eSET on a large scale. The Belgian policy is tabulated below (Dhont et al., 2009; Gerris et al., 2009) (Table 1). This strategy has succeeded in reducing the incidence of multiple pregnancies whilst keeping constant live birth rates in the group of patients with good prognosis, including young women (< 36 years of age), during their first two IVF or ICSI attempts and with a high embryo yield. This "twin-prone" group is the target group for Single-Embryo Transfer (Gerris et al., 2009).

Fertility however, defined as the ability to become pregnant, declines steadily with age for women. The progressive loss of the ability to achieve a pregnancy is a normal biological process that takes place up to ten years before the onset of the menopause (Tarlatzis et al., 2003). In terms of fertility, women of 35 years of age and older are considered women of advanced reproductive age (Dhont et al., 2009).

Table 1. — The Belgian policy for financing IVF (Dhont et al., 2009; Gerris et al., 2009).

< 36 years	First attempt	Single Embryo Transfer (SET)
	2nd attempt	– Single Embryo Transfer (SET), if one or more good-quality embryos are available. – Transfer of two embryos, if no good embryos are available.
	3th to 6th attempt	Maximum of two embryos
≥ 36 and ≤ 39 years	1th and 2nd attempt	Maximum of two embryos
	3th to 6th attempt	Maximum of three embryos
> 39 and ≤ 42 years	No limit to the maximum number of embryos	

The combination of reduced fertility and deliberately postponing pregnancy has resulted in a large group of women aged more than 35 failing to conceive within twelve months. A growing number of these couples are forced to rely on ART to compensate for the effects of their diminished fertility (Leridon, 2004). Unfortunately, IVF in itself cannot compensate for reduced natural fecundity (Klipstein et al., 2005; Spandorfer et al., 2007). The chances of a spontaneous conception occurring in women aged 35 and older are still significant, but in case of failure, ART will never recover the lost years (Leridon, 2004).

Several factors determine IVF/ICSI success rates. Ageing in women has been shown to correlate with poor ovarian response. In addition, developmental competence of embryos tends to lessen with age, both because of genetic factors (aneuploidy, gene mutations) and because of a lack of energy supply to a metabolically very active cell machinery (mitochondrial function) (Eichenlaub-Ritter et al., 2003; ESHRE, 2005; Van Blerkom et al., 2000). Women of older reproductive age are therefore considered as patients with a poor prognosis (Klipstein et al., 2005; Lass et al., 1998, Schieve et al., 1999; Tarlatzis et al., 2003; Templeton et al., 1996). The number of embryos obtained after stimulation and transferred also matters. Indeed, the number of live births in each age group increases with each additional embryo transfer carried out (Elsner et al., 1997; Klipstein et al.,2005; Opsahl et al., 2001; Roest et al., 1996; Widra et al., 1996). A number of previous failed IVF attempts, typically associated with poor embryo quality, also correlates with a poor IVF prognosis (Elsner et al., 1997; Templeton et al., 1998).

The only rationale for improving IVF/ICSI outcome in these patients (older age and multiple failures) is to increase the number of available embryos transferred. Martin and Welch (1998) examined the theoretical pregnancy rates after completion of a single IVF cycle: increasing the number of embryos transferred improves the probability of pregnancy. It follows that lower implantation rates (which is common in older women) (Tarlatzis et al., 2003; Rowe, 2006) demand a higher number of embryos to be transferred in order to achieve a higher pregnancy rate (Martin and Welch, 1998) (Fig. 1). However, when transferring multiple embryos, consideration should be given to the risk of multiple births. Multiple pregnancies typically worsen the prognosis for the mother and babies, inducing high costs for society (Wennerholm, 2009).

On the other side of the clinical spectrum of the earlier discussed twin prone group, there is a never-pregnant group that tends to be undertreated when only eSET is used. Mostly, they are women aged 40 years or older with a low ovarian response to stimulation and who have had multiple failed IVF attempts. If pregnant, the miscarriage rate in these patients is high and multiple births are comparatively rare. This is the group of choice for Heavy Load Transfer. It is important to distinguish between these different groups, as each group requires a different treatment strategy. In the never-pregnant group, HLT implies that multiple embryos are transferred –

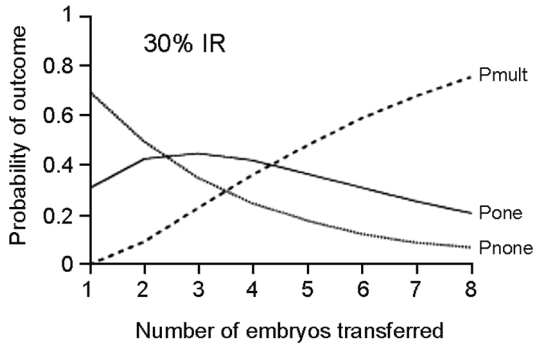

Fig. 1. — The effect of increasing the number of embryos transferred on the probability of pregnancy outcome (assuming an implantation rate of 30%). IR = Implantation Rate; Pmult = probability of multiple births; Pone = probability of a singleton birth; Pnone = probability of no birth. (adapted from Martin et al., 1998).

hoping that at least one developmentally competent embryo is available in a large pool of poor-quality embryos (Opsahl et al., 2001). This approach has often been found to be impaired by the (limited) number of embryos available in these patients (Adonakis et al., 1997).

Based on a literature review and our own research, we investigated which results were obtained after the transfer of a high number of embryos in patients facing final failure of treatment.

Methods

Literature Search

The Pubmed and Elsevier ScienceDirect databases were searched using MeSH (Medical Subject Headings) to retrieve relevant articles matching the search terms: Female age, IVF, ICSI, advanced maternal age, number of embryos transferred, pregnancy outcome, ART, reproductive age, subfertility, fertility, menopause, ageing female, single embryo transfer, multiple embryo transfer, Belgium.

Only studies were included using IVF- and ICSI-cycles with the transfer of fresh embryos with the patient's own oocytes, excluding donated and cryopreserved oocytes in our analysis. Especially was focused on woman of an older reproductive age. Articles included in this study were mainly from Europe and North America published between 1997 and 2010. Our search was limited to articles published in high-impact journals.

Patient Selection Criteria

This retrospective descriptive study was approved by the Ethical Committee of the University Hospital in Ghent (B). We analysed all IVF cycles (n = 7,850) between 13-12- 2002 and 14-12-2009 at the centre for reproductive medicine at Ghent University Hospital. As with our literature study, IVF- and ICSI-cycles with a transfer of fresh embryos with the patient's own oocytes were included, excluding donated and cryopreserved oocytes. We have defined HLT as the transfer of at least three up to all of the available embryos. At an intended last attempt in particular, all embryos could be used. In such cases it must always be convened that fetal reduction may have to be considered in case of a high-order multiple pregnancy.

According to the Belgian legislation (Table 1), HLT is allowed in women younger than 36 years of age from the seventh attempt onwards, considering that only six cycles in a lifetime are financed. Women between 36 and 39 years of age are allowed to have three embryos transferred from the third up to the sixth attempt; following the sixth attempt, there is no limit. As from 40 years old, there is basically no limit as to how many embryos can be transferred, but it would appear that there is a certain reluctance to transfer more than three embryos, although legislation is in place (BELRAP-report, 2007). We specifically included patients who had had at least three attempts elsewhere and had been given up, either because of advanced reproductive age (> 40 years of age) or because of several failed IVF cycles, or both, or patients who had been facing a very strict limitation of the number of embryos to be transferred in their own country, predominantly meaning the Dutch patients. In the majority of cases, these patients had been subjected in our centre to a number of diagnostic tests to rule out known causes of IVF failure, e.g. hysteroscopy, thyroid gland assessment, auto-immune testing or thrombophilia assessment.

Description of the Stimulation Protocol

A short agonist protocol was used, and after stopping a combined oral contraceptive taken for at least 2 weeks, women had an ultrasound scan to confirm the absence of any ovarian cysts. Triptorelin 0.1 mg/day was administered subcutaneously from day 3 of the withdrawal bleeding until day 9. Subsequently, urinary or recombinant gonadotropins, 150 IU/day, were administered subcutaneously from day 5 up to the day of administration of human chorionic gonadotropin (hCG). A maximum dose of 300 IU per day was never exceeded. Follicular development was followed using transvaginal ultrasound. When the average diameter of more than half of follicles was > 18 mm, ovulation was triggered by an injection of 5,000 IU of hCG.

Oocyte Fertilization, Embryo Culture, Embryo Transfer and Follow-up

Oocyte retrieval was performed 36 hours after hCG-injection under ultrasound guidance and under a combination of local anaesthesia and intravenous sedation. Standard IVF and ICSI laboratory procedures were used, described elsewhere. The morphologically best-looking embryos were transvaginally transferred to the uterus two or three days after retrieval, using a Cook catheter (Cook, Belgium). Belgian legislation lays down the maximum number of embryos that can be transferred, as said above (Table 1) (Dhont et al., 2009; Gerris et al., 2009).

After embryo transfer, vaginal progesterone tablets were administered (Utrogestan® 600 mg/day) until menses occurred or until heart action was observed.

Clinical Outcomes

We examined only the most relevant results in our own data-anlysis: the number of live births and the number of single and multiple births.

Results

Literature study

To examine the usefulness of transferring three or more embryos to women of advanced reproductive age, we started reviewing previous studies which have studied the effect of the number of embryos in this age group. The results are tabulated in Table 2.

Although these studies do not deal with what we have defined in this article as HLT and although they are rather concerned with the effect of transferring multiple embryos in the older age group from the first attempt onwards, they do allow some general conclusions regarding this group of patients that may be useful in the broader context we are addressing.

In this population, a high number of embryos transferred correlates with the outcome. The **literature** confirms that a higher chance for pregnancy and for live births in this specific subgroup of older women is obtained by increasing the number of embryos transferred. The data suggest that transfer of four or more embryos has a positive effect: increasing the pregnancy rates without having a significant effect on the multiple pregnancy rates. This effect is only seen in women aged < 45. In women ≥ 45 years of age, only those with a normal ovarian reserve and a successful response of at least five oocytes have been documented to obtain a pregnancy; the others have no chance.

Women aged > 40 years were studied by Adonakis et al. (1997): twins occurred in respectively 11,1% and 17,2% with the transfer of 1-3 and ≥ 4 embryos. Combelles et al. (2005) described a twin rate of 13,3%, 36,7% and 23,8% after the transfer of respectively < 5, 5 and > 5 embryos at the women of ≥ 40 years. Klipstein et al. (2005) reported a 15,3% chance of multiples after the transfer of an average of 3,3 embryos, of which 92,5% were twins. In the group of women aged ≥ 42, less twins were seen. The risk of multiple pregnancies within the group of women aged 40-44 years has been studied by Schieve et al. (1999). They reported a 24,6%, 24,1%, 38,6% risk of multiples (twins or more) and a 2,1%, 0,9%, 5,3% risk of multiples (triplets or more) per live birth transferring respectively five, six, seven or more embryos. These women had only been selected by age and women who have so much embryos retrieved after stimulation, are not really having a bad prognosis. It is clear that a good patient selection is important.

Retrospective analysis of own data

Our own retrospective study was based on data collected from the Ghent University Hospital's Department of Reproductive Medicine. The results are tabulated in Figure 2 and Table 3.

As a consequence of Belgium's strict legislation on the subject, women who had three or more embryos transferred, all had several previous failed attempts and therefore belong to the never pregnant-prone group which is the group in which we wanted to document the results of intentional HLT.

Our data show that the group of women receiving HLT, and with an a priori low chance of pregnancy, still achieve an average ongoing pregnancy rate of 12.7% per (fresh) treatment attempt (Table 4).

Our data show that women who conceive through ART stand an acceptable chance of achieving pregnancy up to the age of 43. In our study, 16.7% out of all women aged 40 to 42 still had a live birth per cycle after the transfer of at least three embryos. After 43 years, the chances of pregnancy are extremely low.

Within the group younger than 40 years there is an excess of singletons with the transfer of 3 embryos, with the transfer of 4 or more embryos twins are more common than singletons. We could doubt the strength of that observance, because it is based on a low count of absolute numbers. After the age of 40 there is an overweight of singletons both the transfer of 3 as the transfer of 4 or more embryos. Triplets were not seen in both age groups (Table 3).

In table 4 it is remarkable that the transfer of five embryos isn't as successful as the transfer of three or four embryos. A possible explanation is that when the clinic makes the decision to transfer five embryos that the quality of the embryos is very low and/or it is the women's last attempt and/or she has a very bad prognosis a priori to become pregnant. In the group of women younger than 36 years, no embryo transfer with five or more embryos occurred. The four cycles with 5 ET that took place in the age group of [36-39] years resulted in no live births. The nine cycles with 5 ET resulted in one singleton at the women aged [40-42] years. After the age of 42, eight cycles took place with the transfer of 5 embryos, none resulted in a live birth.

Discussion

The crucial challenge for doctors in ART is to determine, as accurately as possible, the optimal number of embryos to transfer in order to obtain maximal

Table 2. — Survey **of studies on the relationship between the number of embryos replaced and the outcome of IVF treatment**

Study	Design	Goal	Results	Conclusion
Adonakis *et al.*, 1997	– Belgium (V.U.B.) – Retrospective analysis – 525 ICSI-treatments – women >40 years	Analysing the relationship between the outcome and nET in ICSI-cycles in women >40 years. **Three groups** 112 cycles: no ET 271 cycles: 1ET to 3ET 142 cycles: ≥ 4ET	* Number of pregnancies: NS (7% vs. 12,7%). * Spontaneous miscarriages: NS (25,9% vs. 34,5%) * Twin pregnancies: NS (11,1% vs. 17,2%)	If available, a minimum of four embryos should be transferred to increase chances.
Combelles *et al.*, 2005	– U.S. – Retrospective analysis – 863 ART-treatments – women ≥ 40 years	Does the transfer of several embryos (> 5) raise the pregnancy rates in women over 40 years? **Three groups** 1. < 5ET 2. 5ET 3. > 5ET	* Number live births: (4,3%, 22,6% and 22,3%): every time a NS difference. * Number of live-births after the transfer of 5, 6 and 7 embryos (26.8, 20.7 en 33.7% live births per cycle), every time a NS difference. * The birth of a twin (13,3%, 36,7%, 23,8%): every time a NS difference. * No triplets in the three groups.	Optimal = 5ET; > 5ET brings no added benefit for the clinical outcome (no significantly increased birth rates).
Klipstein *et al.*, 2005	– U.S. – Retrospective analysis – 2705 ART-treatments – women ≥ 40 years – an average of 3.3 embryos transferred	Describes number of live births and predictors of success for women ≥ 40 years who start ART. **Four groups** 1. 40 years 2. 41-43 years 3. 44 years 4. > 44 years	* Number of live births per cycle in women ≥ 40 years: 9.7%. Live births in women 40 years of age significantly higher than in 41-43 year old women, 44-year olds: significantly lower, > 44 years: extremely low. * With each additional embryo eligible for transfer, the number of live births increased significantly. * 15.3%: live-born multiples, of which 92.5% twins. At ≥ 42 years: reduced risk of twins. No multiple births at ≥ 44 years.	* From 42 years it is recommended to transfer all available embryos. Exception: women in this age group with a strong response after stimulation and consideration to cryopreservation. * Until the end of the 43th year success rate is positive (> 5%). * If ≥ 44 years: other options (such as donation, adoption).
Spandorfer *et al.*, 2007	– U.S. - Retrospective analysis – 161 IVF-cycles – Women from 45 to 49 years – The number of embryos replaced did not vary with the patients age, an average of 3.2 (SD ± 1.5) embryos were transferred.	Determine the IVF outcome in women > 44 years.	* Miscarriages are very frequent (85.3%) and average number of births is very low (3.1%). * Pregnancies only in the group of women with ovarian response of more than five eggs. No pregnancies in women ≥ 46 years.	At > 45 years, IVF makes only sense in women with normal ovarian reserve and response of ≥ 5 oocytes.
Schieve *et al.*, 1999	– U.S. – Retrospective study – 35.554 IVF-treatments	Find associations between the nET in IVF and number of live births and multiple births classified according to maternal age and to availability of extra embryos. Discusses only women between 40 and 44 years of age (n = 5016).	* For women aged between 40 and 44: highest live births with 5ET/6ET. * *5ET vs. 6 ET:* – live births (20.3 vs. 20.2) very similar. – Multiple births (≥ 2) (24.6% vs. 24.1%) almost the same – Multiple births (≥ 3) relatively low (2.1% vs. 0.9%). * In women whose embryos could be frozen, the number of live births with fewer embryos transferred was higher.	5ET and 6ET for women between 40 and 44, gets the highest percentage of live births, with a risk of multiple pregnancy of about 24% and a low risk of multiple births (three or more) (between 0.8 and 2.1%). The results were further verified in this study including only those women who underwent the first IVF attempt, no difference noted from current findings.
Stern *et al.*, 2009	– U.S. - Retrospective study - 36.103 treatments – Women ≥ 38 years – First IVF-treatment	Determine the optimal nET in women ≥ 38 years.	* Number of pregnancies and births increased in 38-year-olds after 3ET and in 39-year olds after 4ET. When nET was stepped up, only the number of multiple births increased but not the number of live births. * In women > 40 years, the number of pregnancies and multiple births increased to 5ET. In 40-year-old women with 4ET or 5ET no increase was seen after this nET. In the 41-42 year olds 5ET or 6ET.	Protocol for patients receiving their first ART treatment. The number of oocytes obtained by stimulation is used as a criterion for the decision in nET.

(NS = not significant (p > 0,05), S = significant, ET = Embryo Transfer, n = number of embryos transferred, NPP = never pregnant-prone group).

Table 3. — Live births to women with the never pregnant prone-profile in the three age groups who had three, four or five fresh embryos transferred.

	< 36 years		[36-39] years		≥ 40 years	
	3ET	**≥ 44ET**	**3ET**	**≥ 44ET**	**3ET**	**≥ 44ET**
No live birth	53/68 = 77.9%	9/11 = 81.8%	137/162 = 84.5%	9/10 = 90.0%	291/323 = 90.1%	56/63 = 88.9%
Singletons	9/68 = 13.2%	0/11= 0%	22/162 = 13.6%	0/10 = 0%	26/323 = 8.0%	5/63 = 7.9%
Twins	6/68 = 8.9%	2/11 = 18.2%	3/162 = 1.9%	1/10 = 10.0%	6/323 = 1.9%	2/63 = 3.2%

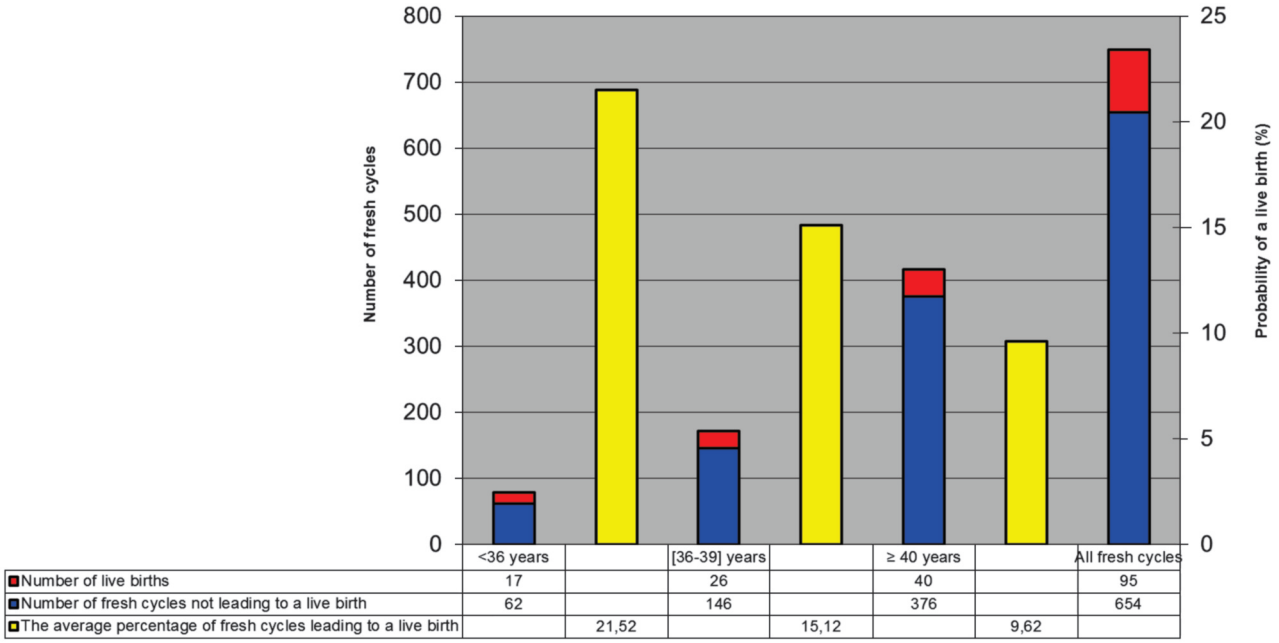

Fig. 2. — The probability of a live birth after the transfer of at least three fresh embryos with IVF/ICSI for the different age groups

chances of pregnancy while trying to minimise the risk of multiple pregnancies (Stern et al., 2009).

Recent clinical data (Garrido et al., 2011) suggest that the total number of transferred embryos rather than the number of attempts should provide guidance whether to stop treatment or to continue. This approach seems very logical and may be implying HLT during the last attempt(s) in these patients.

The subpopulation of patients for HLT remains to be strictly defined. It comprises both patients with a poor prognosis right from the beginning (mainly because of age or low ovarian reserve) and patients who notwithstanding several "good" embryo transfers, did never conceive. The clinical data of Garrido et al. (2011) could help us defining the subgroup.

Although the number of observations is low, we wanted to report objectively what can be expected when continuing to treat patients that were given up elsewhere after several failed IVF cycles, or who had been facing a very strict limitation of the number of embryos to be transferred in their own country (e.g. the Netherlands). For good-prognosis patients, requiring SET, recommendations exist and we fully adhere to them (Jones et al., 2010; Karlström et al., 2007; Ombelet et al., 2005; Saldeen et al., 2005). For the other side of the clinical spectrum, no recommendations exist or are rather vague. Undertreatment is a real risk in these patients. Both the literature study and our own data analysis show that SET clearly is not to be regarded as an ideal option for women of older reproductive age. The transfer of several embryos in the older reproductive age group has shown to have a beneficial effect on pregnancy rates right from the beginning of treatment. The present observation is that in the group of women facing final failure, applying HLT still results in an acceptable pregnancy rate, probably at the price of a significant percentage of twins.

We suggest a clinical protocol (Table 5) enabling clinicians to guide shared decision-making together with their patients, without undermining in the least the spirit of SET or existing legal regulations, but just fine-tuning it in this specific, not altogether small, patient group. This protocol is open to discus-

Table 4. — The probability of a live birth in different age groups classified by the number of embryos transferred.

Probability of a live birth (%)	< 36 years	36-39 years	40-42 years	≥ 43 years	All fresh cycles
3 ET	22.1	25.4	17.3	4.4	13.3
4 ET	18.2	16.7	16.7	0,0	12.4
5 ET	/	0.0	11.1	0,0	3.7
Average chance with ≥ 3ET	21.5	15.1	16.8	3.2	12.7

sion but takes into account Belgium's legal framework as well as a perpetuous concern to avoid multiple pregnancies in early attempts, even in women older than 36 years of age.

We have a few comments on the studies discussed. In the study of Adonakis et al. (1997) the findings should be seen in the context of the then current clinical approach, that was before eSET became common. The patients included could have a less bad prognosis. The study of Combelles et al. (2005) has a low impact due to the small number of live births (= 108). Also there is a restriction within the NPP group: one must of course have five embryos. Study starts from the first treatment cycle, which is a different starting point than we use for the HLT concept. The study of Stern et al. (2009) included only women with their first ART-treatment therefore the effect on the outcome of previous failed IVF-cycles could not be determined. They reported themselves that the analysis misses embryo quality and previous cycle failure.

Both the studies discussed and our own data are retrospective and of a purely descriptive character. Only a large randomized trial can prove the usefulness of transferring many versus just one or two embryos in poor prognosis patients. However, it will be ethically and technically very difficult to conduct such a trial because we are dealing with poor prognosis patients who might not be willing to be randomized into a group receiving one or two embryos and a group receiving at least three and up to all embryos. Taking account of the fact that the correlation between the number of embryos implanted and pregnancy rates has repeatedly been demonstrated (Schieve et al., 1999), it follows that the first group of women would have even smaller chances of becoming pregnant.

We suggest that HLT can be considered for the poor prognosis group of women. Limiting the number of embryos to transfer to a maximum of two in all cases probably leads to undertreatment of this subpopulation. It will be difficult to prove this, nevertheless further studies are needed to strengthen this finding.

Acknowledgements

Petra De Sutter is holder of a fundamental clinical research mandate of the Flemish Foundation of Scientific Researchh (FWO-Vlaanderen).

Table 5. — Suggested clinical protocol. For whom would heavy load transfer (HLT) be a good option (within Belgium's legal framework)?

< 36 years	From the 7th attempt: HLT
[36-39] years	• From the 3rd to the 6thattempt: 3ET • From the 7th attempt: HLT
[40-42] years	• First attempt: 2ET • 2ndattempt: 3ET • 3rd – before last attempt: HLT (5ET) • Last attempt: transfer of all embryos
≥ 43 years	• HLT • Last attempt: transfer of all embryos
> 44 years	Alternatives: egg donation, adoption, abandoning the desire to have children, …

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