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Pregnancy Outcomes of Different Methods for Multifetal Pregnancy Reduction: A Comparative Study

The purpose of this study was to evaluate the outcomes of various methods of multifetal pregnancy reduction (MFPR) and to determine which method produces better outcomes. One hundred and forty-eight patients with multiple pregnancies resulting from assisted reproduction programs and underwent MFPR were included. According to the use of potassium chloride (KCl), patients were divided into 'KCl', and 'non-KCl' groups, and based on gestational age at the time of procedures, patients were divided into 'Early' (before 8 weeks of gestation) and 'Late' (at 8 weeks or later) groups. Firstly, to clarify the effect of each component of MFPR procedure, data were analyzed between 'KCl' and 'non-KCl' groups, and between 'Early' and 'Late' groups with adjustments. Secondly, comparison between 'Early, non-KCl' and 'Late, KCl' groups was performed to evaluate the combinative effect of both components. Non-KCl groups showed a significantly higher take-home-baby rate, and lower risk of extreme prematurity and preterm premature rupture of membranes (PPROM) than KCl groups. Early groups showed a lower immediate loss rate than Late groups. As compared with 'Late, KCl' group, 'Early, non-KCl' group was superior in terms of immediate loss, pregnancy loss, take-home-baby, and PPROM rates. Our data suggest that the 'early, non-KCl' method may be a better option for MFPR.

Key Words : *Pregnancy Reduction, Multifetal; Pregnancy, Multiple; Reproductive Technology, Assisted; Pregnancy Outcome*

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INTRODUCTION

Although multiple pregnancy rate in natural conception is less than one percent, this increases significantly in assisted reproductive cycles. The number and rate of multiple pregnancies have increased over the past two decades, during which the number of twin deliveries rose by 65 percent, and the rate of triplet and higher-order multiple pregnancies increased by more than 400 percent (1). In the majority of developed countries, 30-50% of all twin pregnancies occur as a result of infertility treatment (2). In the United States, multiple pregnancies were reported to occur in 35.4% of in vitro fertilization cycles, which is more than ten times the natural multiple pregnancy rate (3).

The majority of perinatal morbidities associated with multiple gestations are related to preterm delivery. Gestational age at delivery and birth weight are the two most important factors that affect perinatal, neonatal, and infant morbidity and mortality (4). In addition to the morbidity and mortality attributable to preterm delivery, fetuses in multiple gestations are vulnerable to a variety of complications, such as malformations and twin-to-twin transfusion syndrome. Maternal complications such as preeclampsia, gestational diabetes, postpartum hemorrhage, and maternal death are also increased in multiple gestations, and these risks are further increased in triplet or higher-order multiple pregnancies (5).

Multifetal pregnancy reduction (MFPR) is a procedure that reduces higher-order pregnancies, which improves perinatal outcomes. Multifetal pregnancy reduction in triplet and higher-order multiple pregnancies significantly reduces the risks of prematurity and a low birth weight, and may also be associated with a reduction in overall pregnancy loss and obstetric complications (6-8). Moreover, no significant differences were found in mean gestational age at birth, birth weight, and perinatal mortality rates between reduced and non-reduced twins (9). A recent meta-analysis concluded that multifetal pregnancy reduction to twins, compared with expectant management, seems to be an effective treatment option for women with a triplet pregnancy (10). These results suggest that MFPR of higher-order pregnancies to twins is a medically justifiable procedure.

Since the MFPR was first introduced in the mid-eighties (11), several modifications of methods have been developed. Various options are available in regard to the route of approach, the timing of procedure, and the use of embryotoxic agents such as potassium chloride (KCl). However, controversy still exists regarding which method is better than the

others (12-15). We retrospectively analyzed different methods for MFPR to evaluate the outcomes of procedure, and to determine which produces the better outcomes.

MATERIALS AND METHODS

Patients

One hundred and forty-eight patients with multiple pregnancies, resulting from in vitro fertilization-embryo transfer or intrauterine insemination, and underwent MFPR between January 2000 and December 2005 were included in this study. We retrospectively reviewed medical records of the patients. One hundred and one gestations were triplets (68.2 %), 33 were quadruplets (22.3%), 11 were quintuplets or higher-order pregnancies (7.5%), and 3 were twins (2.0%). All cases of MFPR were performed for the purpose of reducing the fetal number. Patients were counseled regarding the risk of miscarriage and preterm delivery in multiple pregnancies and offered the option of MFPR. If the patients chose the option, possible risks of the procedure were explained and informed consents were obtained. This study was approved by our institutional review board.

MFPR procedure

All MFPR procedures were performed at 6+0 to 15+6 weeks of gestational age and were performed by experienced operators. Before procedure, an ultrasound scan was performed using a 5.0 MHz transducer (Panavista-VA GM-2600A, Matsushita, Japan) to determine the number, locations, and sizes of fetuses and gestational sacs. Fetal heart beats were confirmed in each fetus before starting the procedure. After patients had been placed into the lithotomy position, the vagina was prepared with 10% povidone iodine and then thoroughly rinsed with sterile saline solution. Antibiotic prophylaxis with intravenous injection of cefazolin 2.0 g was administered one hour prior to each procedure. Under sonographic guidance with on-screen guideline, the selected fetus was approached transvaginally with a 19-gauge needle. Most easily accessible fetuses were selected for embryo reduction. Alternatively, embryos with a smaller fetal or sac size were selected.

Cardiac puncture and aspiration of amniotic fluid were performed, and the aspiration of fetus was done if possible. Suction was applied using a 50 mL syringe, which resulted in complete or partial aspiration of the embryo and amniotic fluid. An intracardiac (or intrathoracic) injection of 2 mEq/mL of KCl was performed at the operator's discretion. After ensuring that the fetus concerned had been completely aspirated, or if not, that no fetal heart beat occurred over one minute, the needle was withdrawn. The above procedure was repeated for other gestational sacs in cases of quadruplet or higher-order pregnancies. Follow-up ultrasound examination was carried out after one week. All patients underwent subsequent prenatal routine follow-up.

Study design

According to the use of KCl, patients were divided into 'KCl' and 'non-KCl' groups, and based on gestational age at the time of procedures, patients were divided into 'Early' and 'Late' groups. In the 'Early' groups, reduction was performed before 8 weeks of gestation, and in the 'Late' groups, at 8 weeks or later. Finally, in combination, subjects were divided into four groups according to the use of KCl and gestational age at procedure, i.e., the 'Early, non-KCl' (n=60), 'Late, non-KCl' (n=12), 'Early, KCl' (n=21), and 'Late, KCl' groups (n=55). The choice of cut-offs used to define 'Early' and 'Late' groups was arbitrary and based on the fact that, in the gestational ages earlier than 8 weeks, it is not difficult to aspirate all or most of the fetal parts, since the size of fetuses are relatively small (16). Firstly, to clarify the effect of each component of MFPR procedure, data were analyzed between 'KCl' and 'non-KCl' groups, and between 'Early' and 'Late' groups with adjustments. Secondly, comparison between the four groups was performed to evaluate the combinative effect of both components.

Pregnancy outcomes

The primary outcomes were the results of MFPR, i.e., immediate loss rate, pregnancy loss rate, and take-home-baby rate. Immediate loss was defined as fetal loss within 4 weeks of the procedure and such cases were considered as procedure-related losses. Pregnancy loss was defined as fetal loss up to 24 weeks' gestation, and take-home-baby rate as live birth rate per patient. The secondary outcomes were obstetric outcomes, i.e., the gestational ages, birth weight of babies at delivery, and complications of fetuses and mothers. Extreme prematurity was defined as preterm delivery before 28 weeks' gestation. Preterm premature rupture of membranes (PPROM) was defined as the rupture of amniotic membrane without labor pain during the preterm period.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) and the Student's t-test for continuous variables, whereas chi-square and Fisher's exact tests were used for categorical variables, as appropriate. Probability values were adjusted for gestational ages when analyzing differences between KCl and non-KCl groups, and for KCl use when analyzing differences between Early and Late groups. Analysis of covariance (ANCOVA) or Mantel-Haenszel chi-square test was used for adjustment. The statistical software package SPSS version 12.0 (SPSS Inc., Chicago, IL, U.S.A.) was

used for statistical analysis, and results were considered statistically significant at $p<0.05$.

RESULTS

The mean age of the subjects was 30.6±2.9 yr. Eighty-one MFPRs were performed before 8 weeks' gestational age (54.7%) and 67 procedures were performed at 8 weeks or later (45.3%). In 76 patients, KCl was used as the embryo toxic agent (51.4%) and not used in the other 72 patients (48.6%). Maternal ages and characteristics of the MFPR procedures such as starting number of fetuses, finishing number of fetuses, and the number of procedures required to complete the procedure were not different among the four groups. Gestational ages at the times of procedure were different between the groups (Table 1).

Mean gestational ages at delivery and birth weights were not different between KCl and non-KCl groups and between Early and Late groups. As compared with KCl groups, non-KCl groups showed a significantly higher take-home-baby rate (86.1% vs. 69.7%, $p=0.045$). Immediate loss and pregnancy loss rates were lower in non-KCl groups than in KCl groups, but the differences were not statistically significant (5.6% vs. 10.5% and 12.5% vs. 23.7%, respectively). A significant difference was found between Early and Late groups in terms of immediate loss rates (2.5% vs. 14.9%, $p=0.019$). Early groups showed a lower pregnancy loss rate and a higher take-home-baby rate compared with Late groups, but the differences were not statistically significant (12.3% vs. 25.4% and 81.5% vs. 73.1%, respectively) (Table 2).

Fetal and maternal complications, such as fetal growth restrictions, discordant twins, congenital anomalies, gestational hypertension, and cervical incompetence were not different between KCl and non-KCl groups or between Early and Late groups. Extreme prematurity rates were significant-

Table 1. Comparison of the maternal ages and characteristics of multifetal pregnancy reduction procedures between groups

	Early, non-KCl group (n=60)	Late, non-KCl group (n=12)	Early, KCl group (n=21)	Late, KCl group (n=55)	p^*
Maternal age (yr)	30.6±2.6	31.2±2.5	30.5±2.8	30.7±2.9	NS
Gestational age at procedure (wks)	7.3±0.4	8.8±1.3	7.6±0.3	9.1±1.2	<0.001
Starting number of fetuses	3.3±0.6	3.2±0.4	3.8±0.9	3.3±0.8	NS
Finishing number of fetuses	2.0±0.1	1.8±0.4	2.0±0	1.9±0.3	NS
Number of trials	1.1±0.3	1.0±0	1.2±0.4	1.2±0.5	NS

All values are means±SD. *by ANOVA.
NS, not significant.

Table 2. Pregnancy outcomes according to the different methods of multifetal pregnancy reduction

	KCl groups (n=76)	Non-KCl groups (n=72)	$p^\dagger$	Early groups (n=81)	Late groups (n=67)	$p^\ddagger$
Gestational age at delivery (wks)*	35.5±3.8	35.9±2.0	NS	35.4±3.0	36.1±3.0	NS
Birth weight (g)*	2,297.3±648.1	2,314.0±396.6	NS	2,247.3±493.8	2,389.1±579.2	NS
Immediate loss rate (%)	10.5 (8/76)	5.6 (4/72)	NS	2.5 (2/81)	14.9 (10/67)	0.019
Pregnancy loss rate (%)	23.7 (18/76)	12.5 (9/72)	NS	12.3 (10/81)	25.4 (17/67)	NS
Take-home-baby rate (%)	69.7 (53/76)	86.1 (62/72)	0.045	81.5 (66/81)	73.1 (49/67)	NS

*Values are means±SD; †Adjusted for gestational ages at procedure; ‡Adjusted for the use of KCl.
NS, not significant.

Table 3. Fetal and maternal complications according to the different methods of multifetal pregnancy reduction

	KCl groups (n=76)	Non-KCl groups (n=72)	p^*	Early groups (n=81)	Late groups (n=67)	$p^\ddagger$
Extreme prematurity (%)	8.6 (5/58)	1.6 (1/64)	0.020	6.9 (5/72)	2.0 (1/50)	NS
PPROM (%)	27.6 (21/76)	9.7 (7/72)	0.019	14.8 (12/81)	23.9 (16/67)	NS
Fetal growth restriction (%)	10.3 (6/58)	9.5 (6/63)	NS	9.9 (7/71)	10.0 (5/50)	NS
Discordant twin (%)	5.2 (3/58)	4.8 (3/63)	NS	5.6 (4/71)	4.0 (2/50)	NS
Congenital anomaly (%)	0 (0/58)	4.8 (3/63)	NS	4.2 (3/71)	0 (0/50)	NS
Gestational hypertension (%)	9.2 (7/76)	2.8 (2/72)	NS	2.5 (2/79)	10.4 (7/67)	NS
Cervical incompetence (%)	5.3 (4/76)	5.6 (4/72)	NS	4.9 (4/81)	6.0 (4/67)	NS

*Adjusted for gestational ages at procedure; †Adjusted for the use of KCl.
PPROM, preterm premature rupture of membranes; NS, not significant.

Table 4. Comparison of pregnancy outcomes and complications among the four groups

	Early, non-KCl group (n=60)	Late, non-KCl group (n=12)	Early, KCl group (n=21)	Late, KCl group (n=55)	$p^{\dagger}$	$p^{\ddagger}$
Gestational age at delivery (wks)*	36.0±1.9	35.5±2.4	33.9±4.6	36.2±3.1	NS	NS
Birth weight (g)*	2,323.5±399.1	2,262.2±401.3	2,040.0±659.2	2,419.2±614.3	NS	NS
Immediate loss rate (%)	3.3 (2/60)	16.7 (2/12)	0 (0/21)	14.5 (8/55)	0.037	0.033
Pregnancy loss rate (%)	11.7 (7/60)	16.7 (2/12)	14.3 (3/21)	27.3 (15/55)	NS	0.034
Take-home-baby rate (%)	86.7 (52/60)	83.3 (10/12)	66.7 (14/21)	70.9 (39/55)	NS	0.038
Extreme prematurity (%)	1.9 (1/53)	0 (0/10)	22.2 (4/18)	2.5 (1/40)	0.016	NS
PPROM (%)	10.0 (6/60)	8.3 (1/12)	28.6 (6/21)	27.3 (15/55)	0.047	0.017

*Values are means±SD; †Among the four groups; ‡Between the 'Early, non-KCl' and 'Late, KCl' groups.
NS, not significant.

ly higher in KCl groups than in non-KCl groups (8.6% vs. 1.6%, p=0.020). PPROM occurred significantly more frequently in KCl groups than in non-KCl groups (27.6% vs. 9.7%, p=0.019) (Table 3).

The 'Early, non-KCl' group showed a significantly lower immediate loss rate, pregnancy loss rate, and PPROM rate as compared with the 'Late, KCl' group (3.3% vs. 14.5%, p=0.033; 11.7% vs. 27.3%, p=0.034; 10.0% vs. 27.3%, p=0.017, respectively). The take-home-baby rate was significantly higher in the 'Early, non-KCl' group than in the 'Late, KCl' group (86.7% vs. 70.9%, p=0.038) (Table 4).

DISCUSSION

Several methods of MFPR are available based on different combinations of three components, i.e., the use of embryotoxic agent, the timing of procedure, and the route of approach. However, it is difficult to determine which one is a better option because prospective randomized studies or simultaneous comparisons among these variable methods are difficult to perform. In particular, no randomized controlled trial has been conducted (17), and few comparative studies have been conducted to compare the effects of different factors on outcomes. The present study is a retrospective comparative study of different modalities comprised of two components of the MFPR procedure, i.e., the use of an embryotoxic agent, and the timing of procedure. To our knowledge, this is the first study to evaluate the impact of those two components simultaneously.

Potassium chloride is widely used for MFPR, but the safety and efficacy of this agent are debatable. Cases of anencephaly and limb amputation have been reported, and total pregnancy loss may be resulted if the KCl solution accidentally reaches the amniotic fluid of remaining fetuses (18). It has been suggested that the development of an inflammatory response to the resorbing dead feto-placental tissue with subsequent release of cytokines and stimulation of prostaglandins is a cause of pregnancy loss, preterm delivery, and other complications following MFPR (19-21). All procedures in those studies were performed by KCl injection. Based on those previous findings and our results, we suggest that the use of KCl for MFPR may cause or aggravate the inflammatory process and induce PPROM and preterm birth.

The reason for the higher PPROM rate for KCl groups in the present study is not clear. However, several reports have demonstrated that PPROM is associated with matrix degrading enzymes, such as, plasminogen activators (PAs) and matrix metalloproteinases (MMPs) (22-24). Moreover, in animal studies, investigators found that KCl can induce a release of tissue plasminogen activator in the hypothalamo-neurohypophysial system (25) and an upregulation in MMP-9 activity in the retina, which promote retinal damage (26). Although no study has been conducted on the roles of KCl in relation to matrix degrading enzyme activity in the intrauterine system, we hypothesize that KCl can cause PPROM by inducing matrix degrading enzymes in the uterus after MFPR.

Optimal MFPR timing remains controversial. There have been studies that suggested that fetal reduction should be delayed until the late first trimester, based on the expectation of a natural reduction of fetus and a detection of anomalies (27, 28). However, studies that have focused on loss rates per gestational sac or embryo, not per patient, observed that the rates of spontaneous fetal demise in multiple gestations during early pregnancy were much lower than those reported by other studies that did not consider this condition (29-31). Moreover, reported rates of malformation after MFPR are lower than expected rates, which is probably due to the tendency to selectively reduce embryos with a higher risk of congenital malformation (12). Several studies reported that MFPR during early gestation, at 7-8 weeks, without KCl injection showed superior results to that performed later (13, 16). In the present study, better outcomes were observed in the Early groups, and we confirm these previous study results.

Our data show that 'early, non-KCl' is the best of the different MFPR methods. This is thought to have both advantages of early MFPR and that of non-KCl MFPR. Performing MFPR in earlier gestational age without KCl injection makes the procedure easier because the targeted fetus is relatively small and easily aspirated. If the procedure is performed in later gestation, the relatively large fetus cannot be

easily terminated. The early, non-KCl method is also easier and faster than the other methods and is probably related to fewer procedure-related complications. The significantly lower immediate loss rate in the 'Early, non-KCl' group in our data supports this suggestion. Another advantage of the early method is that it avoids the use of KCl. Potassium chloride is more needed as gestational age increases because larger fetuses cannot be easily terminated by aspiration without KCl injection. As we have discussed earlier, using KCl is not a safe way to perform MFPR, and thus, the use of KCl should be avoided. Early MFPR favors this approach. The early, non-KCl method has benefits of both early and non-using KCl procedure, and thus it could be the best among the four methods investigated.

In the present study, all procedures were performed via the transvaginal route. Several studies have been conducted on different routes of approach, and shown controversial results (12, 15, 32). Experience and skill are also important factors in addition to the route of approach. Like any other procedures, MFPR has a learning curve, and thus outcomes improve with the number of cases. In a collaborative study of 3,513 cases from 11 centers, Evans et al. found the outcomes improved considerably with increasing experience (33). Therefore, if poor initial outcomes are inevitable due to the learning curve, simpler methods should be adopted. Reproductive physicians are more comfortable with transvaginal MFPR because the procedure is similar to ovum aspiration under transvaginal ultrasonographic guidance. Thus, transvaginal MFPR requires fewer technical considerations than transabdominal MFPR. Moreover, not every center performs several hundreds of MFPR procedures as in the large centers included in the previous studies. As cases of high-order multiple pregnancies become less frequent, the number of cases requiring MFPR will also decrease. In this situation, each center must choose the method that best fits its own situation. Transvaginal MFPR could be a better option for centers that perform relatively few procedures and have relatively less experiences. In a collaborative study involving more than 1,000 cases, Evans et al. (32) observed that transabdominal MFPR was more associated with a poor outcome than transcervical or transvaginal MFPR earlier in the series, which also support our suggestion.

Limitations of the present study should be mentioned. First, this is an observational study and not a randomized controlled trial; hence there may be confounding variables that might have influenced the results. However, the similarity between the groups in clinical characteristics such as maternal age, starting number of fetuses, finishing number of fetuses, and number of trials supported that selection bias would be minimal. Second, regarding sample sizes, 250 patients in each group achieve 80% power at a 5% significance level when the difference of take-home-baby rate is 10%. Sample size calculations with other proportional variables showed similar results. In the present study, there are several proportional data that showed a nonsignificant trend of about 10% difference. We could not rule out the possibility that the nonsignificant differences seen in those data could be attributed to a type II error. Further studies in a larger scale will be necessary.

In conclusion, we suggest that the early transvaginal non-KCl method is a better option for MFPR. We believe that it should be considered as the first choice modality in the majority of centers.

REFERENCES

1. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. *Births: final data for 2002. Natl Vital Stat Rep 2003; 52: 1-113.*
2. Ombelet W, De Sutter P, Van der Elst J, Martens G. *Multiple gestation and infertility treatment: registration, reflection and reaction--the Belgian project. Hum Reprod Update 2005; 11: 3-14.*
3. Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine. *Assisted reproductive technology in the United States: 2000 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. Fertil Steril 2004; 81: 1207-20.*
4. Stone J, Eddleman K. *Multifetal pregnancy reduction. Curr Opin Obstet Gynecol 2000; 12: 491-6.*
5. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap III L, Wenstrom KD. *Williams Obstetrics 22nd ed. New York: McGrow-Hill; 2005.*
6. Smith-Levitin M, Kowalik A, Birnholz J, Skupski DW, Hutson JM, Chervenak FA, Rosenwaks Z. *Selective reduction of multifetal pregnancies to twins improves outcome over nonreduced triplet gestations. Am J Obstet Gynecol 1996; 175: 878-82.*
7. Boulot P, Vignal J, Vergnes C, Dechaud H, Faure JM, Hedon B. *Multifetal reduction of triplets to twins: a prospective comparison of pregnancy outcome. Hum Reprod 2000; 15: 1619-23.*
8. Yaron Y, Bryant-Greenwood PK, Dave N, Moldenhauer JS, Kramer RL, Johnson MP, Evans MI. *Multifetal pregnancy reductions of triplets to twins: comparison with nonreduced triplets and twins. Am J Obstet Gynecol 1999; 180: 1268-71.*
9. Antsaklis AJ, Drakakis P, Vlazakis GP, Michalas S. *Reduction of multifetal pregnancies to twins does not increase obstetric or perinatal risks. Hum Reprod 1999; 14: 1338-40.*
10. Dodd J, Crowther C. *Multifetal pregnancy reduction of triplet and higher-order multiple pregnancies to twins. Fertil Steril 2004; 81: 1420-2.*
11. Dumez Y, Oury JF. *Method for first trimester selective abortion in multiple pregnancy. Contrib Gynecol Obstet 1986; 15: 50-3.*
12. Dechaud H, Picot MC, Hedon B, Boulot P. *First-trimester multifetal pregnancy reduction: evaluation of technical aspects and risks from 2,756 cases in the literature. Fetal Diagn Ther 1998; 13: 261-5.*
13. Coffler MS, Kol S, Drugan A, Itskovitz-Eldor J. *Early transvaginal embryo aspiration: a safer method for selective reduction in high order multiple gestations. Hum Reprod 1999; 14: 1875-8.*

14. Geva E, Fait G, Yovel I, Lerner-Geva L, Yaron Y, Daniel Y, Amit A, Lessing JB. *Second-trimester multifetal pregnancy reduction facilitates prenatal diagnosis before the procedure. Fertil Steril 2000; 73: 505-8.*
15. Timor-Tritsch IE, Bashiri A, Monteagudo A, Rebarber A, Arslan AA. *Two hundred ninety consecutive cases of multifetal pregnancy reduction: comparison of the transabdominal versus the transvaginal approach. Am J Obstet Gynecol 2004; 191: 2085-9.*
16. Mansour RT, Aboulghar MA, Serour GI, Sattar MA, Kamal A, Amin YM. *Multifetal pregnancy reduction: modification of the technique and analysis of the outcome. Fertil Steril 1999; 71: 380-4.*
17. Dodd JM, Crowther CA. *Reduction of the number of fetuses for women with triplet and higher order multiple pregnancies. Cochrane Database Syst Rev 2003: CD003932.*
18. Iberico G, Navarro J, Blasco L, Simon C, Pellicer A, Remohi J. *Embryo reduction of multifetal pregnancies following assisted reproduction treatment: a modification of the transvaginal ultrasound-guided technique. Hum Reprod 2000; 15: 2228-33.*
19. Sebire NJ, Sherod C, Abbas A, Snijders RJ, Nicolaides KH. *Preterm delivery and growth restriction in multifetal pregnancies reduced to twins. Hum Reprod 1997; 12: 173-5.*
20. Silver RK, Helfand BT, Russell TL, Ragin A, Sholl JS, MacGregor SN. *Multifetal reduction increases the risk of preterm delivery and fetal growth restriction in twins: a case-control study. Fertil Steril 1997; 67: 30-3.*
21. Geva E, Lerner-Geva L, Stavorovsky Z, Modan B, Freedman L, Amit A, Yovel I, Lessing JB. *Multifetal pregnancy reduction: a possible risk factor for periventricular leukomalacia in premature newborns. Fertil Steril 1998; 69: 845-50.*
22. Liu YX, Hu ZY, Liu K, Byrne S, Zou RJ, Ny T, d'Lacey C, Ockleford CD. *Localization and distribution of tissue type and urokinase type plasminogen activators and their inhibitors Type 1 and 2 in human and rhesus monkey fetal membranes. Placenta 1998; 19: 171-80.*
23. Maymon E, Romero R, Pacora P, Gervasi MT, Bianco K, Ghezzi F, Yoon BH. *Evidence for the participation of interstitial collagenase (matrix metalloproteinase 1) in preterm premature rupture of membranes. Am J Obstet Gynecol 2000; 183: 914-20.*
24. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P, Menon R. *A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. Am J Obstet Gynecol 1998; 179: 1248-53.*
25. Miyata S, Nakatani Y, Hayashi N, Nakashima T. *Matrix-degrading enzymes tissue plasminogen activator and matrix metalloprotease-3 in the hypothalamo-neurohypophysial system. Brain Res 2005; 1058: 1-9.*
26. Mali RS, Cheng M, Chintala SK. *Intravitreous injection of a membrane depolarization agent causes retinal degeneration via matrix metalloproteinase-9. Invest Ophthalmol Vis Sci 2005; 46: 2125-32.*
27. Lipitz S, Shulman A, Achiron R, Zalel Y, Seidman DS. *A comparative study of multifetal pregnancy reduction from triplets to twins in the first versus early second trimesters after detailed fetal screening. Ultrasound Obstet Gynecol 2001; 18: 35-8.*
28. Dickey RP, Taylor SN, Lu PY, Sartor BM, Storment JM, Rye PH, Pelletier WD, Zender JL, Matulich EM. *Spontaneous reduction of multiple pregnancy: incidence and effect on outcome. Am J Obstet Gynecol 2002; 186: 77-83.*
29. Kol S, Levron J, Lewit N, Drugan A, Itskovitz-Eldor J. *The natural history of multiple pregnancies after assisted reproduction: is spontaneous fetal demise a clinically significant phenomenon? Fertil Steril 1993; 60: 127-30.*
30. Tummers P, De Sutter P, Dhont M. *Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. Hum Reprod 2003; 18: 1720-3.*
31. Ulug U, Jozwiak EA, Mesut A, Berksoy MM, Bahceci M. *Survival rates during the first trimester of multiple gestations achieved by ICSI: a report of 1448 consecutive multiples. Hum Reprod 2004; 19: 360-4.*
32. Evans MI, Dommergues M, Timor-Tritsch I, Zador IE, Wapner RJ, Lynch L, Dumez Y, Goldberg JD, Nicolaides KH, Johnson MP. *Transabdominal versus transcervical and transvaginal multifetal pregnancy reduction: international collaborative experience of more than one thousand cases. Am J Obstet Gynecol 1994; 170: 902-9.*
33. Evans MI, Berkowitz RL, Wapner RJ, Carpenter RJ, Goldberg JD, Ayoub MA, Horenstein J, Dommergues M, Brambati B, Nicolaides KH, Holzgreve W, Timor-Tritsch IE. *Improvement in outcomes of multifetal pregnancy reduction with increased experience. Am J Obstet Gynecol 2001; 184: 97-103.*