Short-term outcome in preterm infants depending on whether they were born from singleton, twin, or triplet pregnancy – data from a tertiary care hospital in Serbia

Gordana Velisavljev-Filipović1,2, Aleksandra Matić1,2, Marina Dragićević1,3, Divna Damjanovski6

1University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; 2Institute for Child and Youth Health Care of Vojvodina, Pediatric Clinic, Novi Sad, Serbia; 3Clinical center of Vojvodina, Department of hematology, Novi Sad, Serbia; 4Clinical center of Vojvodina, Department of Obstetrics and Gynecology, Novi Sad, Serbia

SUMMARY
Introduction/Objective After the introduction of the national program of fertility treatment, increased frequency of multiple pregnancies was noted. The literature has shown controversies regarding the higher risk of morbidity and mortality of the preterm newborns from multiple pregnancies.

Methods Preterm singletons, twins and triplets born within a two-year study period were included in the analysis. Data about preterm twins were extracted first. For each pair of twins, two singletons of the same gestation age were chosen. The set of the examinees was completed by including the triplets born during the same period. The short-term outcomes were compared between these three groups.

Results A total of 210 preterm infants were included in the study, out of which 84 singletons, 84 twins and 42 triplets. Statistical analysis showed significant difference between the three groups regarding type of conception (p < 0.0001), mode of delivery (p < 0.001) and birth weight (p = 0.005). Short-term mortality and morbidity (neonatal death, the need for intubation at birth, respiratory support, surfactant therapy, and intracranial hemorrhage) were significantly increased in triplets comparing to singletons and twins.

Conclusion Preterm triplets have an increased risk for adverse short-term outcomes comparing to singletons and twins of the similar gestation age in our study sample.

Keywords: premature baby; single pregnancy; twins; triplets; morbidity

INTRODUCTION

In Serbia, and especially in the Autonomous Province of Vojvodina, increasing number of infertile couples is one of the biggest health issues, while birthrate is in constant decline. One third of all the couples in Serbia are childless, while half of them are not able to have children. Since 2006, the national program for infertility treatment has been active. Within this program, the biomedical procedures of assisted reproductive technologies or in vitro fertilization (IVF) are used, hence the initial program title One free attempt of IVF for 1,000 couples. [1] After the introduction of this program, an increased number of multiple pregnancies that often end up in premature labor have been observed. A similar expansion of assisted reproductive techniques accompanied by increased frequency of multiple pregnancies has also been noted in the developed countries 10 years prior to the introduction of our national program. [2, 3]

The recent literature findings are controversial with respect to the risk of the increased morbidity and mortality of preterm singletons compared to twins and preterm triplets [4–8].

To the best of our knowledge, no studies have compared outcome between these groups of preterm infants in Serbia. Clarifying the consequences of multiple premature births on a local level can be helpful in the planning of local perinatal health resources as well as closer surveillance of high-risk infants. In this way, an improvement of the perinatal protection could be achieved, with an impact on the decline in adverse short-term outcome of the premature infants, regardless of whether they are from singleton, twin, or triplet pregnancies.

METHODS

This retrospective case-control study was carried out at the Institute for child and youth health care of Vojvodina, Novi Sad, Serbia, a part of which is regional tertiary-level and university pediatric clinic with level III neonatal intensive care unit (NICU). The study was done in accord with standards of the institutional Committee on Ethics. A total of 210 preterm infants were included, who were hospitalized at the Institute during the two-year study period. Although neonatal service of our clinic performs neonatal transport from six remote maternity hospitals in Vojvodina province, only preterm infants born in Novi Sad were included in the study, in order to
to exclude impact of the transport and local protocol differences in the primary newborn care on the overall outcome.

Our study sample was created in the way that we first identified and retrieved medical records of every preterm twin hospitalized during two-year period. Then, for every twin we found singleton of a matched gestation (± two days) born within the same two years. Finally, the set of the examinees was completed by the triplets who were born and hospitalized during the study period.

Stillborn twins and triplets were excluded from the study. Furthermore, twins and triplets from pregnancies that were affected by the embryo reduction were also excluded from the study. Only preterm infants from the twin and triple pregnancies, which all completed with all live-born fetuses, were included in the study sample.

When we completed our study sample, we wanted to check whether the study sample size was of sufficient to meet the research goal. Having in mind the average annual number of live births with approximate percentage of premature births in maternity hospital in Novi Sad, with the margin of error of 5% and the confidence level of 95%, the study sample should have at least 205 examinees. This calculation shows that our study sample of 210 examinees is appropriately sized.

The short-term outcomes of these three groups, single, twins and triplets, were compared in the study. Collected prenatal and perinatal data refers to the conception type, antenatal steroids, mode of delivery, birth weight (BW), gestational age (GA), sex and Apgar score in the first and the fifth minute.

The data about neonatal outcomes that have been gathered were: respiratory distress syndrome (RDS), based on the need for supplemental oxygen as well as on the lung X-ray; need for intubation at birth; respiratory support applied (which was defined as the need for assisted ventilation with continuous positive pressure or mechanical ventilation); surfactant therapy, chronic lung disease (CLD) defined as continuing need for oxygen therapy at 36 weeks corrected GA with abnormal X-ray; intracranial hemorrhage based on ultrasound examination of the brain; retinopathy of prematurity (ROP) based on ophthalmologic examination, according to the International Classification for ROP.

The SPSS software version 21.0 (IBM Corp. Armonk, NY, USA) was used for the statistical data analysis. Categorical variables are presented as frequencies and percentages. All continuous variables are presented as mean ± standard deviation (SD). The significance in differences in perinatal data between three study groups was tested. For quantitative variables, one-way ANOVA was used. Frequencies were tested using Fisher’s exact test or Pearson χ² test. Multivariable logistic regression was used to assess the differences in mortality and morbidities between the groups with adjustment for perinatal data, which showed significance at the level p < 0.1 on univariate analysis. Statistical significance was set at p < 0.05 for all tests. Results of the generalized logistic regression are presented as adjusted odds ratios (OR) with the appropriate 95% confidence intervals (CI).

RESULTS

Our study sample included 210 preterm infants, of which 84 singletons, 84 twins and 42 triplets. Examinees had a mean GA of 31.1 (± 0.29) weeks (range: 26–35 weeks), with 32.14 ± 2.51 in singletons, 32.12 ± 2.51 in twins and 31.14 ± 1.907 in triplets. The difference in mean GA between the groups was not statistically significant, but with the p value of 0.061 had a tendency toward significance. Since this p value is less than 0.1, we included GA in confounding factors when performing multivariate statistical analysis. Mean BW in singletons was 1,788 (± 68.50) grams, in twins 1,681 (± 41.15) grams and in triplets 1,476 (± 68.79) grams (Table 1).

Among singletons, there were only 2.38% IVF pregnancies, while it was the case with 50% studied twins and 92.85% triplets. This difference in the way of conception between singleton and multiple pregnancies is certainly statistically significant (p < 0.0001). Caesarean section births were noted significantly more frequent in triplets compared to singletons and twins (p < 0.001) (Table 1).

There was no significant difference regarding sex, antenatal steroids, and Apgar score in the first and fifth minute between the studied singleton, twin and triplet group (Table 1).

Neonatal death during primary hospitalization was significantly more often recorded in triplets compared to singletons (p = 0.046) as well as to twins (p = 0.44), even after adjustment for gestation, BW and caesarean delivery (Table 2).

Table 1. Comparison of prenatal and perinatal characteristics of studied preterm singletons, twins and triplets.

| Characteristics          | Singletons (n = 84) | Twins (n = 84) | Triplets (n = 42) | p     |
|--------------------------|--------------------|---------------|------------------|-------|
|                          | (min–max)          | (min–max)     | (min–max)        |       |
| Gesational age (weeks)   | 32.14 ± 2.51       | 32.12 ± 2.51  | 31.14 ± 1.907    | 0.061 |
| Birth weight (grams)     | 1,787.61 ± 627.77  | 1,680.71 ± 377.12 | 1,476.19 ± 448.53 | 0.005 |
| Male sex                 | 35 (41.66%)        | 44 (52.38%)   | 22 (52.38%)      | 0.31  |
| Antenatal steroids       | 7 (8.33%)          | 6 (7.14%)     | 3 (7.14%)        | 0.95  |
| Cesarean delivery        | 47 (55.95%)        | 48 (57.14%)   | 39 (92.85%)      | < 0.001 |
| Apgar score in 1st minute| 6.29 ± 2.08        | 6.107 ± 2.17  | 6.76 ± 1.24      | 0.17  |
| Apgar score in 5th minute| 7.66 ± 1.54        | 7.53 ± 1.44   | 7.97 ± 0.84      | 0.247 |
| In vitro fertilization   | 2 (2.38%)          | 42 (50%)      | 39 (92.85%)      | < 0.0001 |

SD – standard deviation
Table 2. Neonatal outcomes in studied preterm singletons, twins and triplets and their comparison between the groups using multivariate logistic regression

| Parameters                  | Singletons (n = 84) | Twins (n = 84) | Triplets (n = 42) | Singletons vs. twins | Singletons vs. triplets | Twins vs. triplets |
|-----------------------------|---------------------|----------------|-------------------|----------------------|------------------------|-------------------|
| Death                       | 1 (1.19%)           | 1 (1.19%)      | 6 (14.28%)        | 1.00                 | /                      | 0.046             |
| RDS (any degree)            | 69 (82.14%)         | 67 (79.76%)    | 38 (90.47%)       | 0.844                | 1.16 (0.54–2.52)       | 0.174             |
| Endotracheal intubation      | 30 (35.71%)         | 24 (28.57%)    | 24 (57.14%)       | 0.408                | 1.39 (0.72–2.66)       | 0.035             |
| Respiratory support         | 37 (44.04%)         | 33 (39.28%)    | 27 (64.28%)       | 0.532                | 1.27 (0.69–2.35)       | 0.038             |
| Surfactant                  | 14 (16.66%)         | 19 (22.62%)    | 19 (45.23%)       | 0.437                | 0.68 (0.31–1.47)       | 0.001             |
| CLD                         | 8 (9.52%)           | 5 (5.95%)      | 5 (11.9%)         | 0.565                | 1.66 (0.52–5.31)       | 0.758             |
| Intracranial hemorrhage     | 16 (19.04%)         | 17 (20.24%)    | 17 (39.09%)       | 1.000                | 0.95 (0.47–2.12)       | 0.057             |
| ROP                         | 15 (17.85%)         | 10 (11.9%)     | 4 (9.52%)         | 0.386                | 1.61 (0.67–3.82)       | 0.294             |

RDS – respiratory distress syndrome; CLD – chronic lung disease; ROP – retinopathy of prematurity; aOR – adjusted odds ratio (adjusted for gestation, birth weight and caesarean delivery); 95% CI – 95% confidence interval

Inconsistency with the findings in our study. However, they also found that triplets and twins had more frequent RDS than singletons of the similar gestation. In accordance with our results, they also did not find a difference in the frequency of chronic lung disease between the studied groups [12]. Ziadeh et al. [13] found a significantly higher mortality rate in triplets compared to twins, while they did not find differences regarding the incidence of RDS and intracranial hemorrhage between twins and triplets. Contrary to the results of the above-mentioned studies, Russell et al. [14] found that mortality of premature infants from multiple pregnancies was lower than those from singleton pregnancies, when it was adjusted to BW.

Obeichina et al. [15] analyzed perinatal data from 3531 deliveries completed in term as well as preterm at the University Hospital in Southeast Nigeria. The authors found that there was a significantly increased mortality in twins compared to singletons. Mathews et al. [16] in their report of infant mortality statistics from the 2013 have also found increased mortality in triplets than in singletons and twins. However, no differences in mortality between preterm singletons and twins have been found.

Data from a large study regarding rate of multiple births in Europe showed that median rate of triplet pregnancies in Europe in 2010 was 0.3 per 1,000 deliveries, regardless of the presence of stillbirths [17]. Overall, the frequency of triplets was similar in European countries included in the analysis. Results from the same study showed that multiple pregnancies had a nine-fold relative risk of preterm delivery compared to singletons. Furthermore, the median neonatal mortality rate among multiples was considerably higher than in singletons [17].

The study dealing with outcomes of multiple births in Korea found that neonatal mortality rate was higher in the triplets compared to singletons in a group of infants born with less than 28 weeks of gestation, while this difference was lost for gestations over 28 weeks. An interesting finding in the same study is that survival rate was higher in triplets compared to singletons and twins. These results are in consistency with the findings in our study. However, they also found that triplets and twins had more frequent RDS than singletons of the similar gestation. In accordance with our results, they also did not find a difference in the frequency of chronic lung disease between the studied groups [12]. Ziadeh et al. [13] found a significantly higher mortality rate in triplets compared to twins, while they did not find differences regarding the incidence of RDS and intracranial hemorrhage between twins and triplets. Contrary to the results of the above-mentioned studies, Russell et al. [14] found that mortality of premature infants from multiple pregnancies was lower than those from singleton pregnancies, when it was adjusted to BW.
in triplets than in twins for gestations of 32 weeks and above [9].

Donovan et al. [18] in their analysis of outcomes of a very low BW twins cared for in the National Institute of Child Health and Human Development Neonatal Research Network’s intensive care units found no differences in frequency of CLD, grade 3/4 intraventricular hemorrhage and in death rate between the very low BW singletons and twins. Wolf et al. [19] did not find differences in morbidity and mortality between a very low BW twins and singletons. These results are in consistency with the results of this study, since we did not find differences in short-term outcomes between singletons and twins either. A recent study comparing the neonatal outcome of late-preterm twins and late-preterm singletons at the university clinic in Toronto found that the risk of RDS in singletons was like that of twins. However, antenatal corticosteroids were administered significantly more frequently in pregnant women with twin pregnancy [20]. The level of respiratory morbidity was the same between the twins and the singles when adjustment for the mode of delivery and exposure to antenatal steroids was made. Contrary to above mentioned studies, opposite results can be found in contemporary literature. Comparing singletons, twins, and triplets of similar GA, Kaufman et al. [21] did not find a significant difference in morbidity and mortality between them. However, this study included only 55 sets of triplets.

In their retrospective study, Spasojevic et al. [22] analyzed the morbidity of preterm twins from IVF pregnancies who were treated in the NICU. They found that the signs of RDS were present in all of them, making RDS the main reason for addition to NICU in this group of infants.

Regarding antenatal steroids administration, we did not find a statistically significant difference between singleton, twin, and triplet pregnancies. In addition, the frequency of RDS of any degree was not different between the studied groups. However, a statistically significant difference in the need for endotracheal intubation and the need for the administration of surfactant in triplets compared to singletons and twins was found. As it was expected, the triplets required significantly more frequent respiratory support compared to singletons and twins, too. These data support the higher incidence of severe early respiratory morbidity in triplets than in singletons and twins in our study sample. However, one should not ignore the possibly that surfactant was sometimes given as a preventative or over-therapy in triplets.

In the study conducted in Japan, the authors have found that the incidence of RDS was significantly lower in preterm twins than in singletons [23]. The other study from Japanese authors showed that the frequency of RDS decreased significantly in twins born with 34 weeks, and at 36 weeks, the twins had a frequency of RDS as singletons at 38 weeks [10]. The authors concluded that lungs matured much faster in twins than in singletons. It is possible that intrauterine stress or fetal growth restriction accelerates lung maturation or leads to abnormal development of the pulmonary vascular bed [23].

In a multicentric study of maternal and neonatal outcomes in 15,194 pregnancies from 15 hospitals in Beijing, the results show that infants from multiple pregnancies had significantly higher admission to NICU compared to infants from single pregnancies. There was a significant association of adverse maternal and neonatal outcomes and multiple pregnancies [24]. In a multicentric, randomized, controlled study conducted in the US, Refuerzo et al. [25] also found an increased risk of admission to NICU of infants from multiple pregnancies. The recent study that analyzed the neonatal outcome of the late-preterm twins and late-preterm singletons found that twins often required resuscitation at birth and were more often admitted to NICU [26]. However, there was no difference between singletons and twins in terms of non-respiratory morbidity, that is, the frequency of ROP and intracranial hemorrhage [4].

The plurality and length of gestation are in inverse correlation, as confirmed by several studies. Assisted reproductive techniques are one of the main reasons for the increase in incidence of multiple births worldwide, and especially in developing countries [6, 7, 27, 28].

Twin and triple pregnancies in low- and middle-income countries give an inherent risk to adverse outcomes at both maternal and neonatal levels [28].

The limitation of our study is a relatively small study sample from just one tertiary-care center. Furthermore, chronic pregnancy-related diseases and socioeconomic status of the mothers, as factors of significant impact on perinatal outcome, were not considered in our study. On the other hand, given the growing problem of infertility and increasing implementation of assisted reproductive techniques with consequent rise in multiple births in Serbia and in Vojvodina in particular, this is the health problem of great local significance.

Large, population-based studies comparing the outcome of preterm singletons, twins, and triplets, as well as higher-order multiples would be of great importance for the improvement of perinatal care [15, 29, 30, 31].

CONCLUSION

The findings of our study suggest that preterm triples have an increased risk of neonatal death, early respiratory morbidity, and intracranial hemorrhage compared to singleton and twin premature infants.

Conflict of interest: None declared.
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САЖЕТАК
Увод
После увођења националног програма лечења не-
плодности запажен је повећан број вишеплодних трудноћа.
Налази из литературе су спорни у погледу постојања по-
већаног ризика морбидитета и морталитета код превремено
рођене вишеплодности.
Методе
У студију су укључена превремено рођена ново-
рођенчад из једноплодних, близаначких и тригемеларних
трудноћа, рођена током двогодишњег периода трајања
студије. Најпре су пронађени подаци о свим превремено
рођеном децом. За сваки пар близанаца одабрано је
dвоје превремено рођене новорођенчади из једноплодних
трудноћа, рођене током истог времена. Сет испитаника је употпуњен укључивањем свих превремено рођене
деце из тригемеларних трудноћа. Поређени су здравствени исходи ове три групе – сингли, близанци и тројке.

Резултати
У студију је укључено укупно 210 превремено рођене деце, од којих 84 из једноплодних трудноћа, 84 близанца и 42 тројке. Статистичка анализа је показала значајну разлику мора-
тритета и морталитета код превремено рођене вишеплодности.
Краткорочни исходи били су значајно чешћи код тројки у поређењу са синглима и близанцима.
Закључак
Превремено рођена тројка имају већи риск за неповољни краткорочни исход у поређењу са синглима и близанцима.

Кључне речи: превремено рођена деца; једнострука трудноћа; близанци; тројке; морбидитет; морталитет