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Outcome of pregnancies with a very recent primary cytomegalovirus infection in the first trimester treated with hyperimmunoglobulin: an observational study

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Cytomegalovirus, pregnancy, hyperimmunoglobulin, outcome

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CONTRIBUTION

What are the novel findings of this work?

Primary CMV infection in the first trimester carries a substantial risk of developmental disorder after birth. For the prevention of transmission, hyperimmunoglobulins (HIG) are discussed but with inconclusive results. We found that treatment with HIG is beneficial if patients are well selected.

What are the clinical implications of this work.

Administration of HIG should be discussed in case of a primary CMV infection in the first trimester. However, the treatment can only be successful in women with a very recent primary infection in the first trimester or during the periconceptual period, a timely start and an appropriate treatment interval.

ABSTRACT

Objective:

In this study we set out to examine the efficacy of the hyperimmunoglobulin (HIG treatment in women with a recent primary CMV infection up to 14 weeks' gestation.

Methods:

Ongoing observational study at the prenatal medicine departments of Tuebingen, Bonn, Cologne and Erlangen, Germany as well as the laboratory Prof. Gisela Enders and Colleagues in Stuttgart, Germany and the Institute for Medical Virology at the University of Tuebingen. Enrollment criteria were the presence of confirmed, very recent primary CMV infection in the first trimester and a gestational age at first HIG administration of ≤ 14 weeks. The following inclusion criteria indicated a recent primary infection: low anti-IgG levels, low anti-CMV-IgG avidity in the presence of a positive CMV IgM test and no or just seroconversion anti-gB2-IgG-reactivity. The HIG administration (Cytotect CP®, Biotest, Germany) was started as soon as possible within few days after the first visit in the four units. HIG was administered at 200 IU per kilogram bodyweight intravenously and repeated every two weeks until about 18 weeks' gestation. Maternal-fetal transmission at the time of amniocentesis was considered as relevant primary outcome measure. Multivariate logistic regression analysis was used to determine significant covariates that could be used for the prediction of maternal-fetal transmission.

Results:

149 pregnant women and 153 fetuses were treated. Median maternal age and weight was 32.0 years and 65.0 kg, respectively. Median gestational age at the time of the first referral to one of the four centers was 9.4 weeks. Median anti-CMV-IgG levels, the anti-CMV-IgM index and the CMV IgG avidity was 5.7 U/ml, 2.5, and 22.3%, respectively. Treatment with HIG started at a median gestational age of 10.6 weeks and ended at 17.9 weeks. Within this time frame, HIGs were administered on average 4 times. Amniocentesis was carried out at a median gestational age of 20.4 weeks.

In 143 (93.6%) of the 153 cases, the fetus was not infected. Maternal-fetal transmission occurred in 10 cases (6.5%, [95% CI 3.2 – 11.7]). In the uni- and multivariate logistic regression analysis, only the level of the anti-IgM index was significantly associated with maternal-fetal transmission at the time of the amniocentesis. However, only four (40.0%) of the 10 cases with maternal-fetal transmission had an anti-IgM level above 11.4 which corresponds to the 95th centile of the pregnancies without transmission.

Conclusion:

HIG is a treatment option to prevent maternal-fetal transmission in a case of a primary CMV infection. However, treatment is only beneficial in women with a very recent primary infection in the first trimester or during the periconceptional period, a timely start and an appropriate treatment interval.

INTRODUCTION

Fetal cytomegalovirus (CMV) infection is the most frequent and relevant viral infection during pregnancy. It affects about 0.2-2.2% of all live births. It is the leading cause for non-genetic hearing loss and belongs to the most relevant reasons for neurological disability ^{1 2 3 4 5 6}.

However, the risk of sequelae highly depends on the gestational age at the time of the maternal and fetal infection ^{7,8}. In a recent meta-analysis from Chatzkis et al., the authors reviewed the risk of maternal-fetal transmission after a primary CMV infection. They included 10 studies with about 2,900 fetuses and concluded that during the pre- and periconception period as well as the first, second and third trimester, the risk of transmission was 5.5%, 21.0%, 36.8%, 40.3% and 66.2%, respectively ⁸.

The authors also summarized the risk of a fetal insult, defined as prenatal CNS findings leading to termination of pregnancy or presence of CNS symptoms at birth. They found that the risk was 22.8% for a primary infection in the first trimester and it decreased to 0.9% and 0.4% for an infection in the second and third trimester ⁸.

There is an ongoing discussion about whether there is an efficient treatment option after a primary maternal CMV infection ⁹. The aim of the treatment could either be to avoid maternal-fetal transmission or in case of a fetal infection to avoid a developmental impairment. Several studies have focused on the efficacy of hyperimmunoglobulin (HIG) to prevent maternal-fetal transmission ^{9 10}.

In two randomized controlled studies, the HIG treatment failed to reduce the maternal-fetal transmission rate. Revello et al. treated 123 women with either HIG or placebo and did not find a significant difference in the congenital infection rate (30% vs. 44%) ¹¹. In another large RCT from Hughes et al., 399 women with a primary infection were enrolled. Unfortunately, details of this study (NCT01376778) are not fully published, yet. However, the study was stopped as the transmission rate in the HIG and in the placebo group was 22.7% and 19.4% ^{12 13}. Both studies included pregnant women up to 24-26 weeks' gestation and used 100 units HIG per kilogram body weight on a monthly basis.

In contrast, we observed a maternal-fetal transmission rate of 2.5% at the time of amniocentesis and a congenital infection rate of 7.5% at birth in a series of 40 women with a primary infection who were treated with HIG ¹⁴. We included only women with a very recent primary infection up to 14 weeks gestation and treated them with 200 units per kilogram body weight on a 2 weekly basis up to 20 weeks.

In this extension of our previous study we set out to examine the efficacy of the HIG treatment in about 150 women with a recent primary CMV infection up to 14 weeks' gestation.

METHODS

This is an ongoing observational study at the prenatal medicine departments of Tuebingen, Bonn, Cologne and Erlangen, Germany as well as the laboratory Prof. Gisela Enders and Colleagues in Stuttgart, Germany and the Institute of Medical Virology at our institution. The study started in 2013 at the University Hospital of Tuebingen. The other centers joined the study group in 2017. The study presented here is an extension of our 2018 study by adding about four times the initial number of patients and includes the previous 40 cases¹⁴. The last woman who was included in the study started with the HIG treatment in June 2020.

In Germany, there is no antenatal screening program for CMV and as such the CMV test in the first trimester is offered upon patients request or after recommendation as a part of an individualised healthcare service. Pregnant women with a primary maternal CMV infection were referred to one of the four prenatal medicine departments involved in this study.

The following commercial tests were used for the diagnosis of the CMV infection: Elecsys (ECLIA) anti-IgG, anti-IgM and IgG avidity (Roche Diagnostics, Switzerland) using fully automated cobas 6000/cobas e 601 analyzer (Roche, Hitachi) and a recomLine CMV IgG, IgM and IgG avidity assay (Mikrogen, Germany). The virological monitoring studies have already been published^{15 14}. At the prenatal medicine departments of the University of Tuebingen, Bonn and Cologne, the virological studies were done at the virological department of the University Hospital of Tuebingen.

For women who were treated at the University Hospital of Erlangen, the blood samples were analyzed by the laboratory Prof. Gisela Enders and Colleagues. At this center, the anti-IgG ECLIA system, the anti-IgM ELA Test PKS (medac), the VIDAS (ELFA) CMV IgG Avidity I/II (bioMerieux, France) and a recomLine CMV IgG, IgM and IgG avidity assay (Mikrogen, Germany) were used¹⁶.

Enrollment criteria were the presence of confirmed, very recent primary CMV infection in the first trimester and a gestational age at first HIG administration of ≤ 14 weeks.

The following inclusion criteria indicated a recent primary infection: anti-CMV-IgG level (ECLIA) < 60 U/mL, no or just seroconversion reactivity against anti recgB2-IgG, and a borderline or reactive anti-IgM index. In the few cases where the IgM index was below 1.0 or the IgG level was between 60-100 U/ml, an early CMV primary infection was still assumed due to the anti-p150-IgM reactivity in the immunoblot and a low IgG avidity.

The CMV IgG avidity level was examined with both, the ECLIA or ELFA system and with a recomLine immunoblot¹. The following recombinant antigens were included to categorize CMV IgG avidity by an immunoblot analysis: IE1, CM2, p150, and gB2. Cases were included if both avidity levels were low or if one test indicated an intermediate avidity and the other had to be low^{4 10}.

Cases with a very recent primary CMV infection were classified according to the potential timing of the maternal infection. We had to make the decision based on one blood sample that was taken up to 14 weeks' gestations. Therefore, we assumed that if the blood sample was taken prior to 8 weeks' gestation, the onset of the maternal infection had to be during the periconceptual period. In women where the blood sample was taken between 8 and 14 weeks, the decision was based on the avidity. If it was 20% or less or not measurable due to low anti-IgG levels, the onset of the infection was in the first trimester otherwise it was during the periconceptual period.

Before HIG (Cytotect CP®, Biotest, Germany) treatment was started, each woman received detailed information about the off-label use of HIG and the potential side-effects. The HIG administration was started as soon as possible within few days after the first visit in the four units involved in the study and after the healthcare insurance providers agreed to cover the cost of medical expenses.

HIG was administered at 200 IU per kilogram bodyweight intravenously and repeated every two weeks until about 18 weeks' gestation.

Maternal-fetal transmission at the time of amniocentesis was considered as primary outcome measure.

In all cases that are included in this study, amniocentesis was carried out at least six weeks after the first presentation, generally at about 20 weeks. In the virological department of the University Hospital of Tuebingen, the amniotic fluid was tested by two polymerase chain reactions (PCRs), nested PCR (nPCR) and quantitative real-time PCR (q-rt-PCR), short-term microculture and long-term viral culture until generation of a cytopathic effect (CPE) ranging from 18h to 5 days. Short-term 18-h fibroblast microculture, followed by CMV-IE1 immunoperoxidase staining and long-term virus isolation (10d) from amniotic fluid, was performed by a virus concentration step using high-speed centrifugation with of 50000g for 1h at 4°C prior to virus inoculation¹⁷. DNA was purified by spin columns using QIAmp DNA Blood Mini Kit (Qiagen, Germany) and used for qualitative nPCR of the IE1Ex4-target region. The limit of detection (LOD) for nPCR was 200 copies/mL. q-rt-PCR from plasma, serum or whole ethylenediaminetetraacetic acid (EDTA) blood as well as from amniotic fluid was performed using CMV-R-gene™ real-time PCR kits (Argene, France) with a LOD of 600 copies of CMV DNA/mL using target gene CMV UL83. At the Laboratory Prof. Enders and Colleagues, a RealStar® CMV PCR Kit 1.0 (Altona Diagnostics, Germany) was used. These samples were additionally tested by rapid cell culture using embryonic lung fibroblasts and CMV-IE/EA immunoperoxidase staining.

If no maternal-fetal transmission occurred, no further treatment was carried out. In case of a maternal-fetal transmission, a further treatment with Valaciclovir 8g/d was offered¹⁸. In these cases, the medication was given until delivery. Furthermore, ultrasound monitoring was scheduled on a 2 weekly basis and an MRI was offered at about 30 weeks. Termination of pregnancy was discussed but none of the women chose this option. Furthermore, none of the women opted for discontinuation of the treatment.

After delivery, the umbilical cord, urine and/or saliva were tested for CMV-DNA and for viral isolation. In case of a positive virus detection, viral load in EDTA whole blood and urine or saliva was performed. Congenitally CMV infected (cCMV) newborns were included in a neurodevelopmental follow up program as part of the local clinical service. This protocol includes Valganciclovir for symptomatically cCMV infected newborns¹⁹.

Information about the fetal development, the transmission status at the time of the amniocentesis and the outcome data after birth were recorded in the perinatal databases (Viewpoint, Solingen, Germany). From 2017 on, we also recorded all pregnancy complications during and after the HIG treatment and during the subsequent course of their pregnancy as well as for the head circumference of the newborns.

Ethical approval was given the University of Tuebingen, Cologne, Bonn and Erlangen (749/2020BO2, 20-1525, 285/17, 85_19 Bc).

Statistical analysis

Pregnancy and treatment characteristics are compared according to the transmission status at the time of amniocentesis. Data is presented as median (25th – 75th interquartile range) or percentage whatever is appropriate. Differences between the two groups are compared with a student's t-test or a Mann-Whitney-U-test followed after a Kolmogorov-Smirnov test for normal distribution. For proportions, the 95% Confidence Intervals were calculated according to the method of Clopper and Pearson.

Univariate and multivariate logistic regression analysis was carried to identify significant covariates for the prediction of maternal-fetal transmission. Numerical data was included as continuous variables, dichotomous data was used as categorical variables. Birth weight and head circumference centiles were computed according to the reference ranges of Voigt et al.

²⁰ The level of significance was set at 0.05.

RESULTS

Study population

Since 2013, we started the treatment in 165 women. 16 women were excluded due to a missed abortion (n=5) or a chromosomal defect (n=2) detected during the first trimester risk assessment. Another 8 women were excluded as they refused to undergo an amniocentesis. In one case, the therapy was stopped after the second HIG administration due to an allergic reaction directly after the treatment. This case was also excluded from this analysis.

Thus, in 149 pregnant women we completed the treatment. There were four twin pregnancies. In summary, the study group consisted of 153 fetuses.

In 52 women (53 fetuses) the infection occurred in the first trimester and in 97 women (100 fetuses) during the preconceptional period, respectively.

Median maternal age and weight was 32.0 years and 65.0 kg, respectively. 125 (83.9%) women had at least one previous child and in 110 (73.9%) cases, the youngest child in the family was three years old or younger.

Median gestational age at the time of the first referral to one of the four centers was 9.4 weeks. Median anti-IgG levels, the anti-IgM index and the IgG avidity was 5.7 U/ml, 2.5, and 22.3%, respectively. Further details are given in Table 1. The complete dataset is given in Supp Table 1.

HIG treatment

Treatment with HIG started at a median gestational age of 10.6 weeks and ended at 17.9 weeks. Within this time frame, HIGs were administered on average 4 times. Amniocentesis was carried out at a median gestational age of 20.4 weeks.

In 143 (93.5%) of the 153 cases, the fetus was not infected. Thus, maternal-fetal transmission occurred in 10 cases (6.5%, [95% CI 3.2 – 11.7]) (Figure 1).

In a subgroup analysis, we examined the maternal-fetal transmission rate according to the time of maternal infection. In the group of periconceptional infections, there were 5 (5.0% [95% CI 1.6 – 11.3]) infected fetuses and in the group of first trimester infections, we also observed 5 (9.4% [95% CI 3.1 – 20.6]) fetal infections.

The pregnancy and study characteristics of the 10 and 143 cases with and without transmission are shown in table 2. In the transmission group, the anti-IgM index was higher, the anti-IgG levels were lower and there were more cases with a positive DNA PCR positive result at the time of diagnosis. There were no further significant differences regarding the pregnancy and treatment characteristics in the two groups.

In the uni- and multivariate logistic regression analysis, only the level of the anti-IgM index was significantly associated with maternal-fetal transmission at the time of the amniocentesis (Table 2). However, only four (40.0%) of the 10 cases with maternal-fetal transmission had an anti-IgM level above 11,4 which corresponds to the 95th centile of the pregnancies without transmission.

Side effects during or shortly after the HIG administration were examined in a subgroup of 133 women. 109 (82.9% [95% CI 74.4-88.1]) were asymptomatic, the other 24 women noticed flu-like symptoms, tiredness headache or a rash, predominantly after the first administration. The symptoms disappeared in all patients the following day.

Outcome of pregnancies with maternal-fetal transmission

Median viral load in the amniotic fluid of the 10 cases with maternal-fetal transmission was 1,245,000 copies/ml and ranged between 9,810 and 82,000,000 copies/ml. CMV short term culture indicated on average 5,390 (1,550 – 16,000) infected fibroblasts nuclei per ml. The details of these 10 cases are listed in table 3. At birth, we observed two more cases with maternal-fetal transmission. The amniocentesis in these two cases was carried out at 19,4 and 20.3 weeks and were both CMV-negative. The further course of pregnancy was uneventful, and the two newborns were born asymptotically CMV infected.

In the group of 10 fetuses with an abnormal CMV result in the amniocentesis, one fetus died at 21.1 weeks. In total, median gestational age at delivery was 37.4 (36.4 – 38.9) weeks. Median birth weight was 2,910 (2,620 – 3,100) g, corresponding to the 37th birth weight centile (Figure 2 and 3). Four newborns were asymptomatic, three had some degree of hearing loss, one had increased liver enzymes and one had cystic brain lesions with heterotopia. The median head circumference was 34.0 cm (33.0 – 34.0) (Figure 4 and 5).

Outcome of pregnancies without transmission

So far, 145 fetuses from 141 women were born at a median gestational age of 39.6 (38.7 – 40.3) weeks. 11 (7.6%) fetuses were born preterm (one termination of pregnancy due to a chromosomal defect and one late miscarriage due to rupture of membranes. One preterm delivery at 34 weeks, the remaining eight fetuses were born at 36 weeks). Median birth weight was 3,400 (3,100 – 3,750)g, which corresponds to the 44st birth weight centile (Figure 1 and 2). 4 (2.8%) and 9 (6.2%) newborns had a birth weight below the 5th and 10th centile, respectively (Figure 1 and 2). Median head circumference was 35.0 (34.0 – 35.0) cm (Figure 2). There were 3 pregnancies that were complicated by preeclampsia (delivery at 36.5, 38.0 and 38.9 weeks) and 5 women were treated due to preterm contractions. 4 women had gestational diabetes, one women suffered from depression and 3 pregnancies were complicated by an abnormally invasive placenta or placenta praevia. In one case, there were recurrent episodes of vaginal bleeding with unknown origin leading to delivery at 34 weeks' gestation.

DISCUSSION

In this study based on 149 women with a recent primary CMV infection up to 14 weeks gestation, we have used HIG to prevent maternal-fetal transmission. The transmission rate at the time of the amniocentesis was only 6.5%, the HIG administration was predominantly well tolerated and the proportion of women with complications during the subsequent course of pregnancy was similar to the general population. The transmission rate was similar in the group of pregnancies with an primary infection in the first trimester and in the periconceptional period.

Multivariate logistic regression analysis indicates that the only significant predictor for maternal-fetal transmission at the time of amniocentesis was a higher anti-IgM index at the time of the first presentation. Given the fact, that in all pregnancies there was a very recent CMV infection, proven by several test systems, we believe that the increased anti-IgM level in cases with maternal-fetal transmission may indicate a more pronounced immune response compared to the non-transmission group.

The results of the previous studies are inconsistent⁹. In the majority of the studies, HIG was given on a monthly basis and the treatment was continued up to the third trimester. However, we have demonstrated, that the half life time of HIG is much shorter than previously thought, which necessitates a more frequent application²¹.

There are two randomized controlled studies that examined the effectiveness of HIG^{11 12}. Both failed to demonstrate a lower transmission rate after administration of HIG. In the study of Revello et al., 30% of the newborns were infected and in the trial of Hughes et al., the transmission rate was 22.7%. In the placebo arms of the two studies, the transmission rates were 44% and 19.4%, respectively. The study protocol of both RCTs was similar and differs in many aspects from our study.

First, in both RCTs, the HIG dosage was 100 U per kilogram bodyweight and the treatment was repeated on a monthly basis almost up to the end of the pregnancy. In our study, we used 200 U per kilogram bodyweight and administered the medication on a two weekly basis up to 18 weeks. The rationale for this protocol was based on the experience of our previous study and on our pharmacological studies indicating that the half life time of HIG is only about 10 days^{21 14}.

Second, we only included pregnant women with a recent primary infection²². We used several test systems and stricter inclusion criteria than recently proposed by Khalil et al. to make an unequivocal diagnosis of a very recent infection¹. From our understanding, there has to be a lack of IgG antibodies with high avidity in order for the HIG therapy to be useful.

Third, we only included women with an infection up to 14 weeks while the RCTs recruited up to 26 and 24 weeks, respectively. As the risk of sequelae for second and third trimester infections is only 0.9 and 0.4%, we did not see an indication for HIG in these cases⁸.

Fourth, in contrast to the two RCTs, we included only cases where an amniocentesis was carried out - six weeks after the diagnosis of the maternal infection at the earliest. This gave us the chance to assess the infection status of the fetus during the most vulnerable time until 20 weeks' gestation²³.

In contrast to other studies, we did not observe a higher proportion of obstetric complications during the subsequent course of the pregnancy after HIG treatment^{12 24 11}. Revello et al. observed a preterm delivery rate of 7.6%, fetal growth restriction in 3.8% and eclampsia in 1.9% of the women treated with HIG¹¹. In contrast, in our study the preterm birth and preeclampsia rate as well as the proportion of fetuses with a birth weight below the 10th centile was consistent with the complication rates in the general population^{25 26 27 28}. This difference may be explained by fact that the HIG treatment was stopped before 20 weeks while in the two other studies, the treatment was continued up to the third trimester.

We acknowledge that our study has some weaknesses. We report on a large series of women who all fulfilled the inclusion criteria and who were treated in four different centers. However, this is not a prospective randomized study. Unfortunately, a randomized study to compare HIG to placebo is not possible in Germany anymore due to ethical concerns and due to the fact that we would not be able to recruit women for such a study anymore. We also acknowledge that in this study we do not report on the transmission rate of a control group without HIG. In our previous study, we reported on the outcome of a historical cohort of 108 women who had an amniocentesis between 19 and 22 weeks due to a primary CMV infection in the first trimester. In this group, the transmission rate was 35.2% (95% CI, 26.2 – 45.0%)¹⁴.

In a very recent RCT study from Shahar-Nissan et al., the authors used valaciclovir (8g/d) to prevent maternal-fetal transmission. The transmission rate was 30% and 11% in the placebo and in the treatment group²⁹. Further studies are needed to compare these two different methods and to assess if a combination of both therapies could be beneficial.

In conclusion, compared with historical rates of maternal fetal transmission, HIG is associated with lower transmission rates in case of a primary CMV infection. However, treatment is only beneficial in women with a very recent primary infection in the first trimester or during the periconceptional period, a timely start and an appropriate treatment interval.

Acknowledgements

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Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Hospital ethics committees.

Disclosure statement:

K.O. Kagan and K. Hamprecht have given paid presentations for Biotest, a company that produces immunoglobulins preparations.

K. Hamprecht is a member of the Scientific Advisory Board of the Initiative for the Prevention of Congenital Cytomegaly Disorders (ICON). All related honoraria are paid into a UKT Institute for Medical Virology grant account to support the Tuebingen Congenital CMV Study.

Conflicts of interest:

K. Hamprecht is a member of the Scientific Advisory Board of the Initiative for the Prevention of Congenital Cytomegaly Disorders (ICON). All related honoraria are paid into a UKT Institute for Medical Virology grant account to support the Tuebingen Congenital CMV Study.

Author Contributions

KOK and KH: Conceptualization, project development, formal analysis, manuscript writing and editing

ME: Data collection, formal analysis manuscript editing

MH: Data collection, manuscript editing

AG: Data collection, manuscript editing

CS: Data collection, manuscript editing

CB: Data collection, manuscript editing

IG: Data collection, manuscript editing

FF: Data collection, manuscript editing

MS: Data collection, manuscript editing

TG: Data collection, manuscript editing

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FIGURE LEGENDS

Figure 1. Outcome of women treated with HIG

Figure 2. Birth weight distribution in the fetuses without (white circles) and with (black circles) transmission at the time of the amniocentesis

Figure 3. Box and whiskers blot of the birth weight distribution in fetuses with and without transmission at the time of the amniocentesis. The horizontal line in the box indicates the median, the box the 25-75 centile. The whiskers demonstrate the range.

Figure 4. Distribution of the head circumference in the fetuses without (white circles) and with (black circles) transmission at the time of the amniocentesis

Figure 5. Box and whiskers blot of the distribution of the head circumference in fetuses with and without transmission at the time of the amniocentesis. The horizontal line in the box indicates the median, the box the 25-75 centile. The whiskers demonstrate the range.

SUPPL. TABLES

Table 1. Data synopsis of the women treated with HIG

TABLES

Table 1. Pregnancy characteristics and results of the virological studies stratified according to the transmission status at the time of the amniocentesis

	No maternal-fetal transmission n=143 fetuses	Maternal-fetal transmission n=10 fetuses	p
Maternal age (years) Median (25 th -75 th centile)	32.0 (29.6 – 34.8)	34.7 (30.3 – 38.7)	0.974
Maternal weight (kg) Median (25 th -75 th centile)	65.0 (58.0 – 72.0)	66.4 (52.0 – 75.5)	0.635
Gestational age at presentation (wks) Median (25 th -75 th centile)	9.6 (8.1 – 11.4)	8.8 (8.0 – 10.7)	0.365
Child <3 years at home n women (%)	103/139 (74.1)	7 (70.0)	0.890
Gestational age at first HIG administration (wks) Median (25 th -75 th centile)	10.6 (9.1 – 12.3)	9.2 (8.3 – 11.1)	0.123
Gestational age at last HIG administration (wks) Median (25 th -75 th centile)	17.9 (16.7 – 18.7)	18.0 (16.9 – 19.1)	0.465
Gestational age at amniocentesis (wks) Median (25 th -75 th centile)	20.4 (20.1 – 20.9)	20.2 (20.1 – 20.3)	0.591
PCR positive at the time of first presentation n women (%)	27/139 (19.4)	5/10 (50.0)	0.019
ECLIA anti-IgG U/ml Median (25 th -75 th centile)	5.9 (2.4 – 16.5)	2.7 (1.3 – 4.9)	<0.001
ECLIA anti-IgM Index Median (25 th -75 th centile)	2.3 (1.3 – 4.3)	6.4 (4.4 – 20.1)	0.002*
ECLIA IgG avidity (%) Median (25 th -75 th centile)	22.8 (9.9 – 32.0)	15.1 (4.8 – 23.1)	0.537

* Man-Whitney U -Test otherwise t-Test or chi-square test

Table 2. Uni- and multivariate regression analysis for the prediction of maternal-fetal transmission at the time of the amniocentesis.

Covariates	Univariate analysis		Multivariate analysis	
	OR 95% CI	p	OR 95% CI	p
Maternal age (years)	1.000 0.973 – 1.027	0.974		
Maternal weight (kg)	1.018 0.971 – 1.068	0.453		
Gestational age at presentation (wks)	0.868 0.640 – 1.178	0.364		
Child <3 years at home (categorical)	0.906 0.223 – 3.678	0.890		
Gestational age at first HIG administration (wks)	0.776 0.560 – 1.1077	0.129		
Gestational age at last HIG administration (wks)	1.189 0.750 – 1.886	0.462		
Gestational age at amniocentesis (wks)	0.821 0.404 – 1.670	0.587		
PCR positive at the time of first presentation n (%)	4.296 1.161 – 15.898	0.029	2.988 0.735 – 12.157	0.126
anti-IgG (U/ml)	0.836 0.687 – 1.018	0.075		
anti- IgM Index	1.203 1.089 – 1.330	<0.001	1.193 1.076 – 1.323	0.001
ECLIA IgG avidity (%)	0.985 0.938-1.034	0.535		

Table 3. Characteristics of the 10 cases with maternal-fetal transmission

No	Virological data at presentation	Clinical data	HIG application	Examination of AF	Delivery	Current status
	anti-IgM index, anti-IgG (U/ml) IgG Avidity (%), PCR status	MA (yrs), child<3y, weight (kg)	GA first and last HIG, GA amniocentesis (wks)	Viral load (copies/ml), Short term microculture (no inf. fibroblast/ml)	GA (wks),BW (g/centile), HC (cm/centile)	Age of child* (yrs) Outcome
1	5.3, 3.2, 9.4, neg	34.8, yes, 72.0	8.3, 16.4, 20.3	3,740,000, 5390	37.6, 2620/11, 33/22	2.8, asymptomatic
2	11.6, 3.8, 51.1, pos	38.8, no, 48.0	8.4, 18.4, 20.3	875,000, 1550	38.3, 3100/37, 35/62	1.5, asymptomatic
	7.4, 1.3, 23.1, pos	42.2, yes, 98.0	8.9, 16.9, 20.1	82,000,000, 4400	36.7, 2760/46, 33.0/37	2.2, heterotopia, hearing loss
4	20.1, 8.0, 3.8, neg	34.7, yes, 50.0	9.6, 17.6, 20.1	1,420,000, 8000	35.6, 2400/49, 33.0/54	2.4 hearing loss*
5	4.4, 4.9, 40.3, neg	23.8, yes, 52.0	10.1, 18.1, 20.3	27,300, 26	21.1, 311/1 nd	Intrauterine death
6	20.6, 6.3, 4.8, neg	30.3, yes, 60.7	11.1, 19.1, 20.1	9,810, 8	38.9, 3040/38, 34.0/33	1.0 Increased ALT/AST*
	0.6, 1.2, ne, pos	38.7, yes, 59.1	11.9, 17.9, 20.9	8,230,000, 40000	37.3, 3100/50, 34.0/46	0.7, asymptomatic
8	21.0, 1.2, 22.9, pos	28.5, yes, 102.0	6.6, 16.7, 20.7	1,070,000, 16000	40.0, 3170/15, 34.0/18	0.5, hearing loss*
9	4.2, 2.2, 14.2, pos	30.5, no, 75.5	13.1, 19.1, 20.1	31,700,000, 16000	36.4, 2780/44, 32.0/16	0.2 heterotopia*
10	5.0, 1.4, 16.0, neg	35.0, no, 74.0	8.0, 20.0, 20.0	49,000, nd	40.6, 3230/28, 34.0/18	1.2 asymptomatic*

Ne= not evaluable due to too low IgG, nd= not done

MA = maternal age, AF= amniotic fluid, BW = birth weight, HC= head circumference, *= treatment with Valganciclovir after delivery

Age of child = Age of the child at the time of the last examination.

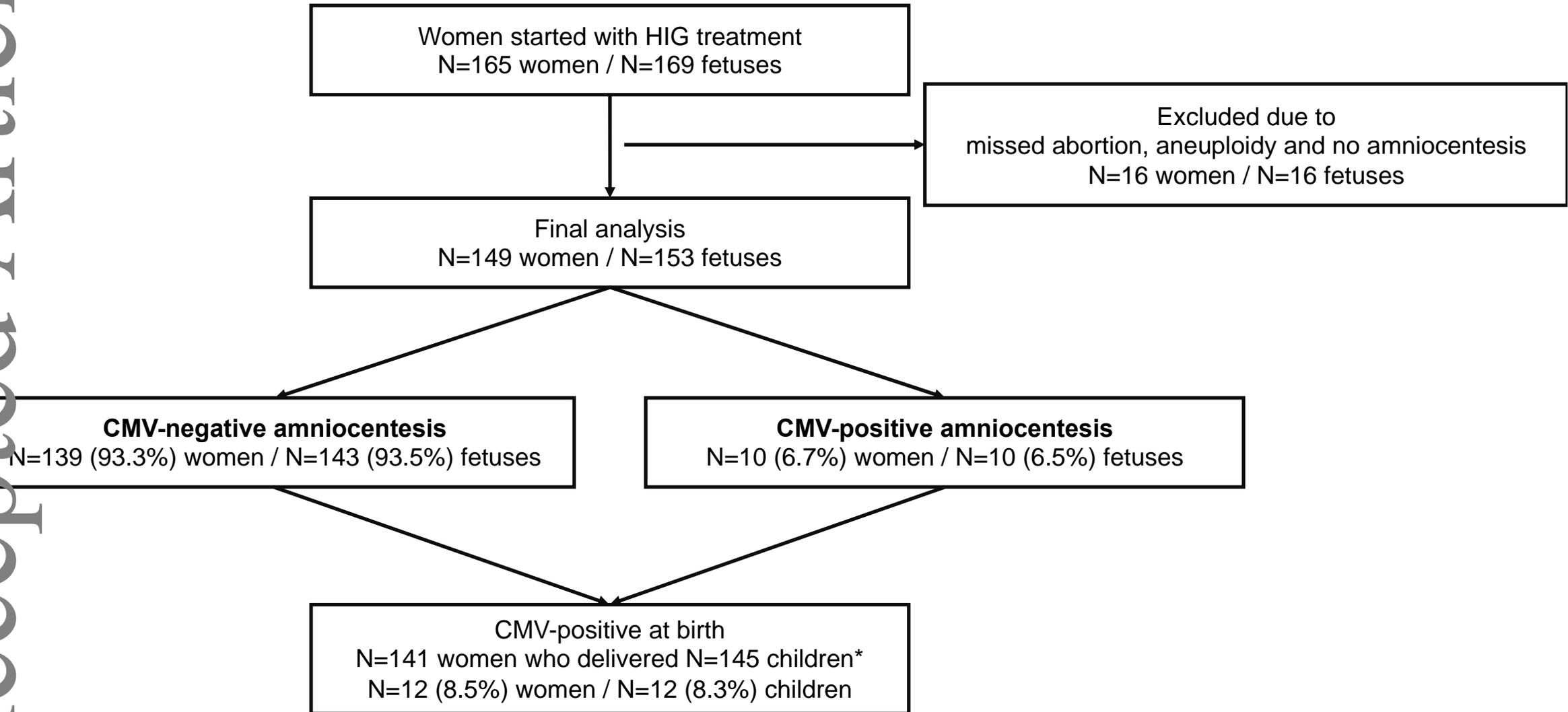


Figure 1. Outcome of women treated with HIG

* = the remaining pregnancies are still ongoing

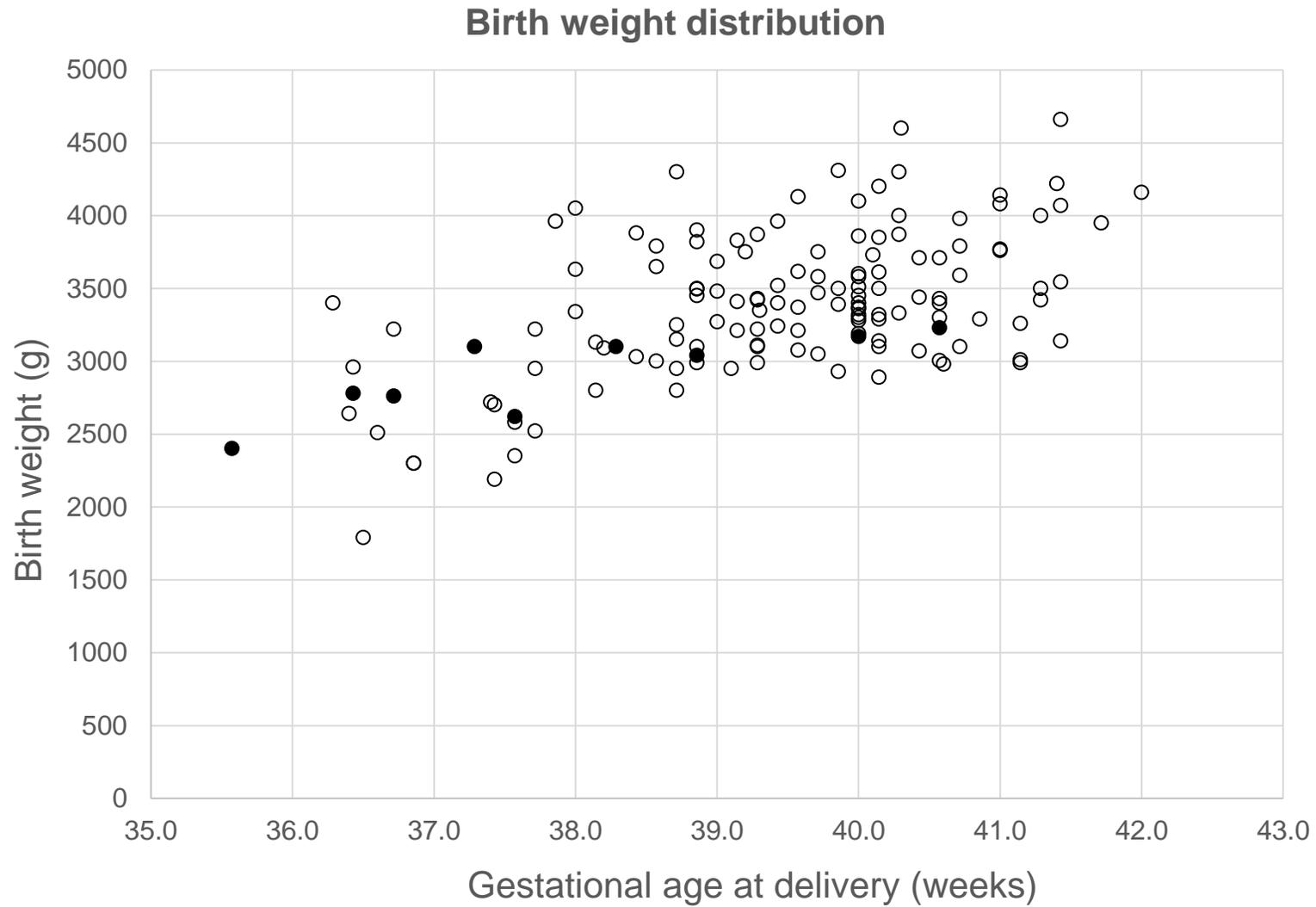


Figure 2. Birth weight distribution in the fetuses without (white circles) and with (black circles) transmission at the time of the amniocentesis

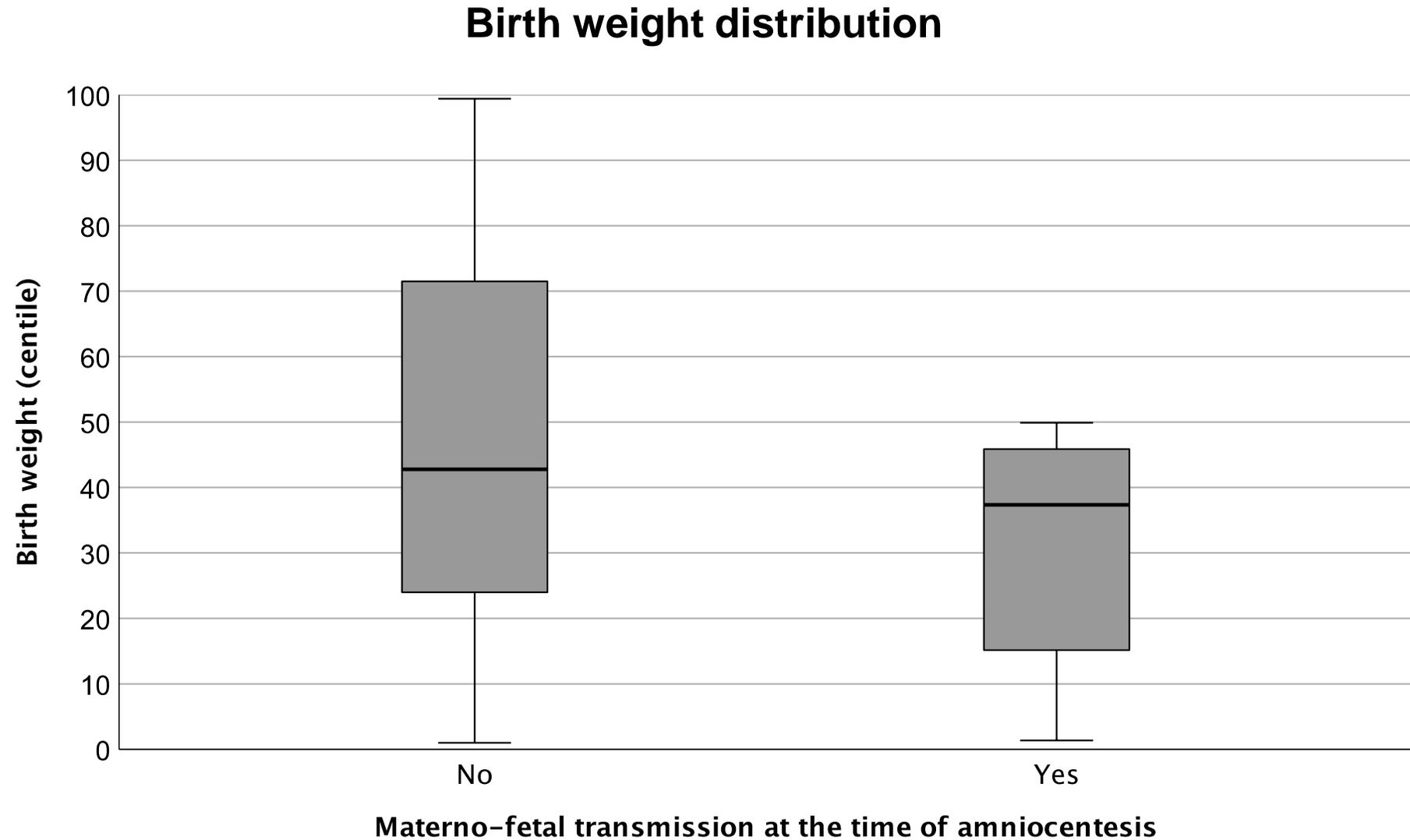


Figure 3. Box and whiskers blot of the birth weight distribution in fetuses with and without transmission at the time of the amniocentesis. The horizontal line in the box indicates the median, the box the 25-75 centile. The whiskers demonstrate the range.

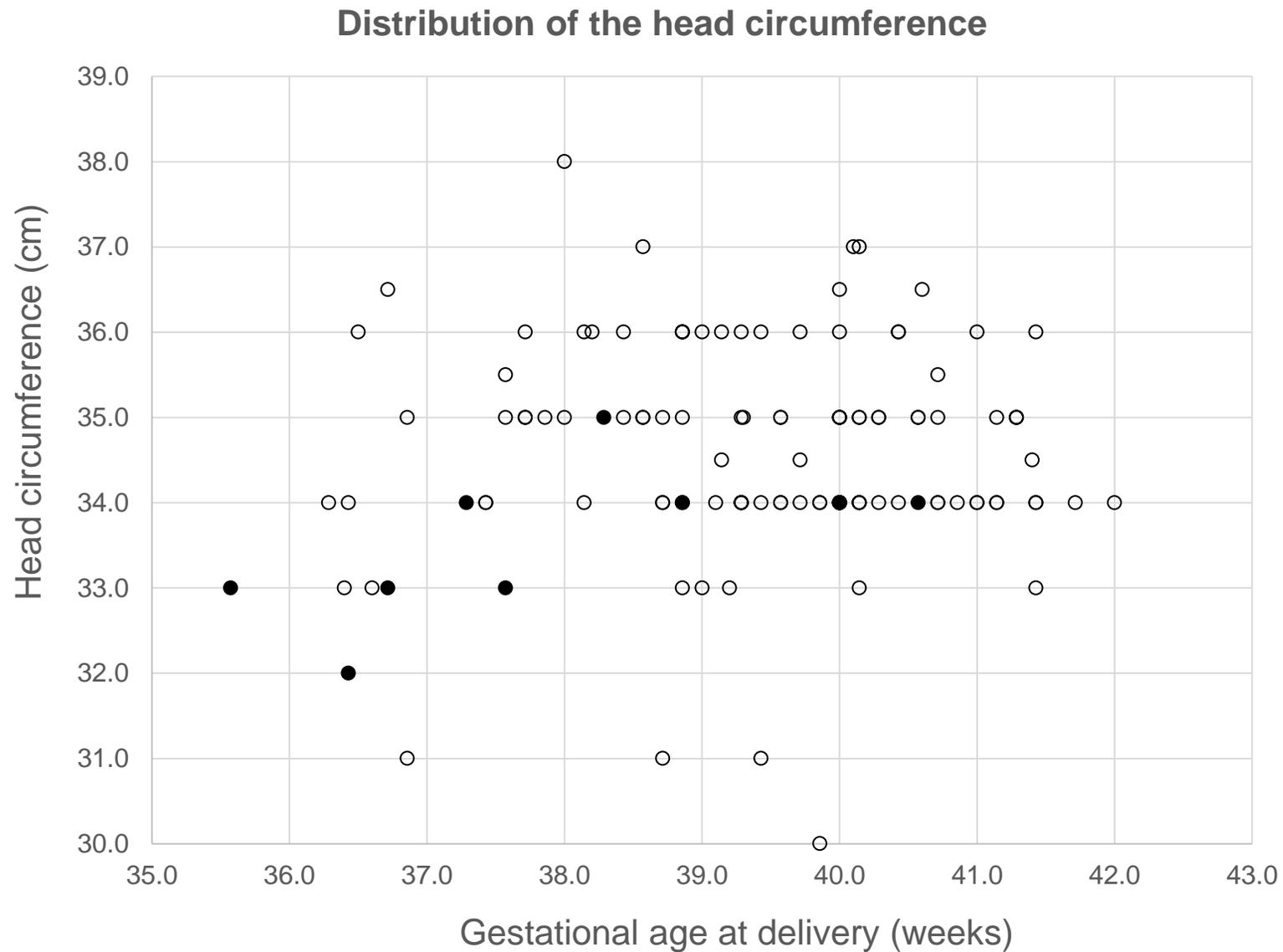


Figure 4. Distribution of the head circumference in the fetuses without (white circles) and with (black circles) transmission at the time of the amniocentesis

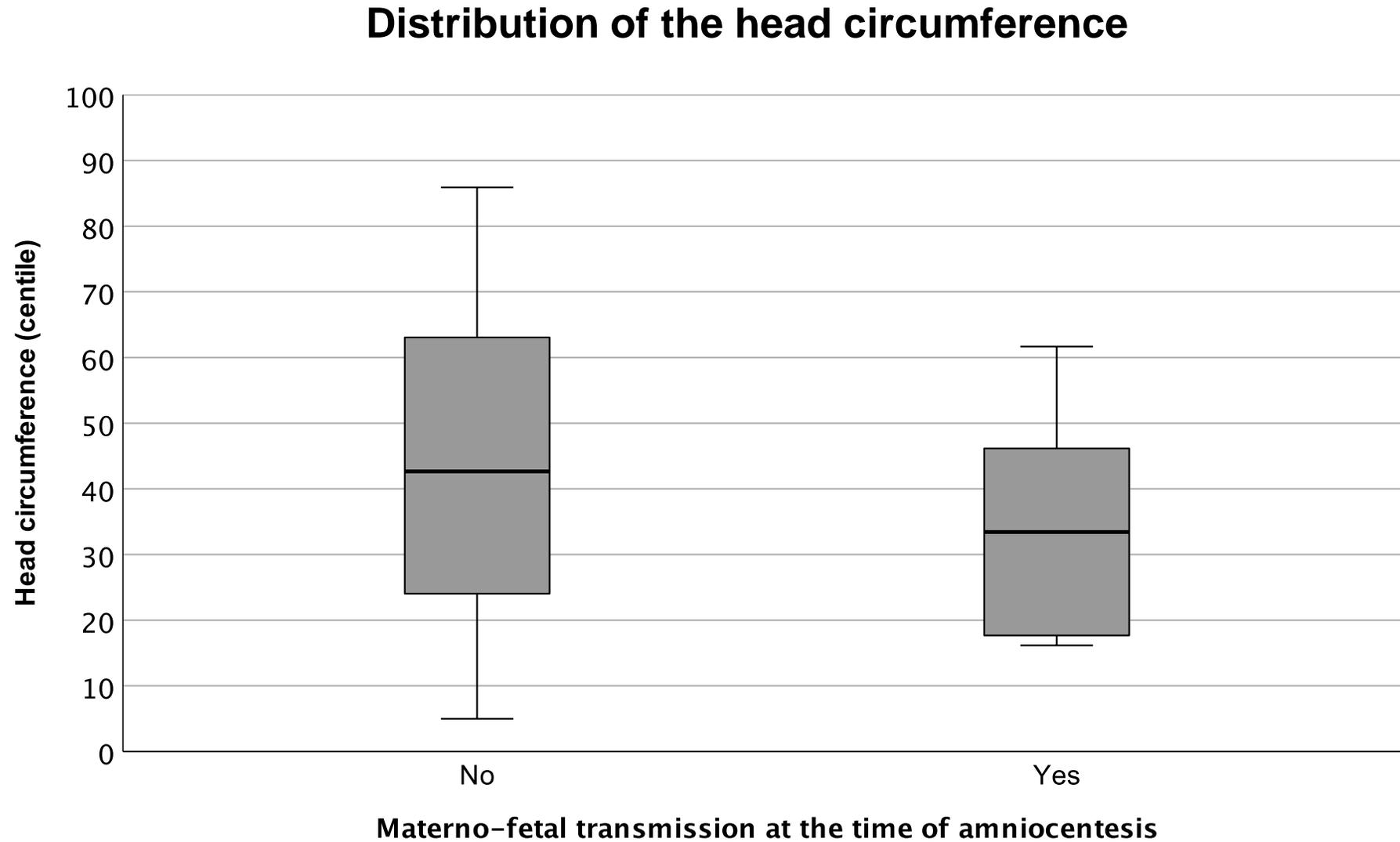


Figure 5. Box and whiskers blot of the distribution of the head circumference in fetuses with and without transmission at the time of the amniocentesis. The horizontal line in the box indicates the median, the box the 25-75 centile. The whiskers demonstrate the range.