Implementation of blastocyst transfer in the routine clinical practice of assisted reproductive techniques. Analysis of 6000 consecutive cycles

Vključitev prenosa blastociste v rutinsko klinično delo pri oploditvah z biomedicinsko pomočjo – analiza 6000 zaporednih ciklov

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Izvleček

Izhodišče: S študijo smo želeli primerjati uspešnost postopkov oploditve z biomedicinsko pomočjo (OBMP) po prenosu zgodnjih zarodkov in po prenosu blastocist pri vseh bolnikih, ki so bili v zadnjih 7 letih vključeni v program zdravljenja s tehnikami asistirane reprodukcije (ART).

Metode: V nerandomizirano retrospektivno študijo smo vključili vse ženske z uspešno aspiracijo jajčnih celic po kontrolirani ovarijski hiperstimulaciji (KOH) med letoma 2002 in 2008. Cikle smo razdelili glede na število osamljenih jajčnih celic na slabo odzivne, pri katerih smo dobili le eno jajčno celico; nizko odzivne, kjer smo aspirirali 2–4 jajčne celice, in dobro odzivne, kjer smo v aspiratu našli več kot 5 jajčnih celic. Izračunali in primerjali smo deleže nosečnosti, porodov in mnogoplodnih porodov pri ciklih, pri katerih smo opravili prenos zarodkov na tretji oziroma peti dan po aspiraciji foliklov.

Rezultati: Med 6098 vključenimi bolniki je bilo 292 (4,8 %) slabo odzivnih, 1450 (23,8 %) nizko odzivnih in 4356 (71,4 %) dobro (normalno) odzivnih. V 5,7 % (350/6098) vseh ciklov smo zabeležili popolno odsotnost oploditve jajčnih celic. V ciklih, v katerih so se jajčne celice oplodile, smo prenos zarodkov na tretji dan razvoja načrtovali pri 1940 (33,9 %) parih, prenos blastocist na peti dan razvoja pa pri 3788 (66,1 %) parih. V 6,8 % (394/6098) ciklov prenosa zarodkov nismo naredili, in sicer pogosteje po petdnevnem kot po tridnevnem gojenju zarodkov (7,8 % oz. 6,1 %). Kljub temu je več žensk zanosilo po prenosu blastocist kot po prenosu zgodnjih zarodkov v skupini z nizko odzivnostjo (9,5 % oz. 6,1 %), slabo odzivnostjo (23,7 % oz. 15,2 %) in dobro odzivnostjo (39,9 % oz. 17,3 %). Tudi delež večplodnih porodov je bil višji po prenosu blastocist kot po prenosu zgodnjih zarodkov, in sicer tako pri nizko odzivnih (24,3 % oz. 13,3 %) kot tudi pri dobro odzivnih ciklih (29,4 % oz. 24,6 %).

Zaključek: Prenos zarodkov, razvitih do stopnje blastociste, poviša delež uspešnosti v postopkih zunajtelesne oploditve pri vseh bolnikih. Za preprečitev večplodnih nosečnosti priporočamo prenos le ene blastociste.

Abstract

Background: All patients who entered our assisted reproductive technology (ART) program during last 7 years were analyzed in order to compare the outcome of *in vitro* fertilization (IVF) cycles after early cleavage stage embryo transfers and blastocyst transfers.

Methods: All patients with successful oocyte pick up (OPU) after controlled ovarian hiperstimulation in the period from 2002 to 2008 were included in this retrospective analysis. The cycles were stratified with respect to the number

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of aspirated oocytes as follows: poor responders in whom only a single oocyte was aspirated, low responders with 2 to 4 oocytes, and good responders with more than 4 oocytes retrieved. Pregnancy, delivery and multiple delivery rates were calculated and compared between the cycles with day 3 and cycles with day 5 embryo transfers.

Results: Among 6098 included patients, 292 (4.8 %) were poor, 1450 (23.8 %) low and 4356 (71.4 %) good responders. Total fertilization failure was observed in 5.7 % (350/6098) of all cycles. Among the cycles with fertilization, embryo transfer on day 3 was planned in 1940 (33.9 %) cycles and blastocyst transfer on day 5 in 3788 (66.1 %) cycles. Transfer was cancelled in 6.8 % (394/5748) of cycles, more frequently after embryo culturing *in vitro* for 5 than for 3 days (7.8 % vs. 6.1 %). However, more women delivered after blastocyst transfer compared to early stage embryo transfers in low (9.5 % vs. 6.1 %), poor (23.7 % vs. 15.2 %) and normal (39.9 % vs. 17.3 %) responders. Multiple delivery rate was also higher after blastocyst rather then after early embryo transfers in low (24.3 % vs. 13.3 %) and normal (29.4 % vs. 24.6 %) responders.

Conclusions: The transfer of blastocyst stage embryos increases the success rate of ART procedures in all patients. To avoid multiple pregnancies, single blastocyst transfer is recommended.

Introduction

Nowadays, multiple gestation is recognized as a major problem associated with assisted reproductive technologies (ART).[1] Despite technological advances, multiple pregnancy rate in most of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) programs is still 20 % and more.[2] In the last decade, public opinion and health policies have come closer to an agreement regarding the transfer of fewer embryos in ART procedures, being the only safe method in order to avoid multiple pregnancies. Because of that, some countries are adopting measures to address this issue either through legislation or the development of clinical guidelines. Some European countries have passed laws aimed at reducing the number of embryos transferred. Some national societies have official regulations set by national scientific societies, restricting the number of embryos transferred to a smaller number than is regulated by law. This is the case in Slovenia where maximum number of transferred embryos was limited to three, but in women under 36 years of age only one top quality embryo is transferred in the first two ART cycles.[3]

The aim of such strategy is to reduce multiple pregnancy rate while maintaining the overall success rate of the entire IVF programme.[4] This is only possible with good embryo selection. Recent advances in cell culture media have led to a shift in IVF practice from early cleavage embryo transfer to blastocyst stage transfer.[5,6] Extension of culture until day 5 facilitates the identification of those embryos likely to have the highest implantation and pregnancy potential.[7,8] However, those reports were restricted to a highly selected group of women with good prognosis and good response to stimulation. Wider implications of adopting such policy on the overall results in an IVF program have not been studied.[9,10] Recent meta-analysis suggests that the probability of live birth after fresh IVF cycle is significantly higher with transfering blastocyst-stage embryo compared to cleavage-stage embryo transfer when equal number of embryos are transferred.[11] But a relative weakness of the current meta-analysis is that the huge variety of ART used among laboratories worldwide cannot be clearly addressed. Therefore it is very important that each ART center analyse and compare their own results after cleavage-stage and blastocyst-stage transfer.

The aim of our study was to compare the outcome of cycles after cleavage-stage embryo transfer with cycles after blastocyst-stage transfer in all patients entering our ART program.

Material and methods

Patient population

All patients undergoing ART (Table 1) from 2002 to 2008 at our department were included in this retrospective analysis. Natural, donor, and cycles with no oocyte

Table 1: Outcome of cycles with fertilization in poor, low and good responders

	Poor respond Oocyte: 1	Low respond Oocytes: 2 do 4	Normal respond Oocytes:≥ 5	Total
Cycles with fertilization, N (%)	200 (3.5)	1299 (22.6)	4249 (73.9)	5748 (100)
No embryo transfer, % (N)	16.0 (32)	7.5 (97)	6.2 (265)	6.8 (394)
Embryo transfer day 3, % (N)	79.0 (158)	73.1 (950)	17.3 (733)	32.0 (1841)
Embryo transfer day 5, % (N)	5.0 (10)	19.2 (250)	76.0 (3231)	60.7 (3491)
Pregnancy rate /cycle with fertilization, % (N)	9.0 (18)	23.6 (307)	45.8 (1946)	39.5(2271)
Delivery rate /cycle with fertilization, % (N)	6.5 (13)	15.9 (206)	36.5 (1549)	30.8 (1768)
Multiple delivery rate, % (N)	0	18.4 (38)	28.9 (447)	27.4 (485)

retrieved as well as cycles using frozen embryos were excluded.

Ovulation induction

During the observed period patients underwent controlled ovarian hyperstimulation (COH) using gonadotropin releasing hormone (GnRH) analog triptorelin (Diphereline, Ibsen Pharma, France) in long protocol or GnRH antagonist cetrorelix (Cetrotide, Merck Serono, Italy) in flexible protocol and either a recombinant FSH (Gonal F, Merck Serono, Italy) or a human menopausal gonadotropin (Menopure, Ferring, Belgium). All cycles were pretreated with oral contraceptives for 18–35 days before COH started. In all down-regulated stimulated cycles follicle development was monitored by ultrasound from day 7 of the menstrual cycle. In cycles controlled by GnRH antagonists (3 mg single dose) follicular measurement and assessment started on day 6. After this period in both groups of patients follicular measurement was performed every second day. Three follicular diameters were measured and the average value was considered for further calculations. When mean diameter of the dominant follicle had reached > 17 mm, recombinant hCG (Ovitrelle, Merck Serono, Italy) was administrated.

Laboratory techniques

Follicle aspiration was done 35–37 hours after hCG administration. Retrieved oocytes were fertilized by either conventional IVF or intracytoplasmic sperm injection (ICSI), as described elsewhere.[12,13] The oocytes and embryos were cultured in groups in sequential media (BlastAssist System, Jillinge, Medicult, Denmark), changing the first medium with the second one on day 3, as described in the manufacturer's manual. The media were overlayid with paraffin oil.

Fertilization was assessed 18–20 hours after insemination and confirmed by the presence of two distinct pronuclei. On day 2–5 of culture, embryo morphology was assessed. The criteria for embryo grading were based on morphological characteristics according to the number of blastomeres, regularity and symmetry of cell division, degree of fragmentation and the dynamics of their development in last three days. The embryos with the cell number close to 8, with the highest cell similarity, absence of multinucleation and with a lesser amount of fragments are selected for embryo transfer on day 3.

Blastocyst quality was determinate on the day of blastocyst transfer (day 5) according to our own criteria.[14] Optimal blastocyst was recognized as expanded blastocyst with compact inner cell mass (ICM) and absence of fragments or necrosis in either ICM or trophectoderm (TE). All suboptimal blastocysts were divided into seven different groups according to the mentioned criteria for scoring of blastocele expansion, ICM and TE.

Embryo transfer and luteal supplementation

All patients undergoing IVF/ICSI were informed about the possibility of having blastocyst transfer. Those who had more than 3 cleavage stage embryos on day three after oocyte pick-up (OPU) were informed about the possibility of natural self-selection of embryos during cultivation prolonged to day 5. Some patients were included in the protocol of prolonged cultivation if they wanted so, even if the number of aspirated oocytes was lower than 5. In cases when fertilization and/or morphology of the embryos on day 3 were not optimal, embryo transfer on day 3 was recommended, regardless of whether they were previously allocated to the group for blastocyst culture.

The number of transferred embryos or blastocysts was individualized based on the patient's age, number of previous failed attempts, embryos/blastocysts availability and patient preferences. Generally, one or two blastocysts were transfered in cycles with more than 4 oocytes obtained by oocyte pick-up. Three embryos were transferred only in patients older than 40 and on their request.

Embryo transfer was performed using a Labotect (Göttingen, Germany) catheter. The catheter was loaded with embryos (blastocysts) directly from the dish covered with paraffin oil.

In both groups of patients (day 3 and day 5 transfer) the luteal phase of the cycle was supported by transvaginal micronized progesterone 200 mg three times daily (Utrogestan, Iscovesco, France) or dydrogesterone 300 mg daily (Dabroston, Duphare, Netherland). All pregnant women continued with luteal supplementation until the eighth week of pregnancy.

Outcome measures and analysis

Pregnancy was confirmed by the determination of serum beta hCG 16 days after OPU. Deliveries were confirmed with medical record or with patient responses to the questionnaire sent from our clinic. In all cycles clinical data were finally analyzed after deliveries.

Pregnancy rate, delivery rate and multiple delivery rate were calculated and compared between patients undergoing day 3 embryo transfer and those undergoing day 5 embryo transfer, separately for poor-, low- and good-responders. Poor responders were those with only one aspirated oocyte, low responders with 2 to 4 punctured oocytes and good responders with 5 or more oocytes. Data are presented in tables and are not statistically evaluated.

Results

A total number of 6098 consecutive IVF/ICSI cycles with successful OPU were analyzed. Among them 292 (4.8 %) were poor-, 1450 (23.8 %) low- and 4356 (71.4 %) normal-responders. Total fertilization failure was observed in 5.7 % (350/6098) of all cycles, 31.5 % (92/292) in poor responders, 10.4 % (151/1450) in low responders and 2.4 % (107/4356) in normal responders. In the cycles where fertilization was achieved, embryo transfer was cancelled in 6.8 % (394/5748) of cycles, 5.1 % (99/1940) on day 3 and 7.8 % (297/3788) on day 5; in all these cases embryos were scored as nonviable and/or not in an appropriate stage of development expected for the day of embryo transfer. In 32.0 % (1841/5748) of cycles embryo transfer was performed on day 3, and in 60.7 % (3491/5748) in blastocyst stage. Pregnancy and delivery rates per cycle with fertilization were 39.5 % (2271/5748) and 30.8 % (1768/5748) respectively. Among deliveries, 485 (27.4 %) were multiple.

Cycle outcomes were compared between three groups of cycles with different response to stimulation and it was found that embryo transfer cancellation was more common in poor responders than in low and good responders. Embryo transfer on day 3 was mainly done in poor and low responders. A lower number of inseminated oocytes is associated with a higher chance for total fertilization failure, lower fertilization rate and lower number of early stage embryos suitable for cultivation to day 5. Extended embryo culture to day 5 was common approach only in normal responders. As we expected, the highest pregnancy, delivery and multiple

Table 2: Outcome of cycles after embryo transfer on day 3 and on day 5 in low responders.

		Number (%)	Pregnancy rate / ET %, (N)	Delivery rate /ET, % (N)	Multiple delivery rate, % (N)
eSET	day 3	32 (3.2)	31.2 (10/32)	31.2 (10/32)	0
	day 5	12 (3.8)	41.7 (5/12)	25.0 (3/12)	0
SET	day 3	283 (28.7)	17.3 (49/283)	10.9 (31/283)	0
	day 5	113 (36.2)	25.7 (29/113)	23.0 (26/113)	0
eDET	day 3	71 (7.2)	28.2 (20/71)	25.3 (18/71)	33.3 (6/18)
	day 5	22 (7.1)	45.5 (10/22)	42.9 (9/22)	66.7 (6/9)
DET	day 3	439 (44.5)	34.2 (105/439)	15.5 (68/439)	13.2 (9/68)
	day 5	101 (32.4)	44.5 (45/101)	35.6 (36/101)	33.3 (12/36)
TET	day 3	125 (12.7)	27.2 (34/125)	18.4 (23/125)	21.7 (5/23)
	day 5	2 (0.6)	0	0	0
All ET	day 3	950 (100.0)	22.9 (218/950)	15.8 (150/950)	13.3 (20/150)
	day 5	250 (100.0)	35.6 (89/250)	29.6 (74/250)	24.3 (18/74)

ET = embryo transfer
SET= single embryo transfer
eSET= elective single embryo transfer
DET= double embryo transfer
eDET= elective double embryo transfer)
TET= triple embryo transfer

delivery rates were observed in normal responders (Table 1).

We also compared the outcomes between day 3 and day 5 embryo transfer policy in these three groups of cycles.

In poor responders, the embryo transfer was cancelled more frequently on day 5 rather than day 3 (52.4 % vs. 11.7 %), but pregnancy rate per transfer was much lower after embryo transfer on day 3 (9.5 % vs. 30 %), so the delivery rate per cycle with fertilization was higher in cycles after transfer on day 5 (9.5 % vs. 6.1 %).

In low responders, embryo transfers were also cancelled more frequently on day 5 than on day 3 (19.9 % vs. 3.7 %), but the delivery rate per cycle with fertilization was also higher in group with blastocyst-stage transfer (23.7 % vs. 15.2 %). After transfer of two or three blastocysts, the implantation and delivery rates were higher, compared to the outcome of the cycles after the transfer of the same number of embryos on day 3, but unfortunately multiple delivery rate was also higher (Table 2).

In good responders, the embryo transfer cancellation rate was similar in both groups (5.2 % on day 3 vs. 6.5 % on day 5). Delivery rate per cycle with fertilization was much higher in cycles after embryo transfer on day 5 compared to day 3 (39.9 % vs. 17.3 %). Extended embryo culture to day 5 increased the pregnancy and delivery rate regardless the number of embryos transferred (one, two or three). The overall multiple delivery rate was comparable in both groups, because of a high proportion of elective single blastocyst transfers. After transfer of two blastocysts, the multiple pregnancy rate was very high, but the pregnancy and delivery rates were not much higher compared to single blastocyst transfer (Table 3).

Discussion

Successful outcome of ART treatment is influenced by many factors. The most impor-

tant one is patient age, with younger women having a greater chance of a successful outcome compared to older ones. Additional factors are: duration of infertility, previous IVF treatments and previous pregnancies. Traditionally, a high number of embryos per transfer manifests poor chances for clinical pregnancy following IVF. Typically, high number embryo transfers are practised in IVF centers where laboratory conditions are not optimal and suboptimal embryo development can be expected, or in centers where a high pregnancy rate is forced by "IVF market competition". With recent advances obtained in this field, milder stimulation protocols followed by the transfer of fewer embryos has become a standard procedure. Multiple pregnancies have been responsible for many adverse pregnancy effects influencing maternal and perinatal mortality and morbidity. Direct and indirect costs related to multiple pregnancy, including neonatal intensive care unit expenses, medication and long-term care disability costs associated with premature births make multiple pregnancies not only a personal problem, but a social burden as well.

Multiple pregnancy rates can only be reduced by reducing the number of transferred embryos i.e. by increasing the proportion of elective single embryo transfers. However, if we want to keep high success rate of ART, this will be possible only by good embryo selection. Therefore, the policy of blastocyst transfer was introduced.[15] The rationale for blastocyst culture and transfer is to improve both uterine and embryonic synchronicity as well as self-selection of viable embryos, resulting in higher implantation rates.[11] Both the advantages and disadvantages of blastocyst over cleavage-stage embryo transfers are described.[16] The advantages are in better correlation with morphology and euploidy status, better synchronization with the endometrium already affected by stimulated cycle and in high implantation potential. On the other hand, blastocysts have certain drawbacks, such as the lack of one day co-culture with endometrial cells (embryo descends into the uterine cavity on day 4); increased possibility of some embryos not developing into blastocysts in *in vitro* conditions having for a consequence the cancellation of embryo transfer; and finally, the reduced success of cryopreservation compared to the early embryo stages.[11]

The final decision about the appropriate embryo stage and the number of embryos for transfer is individualized according to patients' personal characteristics and their wish. In our unit, more than one third of patients come from abroad, usually with several IVF treatments performed previously in their countries. Their expectations and information obtained in their native countries are different compared to those of patients from Slovenia. Usually, they are interested in and demand the transfer of more than one embryo. Furthermore, their treatment is not reimbursed by insurance as for Slovene patients and therefore they believe that by transferring more than one embryo they are increasing the success rate of the cycle. Despite that, we try to convince them to decide for transfer of one or maximum two blastocysts. This is the reason why embryo transfer in 76 % of good responders was performed on day 5 and that elective single blastocyst transfer was quite common in this subgroup of patients. Our analysis has shown that delivery rate per cycle with fertilization in normal responders was much higher in patients with embryo transfer on day 5 compared to day 3 (39.9 % vs. 17.3 %). Moreover, the extended embryo culture to day 5 increases the pregnancy and delivery rate after transfer of one, two or three embryos. Multiple delivery rate was much higher if two or three blastocysts were transfered compared to the transfer of the same number of cleavage stage embryos. These observations have been confirmed by the results of our previous study presenting an unacceptably high multiple pregnancy rate, despite the transfer of only two blastocysts.[17] After introducing prolonged embryo culturing in our center, double blastocyst transfer has become a routine practice for all our patients. However, our data regarding multiple pregnancy rate encourage us to change this policy in younger patients with a favorable prognosis to a single blastocyst transfer, using blastocysts of good quality. Despite the fact that the average number of transferred

Table 3: Outcome of cycles after embryo transfer on day 3 and on day 5 in normal responders

		Number (%)	Pregnancy rate / ET, (N)	Delivery rate /ET, % (N)	Multiple delivery rate, % (N)
eSET	day 3	29 (3.7)	31.0 (9/29)	13.8 (4/29)	0
	day 5	659 (19.1)	60.5 (399/659)	48.0 (316/659)	0.6 (2/316)
SET	day 3	95 (12.0)	9.5 (9/95)	8.4 (8/95)	0
	day 5	355 (10.3)	26.8 (95/355)	19.4 (69/355)	1.4 (1/69)
eDET	day 3	134 (16.9)	47.0 (63/134)	36.6 (49/134)	24.5 (12/49)
	day 5	1135 (32.9)	64.4 (731/1135)	55.0 (624/1135)	46.3 (289/624)
DET	day 3	273 (34.4)	29.7 (81/273)	21.6 (59/273)	23.7 (14/59)
	day 5	957 (27.7)	46.2 (442/957)	35.1 (336/957)	31.2 (105/336)
TET	day 3	222 (28.0)	32.9 (73/222)	23.0 (51/222)	31.4 (16/51)
	day 5	125 (3.6)	35.2 (44/125)	26.4 (33/125)	24.2 (8/33)
All ET Vsi ET	day 3	753 (100.0)	31.2 (235)	22.7 (171)	24.6 (42)
	day 5	3231 (100.0)	52.9 (1711)	42.6 (1378)	29.4 (405)

ET = embryo transfer
SET= single embryo transfer
eSET= elective single embryo transfer
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eDET= elective double embryo transfer
TET= triple embryo transfer

embryos in our center has been the lowest in Slovenia for several years, the percentage of twin pregnancies following ART treatment remains very high.

Therefore, we encourage elective single embryo transfer in patients under 36 years of age. In order to stimulate that, we succeeded to ensure that governmental support for eSET has been available in Slovenia from the beginning of the year 2008. We believe that this policy will reduce the number of embryos transferred, thus reducing the number of twin pregnancies.

From the literature it is evident that blastocyst transfer is not usually used to treat poor responders who have an insufficient number of embryos.[18,19] In our previous study, we found no benefit of blastocyst transfer in natural cycles.[20] However, in this analysis we compared the outcome in low and poor responders comparing day 3 to day 5 embryo transfer. In normal responders the extended embryo culture to day 5 increased the pregnancy, delivery and multiple pregnancy rates after transfer of two or three embryos. Despite the fact that cycle cancellation rate was much higher after blastocyst transfer policy, the delivery rate per fertilization was higher than after cleavage stage embryo transfer. If possible cancellation of embryo transfer and consequent adverse psychological impact on patients is neglected, the prolonged embryo cultivation can also be proposed in low and poor responders. To avoid multiple deliveries, a single blastocyst transfer is recommended.

Blastocyst transfer improves the implantation rate. In meta-analysis it was suggested that, with the same number of embryos transferred, the probability of both live births and clinical pregnancies, is significantly higher with blastocyst raher than cleavage stage transfer.[21] To answer those questions, we compared the outcome of IVF/ICSI cycles with transfer on day 3 and cycles with transfer on day 5 in poor, low and normal responders. Our aim was not a statistical analysis, however, this is a retrospec-

tive analysis performed on a large number of cycles and despite the strong possibility of bias, we believe that it may give an insight into the topic.

Overall, our data suggest that the transfer of blastocyst stage embryos increases the success rate of ART procedures in all patients. The transfer of elective single blastocyst results in pregnancy and delivery rate comparable to the rates obtained with the transfer of two blastocysts. To avoid multiple pregnancies, a single blastocyst transfer should be recommended.

References

1. Ombelet W, De Sutter P, Van der Elst J and Martens G. Multiple gestation and infertility treatment: registration, reflection and reaction—the Belgian project. Human Reproduction Update 2005; 11: 3–14.
2. Nyboe Andersen A, Goossens V, Bhattacharya S, Ferraretti AP, Kupka MS, de Mouzon J, et al. Assisted reproductive technology and intrauterine inseminations in Europe, 2005: results generated from European registers by ESHRE: ESHRE. The European IVF Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod 2009; 24: 1267–87.
3. Vlaisavljević V. Slovenia extends its state support of IVF. Focus on Reproduction 2008; 10–11.
4. Khalaf Y, El-Toukhy T, Coomarasamy A, Kamal A, Bolton V, Braude P. Selective single blastocyst transfer reduces the multiple pregnancy rate and increases pregnancy rates: a pre- and postintervention study. BJOG 2008; 115: 385–390.
5. Gardner DK; Schoolcraft WB, Wagley L, Schlenker T, Stevens J; Hesla J. „A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization." Human Reproduction 1998; 13: 3434–40.
6. Langley MT, Marek DM, Gardner DK, Doody, KM and Doody KJ. Extended embryo culture in human assisted reproduction treatments. Hum Reprod 2001; 16: 902–8.
7. Gardner D. Single blastocyst transfer: a prospective randomised trial. Fertil Steril. 2004; 81: 551–5.
8. Papanikolaou EG. *In vitro* fertilization with single blastocyst-stage versus single cleavage-stage embryos. N Engl J Med 2006; 354: 1139–46.
9. van Montfoort AP, Fiddelers AA, Janssen JM, Derhaag JG, Dirksen CD, Dunselman GA, et al. In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. Hum Reprod 2006; 21: 338–43.
10. Assisted reproductive technology in the United States: 2001 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology registry. Fertil Steril 2007; 87: 1253–66.
11. Papanikolaou1 EG, Kolibianakis EM, Tournaye H, CA. Venetis CA, Fatemi H, Tarlatzis B et al. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. Hum Reprod 2008; 23: 91–99.
12. Vlaisavljević V, Kovačič B, Gavrić Lovrec V, Reljič M. Simplification of the clinical phase of IVF and ICSI treatment in programmed cycles. Int J Gynecol Obstet 2000; 69: 135–42.
13. Vlaisavljević V, Reljič M, Lovrec Gavrić V, Kovačič B. Comparable effectiveness using flexible single-dose GnRH antagonist (cetrorelix) and single-dose long acting GnRH agonist (goserelin) protocol for IVF cycles – a prospective, randomized study. Reprod Biomed Online 2003; 7: 301–308.
14. Kovačič B, Vlaisavljević V, Reljič M, Čižek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online 2004; 8: 687–94.
15. Coetsier T, Dhont M. Avoiding multiple pregnancies in in-vitro fertilization: who's afraid of single embryo transfer? Hum Reprod 1988; 13: 2663–4.
16. Tsirigotis M. Blastocyst stage transfer: pitfalls and benefits—too soon to abandon current practice? Hum Reprod 1998; 13: 3285–3289.
17. Vlaisavljević V, Dmitrović R, Sajko MC. Should practice of double blastocyst transfer be abandoned? A retrospective analysis. Reprod Biomed Online 2008: 16: 477–83.
18. Goto S, Shiotani M, Kitagawa M, Kadowaki T, Noda Y. Effectiveness of two-step (consecutive) embryo transfer in patients who have two embryos on day 2: comparison with cleavage-stage embryo transfer. Fertil Steril 2005; 83: 721–23.
19. Weissman A. Blastocyst culture and transfer: lessons from an unselected, difficult IVF population. Reprod. BioMed Online 2008: 17: 220–228.
20. Vlaisavljević V, Kovačič B, Reljič M, Gavrić Lovrec V, Čižek Sajko M. Is there any benefit from the culture of a single oocyte to a blastocyst-stage embryo in unstimulated cycles? Hum Rreprod 2001; 16: 101–5.
21. Blake D, Farquhar C, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database of Systematic Reviews 2007; (4): CD002118.