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ARTICLE

Accumulation of oocytes: a new strategy for managing low-responder patients

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Abstract Accumulation of oocytes from several ovarian stimulation cycles is currently possible using novel vitrification technologies. This strategy could increase the inseminated cohort, creating a similar situation to normoresponders. This study included 242 low-responder (LR) patients (594 cycles) whose mature oocytes were accumulated by vitrification and inseminated simultaneously (LR-Accu-Vit) and 482 patients (588 cycles) undergoing IVF/embryo transfer with fresh oocytes in each stimulation cycle (LR-fresh). Drop-out rate in the LR-fresh group was >75%. The embryo-transfer cancellation per patient was significantly lower in the LR-Accu-Vit group (9.1%) than the LR-fresh group (34.0%). Live-birth rate (LBR)/patient was higher in the LR-Accu-Vit group (30.2%) than the LR-fresh group (22.4%). Cumulative LBR/patient was statistically higher in the LR-Accu-Vit group (36.4%) than the LR-fresh group (23.7%) and a similar outcome was observed among patients aged ≥40 years (LR-Accu-Vit 15.8% versus LR-fresh 7.1%). The LR-Accu-Vit group had more cycles with embryo cryopreservation (LR-Accu-Vit 28.9% versus LR-fresh 8.7%). Accumulation of oocytes by vitrification and simultaneous insemination represents a successful alternative for LR patients, yielding comparable success rates to those in normoresponders and avoiding adverse effects of a low response.

KEYWORDS: cumulative outcome, live-birth rate, low responder, oocyte vitrification

Introduction

Low responders (LR) to ovarian stimulation represent a substantial proportion of the patients enrolled in assisted reproduction programmes (Jenkins et al., 1991; Pellicer et al., 1987; Surrey and Schoolcraft, 2000). The setbacks of LR are the number of oocytes retrieved and suboptimal oocyte maturation, embryo quality and cycle/transfer cancellation rates with respect to age-matched normoresponders (Karande and Gleicher, 1999).

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LR management is a challenge, as the limited follicular reserve of these patients results in a small cohort of oocytes (Pellicer et al., 1998). A variety of regimens have been employed to optimize the ovarian response of these women, with disappointing results (Garcia-Velasco et al., 2005; Kyrou et al., 2009; Schoolcraft et al., 2008). Moreover, the drop-out rate due to the low probability of becoming pregnant is high, which naturally leads to disappointment and frustration (Verberg et al., 2008). An ideal treatment would provide a similar number of embryos for transfer as that produced by women responding adequately to ovarian stimulation. Therefore, a strategy for obtaining a similar number of oocytes as in normal responders is needed.

A potential alternative to the management of LR is to create a large stock of oocytes by accumulating vitrified metaphase-II oocytes over several stimulation cycles and inseminating them all at the same time. Theoretically, this could help to increase the chances of success by endowing patients with a 'normoresponder-like' status. To confirm this hypothesis, it is necessary to evaluate both the incidence of treatment drop-out among LR patients and the outcome of vitrified–warmed supernumerary embryos.

The alternative of oocyte accumulation was raised recently thanks to the availability of vitrification techniques (Kuwayama et al., 2005) with success rates on a par with those obtained with fresh oocytes (Cobo et al., 2008, 2010; Rienzi et al., 2010). Although slow-freezing procedures have improved greatly over time, the efficiency of vitrification for oocyte cryopreservation seems to be higher, as shown in a very recent meta-analysis (Cobo and Diaz, 2011).

The aim of the current study was to assess the efficiency of a new strategy for managing LR that takes advantage of vitrification as a way of creating larger cohorts of oocytes. A group of LR patients receiving standard treatments was employed as a control.

Materials and methods

Study population

A total of 724 LR patients, defined as women in whom $\leq$5 oocytes were retrieved in a single ovarian stimulation (Surrey and Schoolcraft, 2000), were prospectively included in the study. Eligible patients were selected after evaluating their ovarian function. All patients who met the criteria of having a FSH concentration >11 IU/ml on cycle day 3 (Jayaprakasan et al., 2007; Soldevila et al., 2007) and an antral follicle (2–10 mm during early follicular phase) count <6 among both ovaries (Bancsi et al., 2003; Broekmans et al., 2006) were considered eligible for the study. Additionally, anti-Müllerian hormone blood concentrations <5 pmol/l were also considered as an inclusion criteria (Nelson et al., 2009).

Patient recruitment was conducted between January 2007 and December 2009, after Institutional Review Board approval (No. 1112-C-090-AC). Patients were informed by clinicians about the purpose of the study and were asked to participate. Subjects were usually consulted during a ovarian stimulation cycle in which the number of follicles developed suggested LR status or at an earlier date when LR status had been diagnosed. Thus, the study population was divided into two groups according to the patients' final decisions.

One of these groups included 242 LR-patients whose metaphase-II oocytes were accumulated by vitrification for later simultaneous insemination. This group was named 'low response, accumulation of oocytes and vitrification' (LR-Accu-Vit). After ovarian stimulation and oocyte retrieval, mature oocytes were vitrified and stored for use in a subsequent cycle. After an interval of at least one menstrual cycle, a new ovarian stimulation was performed. Oocytes were either vitrified or inseminated together with those from previous cycles that had been vitrified and later warmed. The procedure was repeated in two or more stimulation cycles. The decision about whether to vitrify the oocytes obtained in a given ovarian stimulation cycle or to inseminate those harvested in previous cycles was based on two factors: (i) the total number of oocytes likely to be available after vitrification (estimated survival rate) (Cobo et al., 2008) and the need for a total number of five embryos to be transferred in consecutive cycles (the number needed to reach a 52% cumulative live-birth rate (LBR), a standard situation for normoresponders (Garrido et al., 2010)); and (ii) the patient's own decision.

The other group, named low response, fresh oocytes (LR-fresh) included 482 LR patients who underwent standard ovarian stimulation including insemination of fresh mature oocytes and subsequent embryo transfer. Patients included in this group refused to accept the vitrification of oocytes mainly due to fatigue and lack of desire to keep trying because of their poor prognosis, in addition to raising doubts about the effectiveness of the strategy of accumulation of oocytes. The interval between a failed ovarian stimulation cycle and the following cycle was the same as in the LR-Accu-Vit group.

Surplus embryos from both groups were vitrified for their subsequent use, as explained below.

Ovarian stimulation

Ovarian stimulation was initiated on cycle day 2 after the absence of ovarian cysts of more than 10 mm had been confirmed via vaginal ultrasound scan. Oral contraceptives (30 μg ethinylestradiol/pill, Yasmin; Bayer, Spain) were administered for 15–21 days when menses initiated. An initial dose of 225–300 IU recombinant FSH (Gonal-F; Merck Serono, Spain) and/or highly purified human menopausal gonadotrophin (Menopur; Ferring, Spain) was administered. Mean doses administered were 272.5 ± 5.4 IU in the LR-Accu-Vit group and 268.7 ± 5.3 IU in the LR-fresh group. From day 6 onwards, the gonadotrophin dosage was adjusted according to serum oestradiol concentration and a transvaginal ultrasound scan. When a leading follicle reached 14 mm, 0.25 μg/day of a gonadotrophin-releasing hormone antagonist (Cetrotide; Merck Serono) were administered. When at least one follicle reached $\geq$18 mm in diameter, 250 μg recombinant human chorionic gonadotrophin (HCG; Ovitrelle; Merck Serono) were administered. Oocyte retrieval was scheduled 36 h after HCG injection and 400 mg/day of intravaginal micronized progesterone (Progefik; Effik, Spain) were administered as luteal support in cycles during which embryo transfer was performed. The same protocol for ovarian stimulation was used for vitrified cycles and fresh cycles in both groups.

Oocyte vitrification—warming

The Cryotop method for oocyte vitrification was employed as described elsewhere (Kuwayama et al., 2005), with slight modifications (Cobo et al., 2010). Vitrification was carried out 2 h after oocyte retrieval and immediately after assessment of nuclear maturity.

Insemination and embryo transfer

Warmed oocytes were cultured for 2 h prior to intracytoplasmic sperm injection (ICSI), while fresh oocytes were cultured for 4 h following oocyte retrieval and prior to denudation. All fresh and warmed oocytes from both study groups were inseminated simultaneously by ICSI. All embryos derived from these inseminations were cultured to day 3, when transfer took place. A mixed cohort was established in the LR-Accu-Vit group due to the different sources of oocytes (vitrified or fresh oocytes). Vaginal progesterone (800 mg/day) was administered starting from the day of fertilization until 12 weeks of gestation or was discontinued if pregnancy was not achieved (Escriba et al., 2006). Embryos were selected for transfer strictly according to their morphological appearance (Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting, 2011).

Embryo cryopreservation and thawing

Surplus embryos suitable for additional cryopreservation obtained in both groups were vitrified using the protocol described above.

Outcomes

The main outcomes were (i) LBR per patient and cumulative LBR considering that additional embryo cryotransfers were available and (ii) drop-out rate, considered as the number of women who did not return for further treatment during the year following failure.

The secondary endpoints were assessment of oocyte survival rate, fertilization and embryo-cleavage rates, embryo-transfer cancellation rate, mean number of embryos transferred, implantation rate and the influence of patient age.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences version 17 (SPSS, Chicago, IL, USA). Chi-squared tests and t-tests or analysis of variance (followed by Bonferroni's post-hoc tests) were applied to detect statistical differences in either proportions or means among groups. *P*-values <0.05 were considered significant.

The cumulative probability of a first live birth for each couple undergoing treatment during the study period was estimated by means of the Kaplan—Meier method and employing 95% confidence intervals. Censored data were considered when patients did not return for treatment. Data were stratified by group, and Log-rank, Breslow and Tarone—Ware tests were employed to compare the survival curves.

Results

The mean values for basal blood FSH concentration were 11.8 IU/ml (95% CI 11.2—12.5) and 12.7 IU/ml (95% CI 12.1—13.4) in the LR-fresh and LR-Accu-Vit groups, respectively, and showed no statistical difference. Mean anti-Müllerian hormone blood concentration were 2.3 pmol/l (95% CI 1.7—2.9) and 3.1 pmol/l (95% CI 2.4—3.5) in the LR-fresh and LR-Accu-Vit groups, respectively, and also showed no statistical difference.

Table 1 shows the ovarian stimulation cycle distribution in the LR-fresh group and the drop-out rate depending on the number of ovarian stimulation cycles performed. A total

Table 1 Ovarian stimulation cycles and drop outs in the LR-fresh group.

	Ovarian stimulation cycle					
	1	*2*	*3*	*4*	*5*	*6*
Patients (*n*)	397	70	12	2	0	1
Ovarian stimulation cycles (*n*)	397	140	36	8	0	6
Live births						
n/total	93/397	13/70	3/12	0	0	0
% (95% CI)	23.4 (19.2—27.6)	18.6 (9.4—27.6)	25.0 (0.5—49.5)			
Patients eligible for further treatment (*n*)[a]	304	57	9	2	2	1
Drop outs[b]						
n/total		234/304	45/57	7/9		—
% (95% CI)		77.0 (72.3—81.7)	78.9 (68.3—89.5)	77.8 (50.6—100)		100 %

[a] Defined as those not achieving an ongoing pregnancy in a cycle, thus representing candidates for a subsequent ovarian stimulation cycle.
[b] Calculated according to the women eligible for a subsequent cycle that did not return.

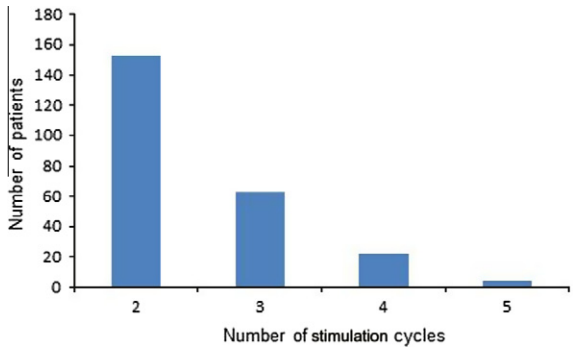

Figure 1 Distribution of stimulation cycles in the low response, accumulation of oocytes and vitrification group: 153 patients underwent two stimulation cycles, 63 patients underwent three stimulation cycles, 22 patients underwent four stimulation cycles and one patient underwent five stimulation cycles.

of 482 women underwent 587 stimulation cycles, resulting in a mean per woman of 1.21 cycles (95% CI 1.14—1.28). Drop-out rates were >75% after each attempt. The mean age of patients included in this group was 36.7 years (95% CI 36.4—37.0). A total number of 1802 oocytes (3.1 oocytes/ovarian stimulation, 95% CI 2.9—3.3) were obtained after oocyte retrieval and 1170 mature oocytes (2.0 oocytes/ovarian stimulation, 95% CI 1.9—2.1) were finally submitted to ICSI.

The LR-Accu-Vit group consisted of 242 patients with a mean age of 36.5 years (95% CI 36.1—36.9). There were no differences between the two groups with respect to age. A total of 594 ovarian stimulation cycles (mean 2.5, mean per woman 2.40—2.60) were performed in the LR-Accu-Vit group, from which a total of 1881 oocytes (3.2 per ovarian stimulation, 95% CI 3.1—3.3) were obtained. The distribution of stimulation cycles in the LR-Accu-Vit group is shown in **Figure 1**. Of these, 1192 mature oocytes (4.2, 95% CI 4.12—4.23) from 384 cycles (1.6 ± 1.2) were vitrified. All of these oocytes were warmed ($n = 1012$; survival rate of 84.9%, 95% CI 82.7—87.1) and added to the 689 oocytes (3.3 per ovarian stimulation, 95% CI 2.8—3.6) obtained from 210 fresh cycles (0.8 per patient, 95% CI 0.7—0.9). In this way, a final mixed cohort of a total of 1701 oocytes (7.02 per patient, 95% CI 6.7—7.4) was inseminated.

All of the participants' oocytes underwent ICSI (242 patients and 242 ICSI procedures in the LR-Accu-Vit group and 482 patients and 587 ICSI procedures in the LR-fresh group). As shown in **Table 2**, fertilization rates were similar for LR-Accu-Vit group and LR-fresh groups (66.1%, 95% CI 63.9—68.4 versus 64.9%, 95% CI 62.2—67.6, respectively). Both cleavage rate (73.7%, 95% CI 70.6—76.6 versus 73.3%, 95% CI 70.3—76.3, respectively) and proportion of top-quality embryos on day 3 (28.4%, 95% CI 24.4—32.4 versus 29.6%, 95% CI 25.6—33.4) were also found to be statistically comparable. In the LR-Accu-Vit group, the results were statistically similar regarding the source of the oocytes inseminated: vitrified or fresh (**Table 2**).

Table 3 shows the clinical outcomes. The embryo-transfer cancellation rate was approximately 4-fold higher in the LR-fresh group than in the LR-Accu-Vit group. In addition, the mean number of embryos transferred was statistically higher in the LR-Accu-Vit group ($P < 0.05$). Although the LBR per patient was statistically similar between groups, the cumulative LBR was statistically higher among patients belonging to the LR-Accu-Vit group (36.4% versus 23.7%, $P < 0.05$). Additionally, no statistical differences were found between the groups with regards to implantation rate or LBR when calculated on a per embryo-transfer basis.

Table 4 shows the LBR/patient according to age. Both treatment groups were categorized in three groups with regards to patient age. A differential age-related pattern was observed when analysing the rates of embryo-transfer cancellation: in the LR-Accu-Vit group rates were 4-times lower among the youngest patients and 13-times lower in women aged 40 or older than in the LR-fresh group.

Table 2 Fertilization and embryo quality on day 3.

	LR-Accu-Vit			*LR-fresh*
	Vitrified	*Fresh*	*Total*	*Fresh*
Injected MII oocytes (*n*)	1012	689	1701	1802
Fertilization of injected oocytes				
n	664	460	1124	1170
% (95% CI)	65.6 (62.6—68.5)	66.8 (62.4—71.0)	66.1 (63.9—68.4)	64.9 (62.2—67.6)
Cleavage on day 3 of fertilized oocytes				
n	484	344	828	858
% (95% CI)	72.9 (68.8—78.8)	74.8 (70.1—79.3)	73.7 (70.6—76.6)	73.3 (70.3—76.3)
Top-quality day-3 embryos from injected oocytes				
n	273	210	483	533
% (95% CI)	27.0 (21.6—32.2)	30.5 (24.1—36.62)	28.4 (24.4—32.4)	29.6 (25.6—33.4)

There were no statistically significant differences between the two groups.
LR-Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes; MII = metaphase II.

Table 3 Live birth rate per patient and per embryo transfer.

	LR-Accu-Vit	*LR-fresh*
Embryo transfers (*n*)	220	318
Transfer cancellations/patient (%, 95% CI)	9.1 (6.8–11.4) [a]	34.0 (29.8–38.2) [a]
Implantation rate		
n/total	110/440	138/540
% (95% CI)	25.0 (20.7–30.0)	25.6 (21.9–29.3)
Embryos transferred (mean, 95% CI)	2.0 (1.9–2.1) [b]	1.7 (1.6–1.8) [b]
Live-birth rate/embryo transfer		
n/total	73/220	108/318
% (95% CI)	33.2 (25.7–38.0)	34.0 (28.7–39.1)
Live-birth rate/patient		
n/total	73/242	108/482
% (95% CI)	30.2 (24.3–35.9)	22.4 (18.7–26.1)
Cumulative live-birth rate/patient[c]		
n/total	88/242	114/482
% (95% CI)	36.4 (30.3–42.4) [d]	23.7 (19.9–27.4) [d]

LR-Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes.
[a,b,d]Same superscript letters in a row indicate a statistically significant difference ($P < 0.05$).
[c]Calculated considering the additional number of babies born after subsequent embryo cryotransfers.

The mean number of embryos transferred was higher among patients aged under 35 years in the LR-Accu-Vit group than among patients of the same age in the LR-fresh group. The same pattern was observed in patients aged 40 or older. Significantly higher outcomes with respect to LBR/patient were observed in the LR-Accu-Vit group (30.2% versus 22.4%). Additionally, in women aged 40 or older, a 2-fold higher LBR per patient was observed in the LR-Accu-Vit group (15.8% versus 7.1%), although the difference was not found to be statistically significant.

Table 5 shows information about the outcome achieved after cryotransfers of surplus embryos in both groups after failing a fresh embryo transfer. The frequency of vitrification of supernumerary embryos was statistically higher ($P < 0.05$) in the LR-Accu-Vit group (28.9%, 95% CI 23.2–34.6) than in the LR-fresh group (8.7%, 95% CI 6.4–11.0). There were no differences between the LR-Accu-Vit group and the LR-fresh group with regards to LBR per warming cycle (28.8%, 95% CI 16.5–51.1 versus 13.3%, 95% CI 3.4–23.2) or per cryotransfer (31.3%, 95% CI 18.2–44.4 versus 17.6%, 95% CI 4.8–30.4).

Figure 2 shows the survival curve analysis of the cumulative LBR for both study groups, including the outcome after embryo transfer and cryotransfer of surplus embryos per intention to treat. In this way, one can appreciate the effects of cancellation due to embryo quality and the higher probability of a live birth thanks to the supernumerary embryos available for each ICSI procedure. Additionally, the cumulative LBR was statistically higher in the LR-Accu-Vit group ($P < 0.05$).

Discussion

Given the clinical challenge represented by LR and the failure of currently employed ovarian stimulation protocols to successfully increase the diminished ovarian reserve of these women (Kyrou et al., 2009; Pellicer et al., 1998), the current study set out to assess the accumulation of vitrified oocytes as a new strategy for managing these patients.

First, this study confirmed the high drop-out rate in women with a poor prognosis in a regular assisted reproduction cycle. It is worth mentioning that all these patients were encouraged to try an additional attempt with their own oocytes, even though they refused. The great majority of them switched to ovum donation (around 80%) while the others quitted. In addition, the fatigue due to the failure makes the excellent outcome achievable in a donation programme an attractive choice for these patients.

Considering the non-existent drop-out rate in the LR-Accu-Vit group, this study achieved one of its goals: to avoid patients abandoning treatment due to negative results that impair their ability to cope with the situation (Domar, 2004; Domar et al., 2010; Olivius et al., 2004a,b; Rajkhowa et al., 2006; Sharma et al., 2002) while obtaining an increased number of oocytes for insemination. Similar observations have been made by other authors. The application of a non-conventional treatment strategy (mild stimulation protocols) in patients considered to be considerably at risk of dropping out due to high pre-existing levels of anxiety has a positive effect in that it reduces this rate (Verberg

Table 4 Live-birth rate according to patient age.

	Patient age (years)							
	LR-Accu-Vit				*LR-fresh*			
	<35	*35–39*	*≥40*	*Total*	*<35*	*35–39*	*≥40*	*Total*
Patients	67	137	38	242	150	178	154	482
Age (years, mean, 95% CI)	32.5 (31.9–33.1)	37.2 (36.7–37.7)	41.7 (40.7–42.7)	36.5 (36.1–36.9)	30.3 (2.7–30.9)	37.1 (36.9–37.3)	42.7 (42.3–43.1)	36.7 (36.4–37.0)
ICSI procedures	67	137	38	242	181	216	190	587
Embryo transfers	59	125	36	220	99	158	61	318
Transfer cancellation rate/ICSI procedure	11.9 (4.2–19.7)[a]	8.8 (4.1–13.5)[b]	5.2 (0–12.3)[c]	9.1 (6.8–11.4)[d]	44.8 (37.6–52.0)[a]	26.9 (21.0–32.8)[b]	67.9 (61.2–74.5)[c]	45.9 (41.9–49.9)[d]
Transfer cancellation rate/patient	11.9 (4.2–19.7)[a]	8.8% (4.1–13.5)	5.2 (0–12.3)[b]	9.1 (6.8–11.4)[c]	34.0 (26.5–41.6)[a]	10.7% (6.2–15.2)	60.3 (52.6–68.1)[b]	34.0 (29.8–38.2)[c]
Embryos transferred (mean, 95% CI)	1.9 (1.8–2.0)[a]	2.0 (1.9–2.1)	1.9 (1.6–2.2)[b]	2.0 (1.9–2.1)[c]	1.6 (1.5–1.7)[a]	1.8 (1.7–1.9)	1.3 (1.1–1.5)[b]	1.7 (1.6–1.8)[c]
Live-birth rate/ patient								
n/total	21/67	46/137	6/38	73/242	42/150	55/178	11/154	108/482
% (95% CI)	31.3 (20.2–42.4)	33.6 (23.5–39.1)	15.8 (4.2–27.4)	30.2 (24.3–35.9)[a]	28.0 (20.8–35.2)	30.9 (24.0–37.6)	7.1 (3.0–11.2)	22.4 (18.7–26.1)[a]

Values are *n* or % (95% CI), unless otherwise stated.
Same superscript letters in a row indicate statistically significant differences ($P < 0.05$).
LR-Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes.

et al., 2008). Additionally, concentrating efforts and decreasing the need for further treatments (as only one ICSI procedure is performed) encourage the patients to continue to 'build' a larger cohort in order to mimic the situation of normoresponder patients.

It is true that one of the limitations of this study was the absence of randomization, i.e. the patients decided whether to undergo the vitrification option. Nonetheless, women who underwent fresh cycles and had an IVF failure, were completely free to try once again. In the study centre's opinion, this does not represent a bias of the study itself and the study denotes a true reflection of the actual drop-out rate in the LR population.

Another positive effect observed in this study was the low percentage of transfer cancellations in the LR-Accu-Vit group, which was especially striking among women aged 40 or older. There is evidence that failure to undergo embryo transfer is significantly related to the probability of discontinuing the treatment; thus, strategies which increase the success of IVF/embryo transfer are also of benefit in terms of anxiety levels (Verberg et al., 2008).

The oocyte survival rate after warming was slightly lower than that described for young normoresponders (Cobo et al., 2008), but was high nonetheless. The embryo quality of these zygotes was similar to that of fresh oocytes, thus providing the rationale for a strategy of accumulation and selection of embryos for transfer regardless of their origin (fresh or cryopreserved cohorts). As a result, more embryos were obtained and transferred in the LR-Accu-Vit group. Only LBR was considered as a clinical outcome.

LBR/patient was higher in the LR-Accu-Vit group, which confirms the efficiency of this method for managing LR patients. Indeed, this study was able to treat all LR patients who chose the option of oocyte accumulation, achieving a very low embryo-transfer cancellation. The positive effects of this strategy are even more evident when cumulative outcome is considered. Larger oocyte/embryo cohorts in LR patients allow the cumulated success rate after cryotransfer of surplus cryopreserved embryos to be increased. In accordance with this, this study has recorded a significantly higher incidence of cryopreservation of surplus embryos after embryo transfer in patients whose oocytes had been vitrified. As shown herein, the cumulative outcome in terms of LBR per IVF treatment and cryotransfer of surplus embryos was significantly increased by the oocyte-accumulation strategy. In the study centre's opinion, this highlights the advantage of applying oocyte vitrification in LR patients, since the women in this study is approximately 55% cumulative LBR after two embryo transfers **Figure 2** which is comparable to success rates in normoresponder patients (Garrido et al., 2010; Malizia et al., 2009).

Table 5 Clinical outcomes after additional cryotransfers.

	LR-Accu-Vit	*LR-fresh*
No. of surplus embryo vitrification cycles	70	51
Vitrification rate/patient		
n/total	70/242	51/588
% (95% CI)	28.9 (23.2–34.6)[a]	8.7 (6.4–11.0)[a]
Warming cycles	52	45
Transfers		
n/total	48	34
% (95% CI)	92.3 (85.1–99.6)	75.6 (62.9–88.1)
Implantation rate		
n/total	21/73	7/34
% (95% CI)	28.8 (19.1–35.5)	20.6 (9.0–34.2)
Embryos transferred (mean, 95% CI)	1.5 (0.5–2.5)	1.0 (0.1–2)
Clinical pregnancies/warming cycle initiated		
n/total	20/52	10/45
% (95% CI)	38.5 (25.3–51.7)	22.2 (10.1–34.3)
Live births/warming cycle initiated		
n/total	15/52	6/45
% (95% CI)	28.8 (16.5–51.1)	13.3 (3.4–23.2)
Live births/cryotransfer		
n/total	15/48	6/34
% (95% CI)	31.3 (18.2–44.4)	17.6 (4.8–30.4)

Values are *n* or % (95% CI), unless otherwise stated.
Same superscript letters in a row indicate statistically significant differences ($P < 0.05$).
LR-Accu-Vit = low response, accumulation of oocytes and vitrification; LR-fresh = low response, fresh oocytes.

Although some concerns have been expressed related to the possible negative effects of the double round of vitrification, in the study centre's experience with nearly 800 revitrified embryos, neither the clinical outcome nor the perinatal data of babies born is impaired (Cobo et al., 2011).

It should be pointed out that outcome calculations per embryo transfer were similar in both groups. This means that if this study had continued to treat patients in the LR-fresh group after every single failure, it would have achieved the same success rate as if the strategy of oocyte accumulation had been applied. Although at first glance it would appear that this strategy does not involve any clinical advantage, it does bring two remarkable benefits related to the previously discussed drop-out rate in the LR population and is sure to palliate the psychological distress caused by repeated failures. This should bring these patients closer to achieving their goal of a healthy new-born without having to resort to using donated gametes. Moreover, the similar outcome per embryo transfer was available for a significantly lower population in the LR-fresh group due to the statistically higher embryo-transfer cancellation rate in these patients. In other words, the chance of achieving such outcome was taken by the patients in the LR-Accu-Vit group.

Certainly some ovarian stimulation treatments may have been unnecessary in the vitrification group as some patients could have achieved a pregnancy from the first cycle (23%, **Table 1**). However, this negative effect of the strategy could be alleviated by the fact this population is most likely to drop out, meaning that this outcome was available for a statistically lower population. Furthermore, the higher number of surplus embryos for additional cryopreservation that was achieved in the LR-Accu-Vit group definitely helped to significantly increase the cumulative LBR in this group. On the other hand, it is logical to think that, if the situation of normoresponders was being simulated in this study, surplus embryos for cryopreservation may be relied on, a situation that occurs very often in IVF programmes which is not considered as a serious shortcoming. On the contrary, it could be assumed as a 'second chance' for patients, an additional advantage, considering the situation for poor responders. In addition, the negative extra manipulation linked to the stimulation itself and the surgical intervention is a matter to be considered. This is an incontestable shortcoming, but this drawback is in fact offset by the statistically higher chance of achieving a live birth in the LR-Accu-Vit group, since this population did not drop out and the embryo-transfer cancellation rate was lower than 10%, whereas it was 4-fold higher in the LR-fresh group.

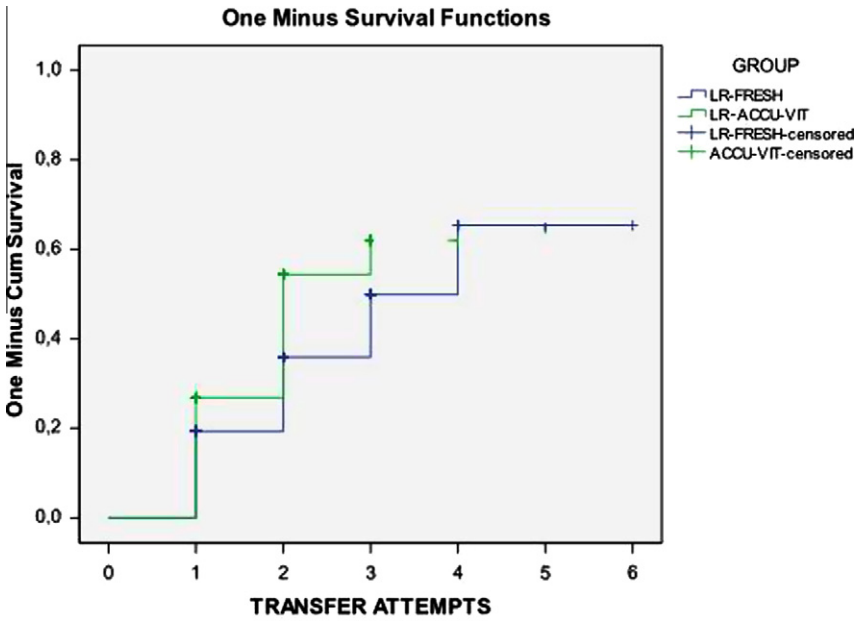

Figure 2 Cumulative live-birth rate in the low response, accumulation of oocytes and vitrification group (LR-Accu-Vit) versus low response, fresh oocytes group (LR-fresh), including embryo transfers and cryotransfers. Overall comparisons: Log rank (Mantel—Cox), $P = 0.03$; Breslow (generalized Wilcoxon), $P = 0.010$; Tarone—Ware, $P < 0.005$.

In addition, cost-related issues should be considered. It may seem clear that the cost of the therapy could be higher in the LR-Accu-Vit group considering the spending on the medication. This analysis may be debatable depending on how it is approached. In the current study, the cost per live births in the LR-fresh group was around 5% lower compared with the LR-Accu-Vit group (data not shown). It is worth mentioning that in the LR-fresh group the population which underwent more than two stimulations is significantly lower than in the vitrification group. In consequence, the analysis is made in a population which was subjected to mainly one stimulation cycle; hence, the cost per new-born seems to be lower. It is needless to say that, within the same group for patients undergoing two or more cycles, the cost per new-born will at least be double that observed in the LR-Accu-Vit group. The situation is not the same for the vitrification group since beyond two or three ovarian stimulation cycles the costs are lower than if they had undergone two or three cycles separately. In this case, although the medication expenditures are doubled or tripled each time, the whole cost of the cycle is cheaper seeing that, among other considerations, they have to pay for just one ICSI procedure and one embryo transfer. Even considering the possibility of an additional cryotransfer of surplus embryos in the LR-Accu-Vit group, far from affecting the total computation of the costs for achieving a new-born, it helps to increase the efficiency of the strategy, since a cryotransfer is much less expensive than a full IVF cycle. In other words, the fresh strategy is slightly more cost-effective for those patients who achieve a baby in the first cycle; however, from here onwards this strategy is much more expensive. This study considers that the 5% additional costs in the LR-Accu-Vit group are justified by the 34% higher outcome rate per patient achieved in this group.

It is also important to mention that, in order to accumulate oocytes for management of LR, a highly efficient and well-established oocyte cryopreservation method is mandatory. The Cryotop method for oocyte vitrification has been demonstrated to be highly efficient (Cobo et al., 2010; Kuwayama et al., 2005; Rienzi et al., 2010). Following a pilot study (Cobo et al., 2008), this study centre has introduced this technology into daily clinical practice. Indeed, a very recent study designed as a prospective randomized clinical trial failed to detect any difference between fresh oocytes and vitrified oocytes used for donation (Cobo et al., 2010), which shows this technology to be an extremely valuable tool for the management of different situations in assisted reproduction. A future study will address the number of vitrified oocytes necessary before performing insemination in order to increase success in LR patients.

In conclusion, the results obtained in the current study demonstrate that the accumulation of oocytes by vitrification for low responders is associated with a lower drop-out rate, fewer transfer cancellations, higher LBR per intention-to treat patient, more cycles with vitrified embryos and higher cumulative LBR, which endorses the treatment as a successful alternative for LR patients.

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