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Supplementary appendix

This online publication has been corrected. The corrected version first appeared at thelancet.com/respiratory on April 28, 2022

This appendix formed part of the original submission. We post it as supplied by the authors.

Supplement to: Stoecklein S, Koliogiannis V, Prester T, et al. Effects of SARS-CoV-2 on prenatal lung growth assessed by fetal MRI. *Lancet Respir Med* 2022; published online March 16. https://doi.org/10.1016/S2213-2600(22)00060-1.

Supplemental Information

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Imaging protocol

MRI was performed at 1.5T MRI (Magnetom Aera, Siemens Healthineers, Erlangen, Germany) using an 18-channel body coil. For detailed information on the scan protocol and sequence parameters see Table S1.

	Orientation	TE (ms)	TR (ms)	ST (mm)	Matrix	Resolution (mm)	Phase resolution
Placenta							
T1w VIBE	sag	1·36	3·46	3·5	0/256/230/0	1·6×1·6	90 %
T2w HASTE	sag	80·00	800·00	4·0	0/256/256/0	1·3×1·3	100 %
DWI (b50+b800)	sag	87·00	8100·00	5·0	0/192/115/0	2·1×2·1	80 %
Fetal brain							
T2w TSE	tra, sag, cor	140·00	11380·00	4·0	0/256/192/0	1·3×1·3	75 %
T2 HASTE	tra, sag, cor	83·00	800·00	3·0	256/0/0/256	1·1×1·1	100 %
GRE tra Head	tra	30·00	1650·00	5·0	128/0/0/128	2·5×2·5	100 %
TIRM	cor	5·60	13270·00	5·0	384/0/0/307	1·0×1·0	80 %
DWI (b0+b600)	tra	69·00	5400·00	5·0	0/158/126/0	2·4×2·4	80 %
T1w FLASH	tra	4·60	160·00	4·5	0/256/205/0	1·4×1·4	80 %
Fetal body							
TRUFI	tra, sag, cor	1·85	4·53	5·0	0/256/256/0	1·2×1·2	100 %
T1w FLASH	cor	4·60	160·00	4·5	0/256/205/0	1·4×1·4	80 %
DWI (b50+b400)	cor	69·00	5400·00	5·0	0/126/158/0	2·4×2·4	80 %

Table S1: Imaging protocol and sequence parameters. VIBE (Volume Interpolated Breathhold Examination), HASTE (Half-Fourier Acquisition Single-Shot Turbo Spin Echo), DWI (Diffusion Weighted Imaging), TSE (Turbo Spin Echo), GRE (Gradient Echo), TIRM (Turbo-Inversion Recovery-Magnitude), TRUFI (True Fast Imaging With Steady-State Free Precession)

Characterization of SARS-CoV-2+ study group
40 pregnant women were recruited between December 4th 2020 and June 25th 2021. Written informed consent was obtained from all subjects in accordance with the guidelines of our institutional review board (ethic approval #20-733). For the current analysis, n=6 cases had to be excluded (twin pregnancy (n=2), uncompleted MRI protocol (n=4)). SARS-CoV-2 was diagnosed by PCR at symptom onset (median 76·5 days, range 22-220 days prior MRI). All women had experienced only mild symptoms including anosmia/hypoosmia (79 %), ageusia (66 %), fatigue (79 %), headache (59 %), cold (56 %), and dry cough (50 %). SARS-CoV-2 infection was confirmed by PCR test during the 1st (n=11), 2nd trimester (n=15), or 3rd trimester (n=8). One woman had received a first dose of COMIRNATY vaccine in the fourth week of pregnancy. None of the women had received steroid treatment as respiratory distress prophylaxis prior to MR imaging. All women were Caucasian. Median birth weight in n=21 infants with available neonatal follow-up ranged from 2202 g to 4430 g (median birth weight 3410 g), with only n=2 infants ranging below the 5th percentile reference value of 2500 g. For information on co-morbidities and history of smoking please refer to table S2.

	1st trimenon infection (n=11)	**2nd trimenon infection (n=15)**	**3rd trimenon infection (n=8)**
Smoking during pregnancy	0/11	0/15	0/15
History of smoking	4/11	7 /15	3 /8
Pack years (median and range)	2.5 (1-5)	5.0 (1-17)	1.0 (1-2)
Diabetes (n)	0/11	0/15	0/8
Chronic pulmonary disease (n)	0/11	1/15 (asthma)	0/8
Hypothyroidism (n)	4/11	2/15	3/8
PCOS (n)	0/11	1/15	0/8
Iron deficiency anemia (n)	0/11	0/15	1/8
Endometriosis (n)	0/11	1/15	0/8
Migraine (n)	1/11	1/15	1/8

Table S2: Co-morbidities and history of smoking. PCOS (polycystic ovary syndrome)

Characterization of SARS-CoV-2- control group
The SARS-CoV-2-negative (SARS-CoV-2-) control group consisted of n=15 cases (median gestational age (GA) at MRI 30·0 weeks, range 24-35 weeks, 6 female fetuses). MRI was performed for clinical indications pertaining to central nervous system development in order to rule out agenesis of the corpus callosum (n=2), asymmetry of the lateral ventricles (n=9), arachnoid cyst (n=2), plexus cyst (n=1), and megacisterna magna (n=1) with none of the MRIs presenting with the indicated pathologies. Study and control group followed the identical imaging protocol.

Estimation of fetal lung volume
Fetal lung volumes (LV) were independently determined by two board-certified radiologists with advanced experience in fetal imaging. Fetal lungs were segmented on bright-fluid true fast imaging with steady-state free precession (TRUFI) images acquired in fetal axial plane. Segmentation was performed using Horos version 3.2.1 (https://horosproject.org). Lung images of SARS-CoV-2+ and SARS-CoV-2- were included in the reading sample. The readers were blinded to the diagnosis (SARS-CoV-2+/-), as well as to all other clinical parameters such as time point and severity of infection.

Estimation of fetal body weight
Fetal body weight was estimated following a formula recommended by Shepard and colleagues[1] that was validated for MRI by Matthew and colleagues[2]: Fetal body weight (in kg) = 10^ (-1,7492+0,166*BPD+0,046*AC)-(2,646*(AC*BPD)/1000); BPD = biparietal diameter, AC=abdominal circumference.

Placental reading
Placental heterogeneity and thrombosis were scored in a consensus reading by two board-certified radiologists with advanced experience in fetal and placental imaging. Readers were blinded to GA of the fetus and SARS-CoV-2 status of the pregnancy. For the assessment of placental heterogeneity, we referred to MRI-based placental grading described by Blaicher and colleagues,[3] which suggests 4 grades in accordance with the Grannum states. Grading ranged from 0, indicating no heterogeneity to 3, indicating severe heterogeneity. Grading of thrombotic

changes ranged from 0, indicating no thrombotic changes to 3, indicating severe thrombotic changes.

Statistical analyses
Fetal LV, normalized to estimated fetal body weight, was described as percentage of the respective 50th percentile reference values[4] and compared to a location mean of 1 using a one-sided t-test. Group comparison used two-sided t- or Mann-Whitney-test, as applicable. Multivariate analyses of normally distributed parameters were done using generalized linear models (GLMs), the traditional linear model with identity link function for normal distribution. Normality of data was checked by looking at histograms, QQ-plots, as well as tests for normality (Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling). For brevity reasons, here only Shapiro-Wilk results are quoted. Fetal lung volume normalized by fetal weight (expressed as percentile of 5th percentile reference value) (p=0.4540), absolute lung volume (p=0.6300), fetal weight (p=0.1235) and signal ratio lung to liver (p=0.1294) followed a normal distribution, whereas GA at scan (p=0.0178) and duration of infection (p=0.0047) were not normally distributed.

Replication of findings based on segmentations results of a second, independent reader
Based on the results of a second independent lung segmentation and volumetry, total fetal LV was below the 50th percentile in comparison to GA adjusted reference values[4] in the majority of SARS-CoV-2+ pregnancies (p<0·0001, mean=82 % +/- 25 % of the 50th percentile reference, range 39 % - 136 %). In order to account for fetal growth, we estimated fetal body weight as an indicator of somatic growth and used the estimate in each case to normalize absolute LV. Indicating a decline in LV unexplained by impaired somatic growth, we confirmed normalized LVs below the 50th percentile in the majority of cases (p<0·0001, mean=79 % +/- 23 % of 50 % percentile reference, range: 37 % - 127 % of 50th percentile reference). The finding was most pronounced in infants of the third trimester (age at scan). To investigate the impact of the estimated time point and anticipated duration of SARS-CoV-2 infection on fetal LV, we performed a general linear model using normalized LV expressed as percent of the 50th percentile reference value. By the use of this model, we found a significant impact of the time of infection (first and second vs. third trimester) indicated by the time point reported for the positive PCR result, on normalized fetal LV (p=0·0173). In contrast, time between test and scan as an estimate of the duration of infection, did not reveal a significant impact on normalized LV (p=0·5511), nor did the interaction term 'time between test and scan' and 'time of infection' (p=0·4356). When adding GA at scan and gender to the model, neither parameter showed a significant effect on normalized fetal LV (p=0·3718 and p=0·4978), although the interaction term 'GA at scan' and 'time of infection' reached marginal association (p=0·0956).

Replication of main finding independent of reference values
In order to test whether our main finding of reduced LV in fetuses of 3rd trimester infection is reproducible while using a comparison independent of reference values, we repeated the analysis using fetal LV normalized to fetal body weight obtained from SARS-CoV-2+ pregnancies (infected in the 3rd trimester) in comparison to values obtained from our local SARS-CoV-2- control group. The two-sided t-test revealed a significant group difference (p=0·0225, mean ratio SARS-CoV-2+ = 0·0260 ± 0·0058, mean ratio SARS-CoV-2- = 0·0320 ± 0·0067).

Association of placental changes and normalized fetal lung volume
As effects of physiologic maturation and effects of infection are hard to disentangle, we defined a SARS-CoV-2- control group (n=15) age-matched to a subsample (n=15) of our SARS-CoV-2+ study group (maximum of 2 days difference in GA per pair). In the control group, we observed a strong correlation of placental heterogeneity and GA (r=0·6970, p=0·0027) explained by physiologic maturation. In the SARS-CoV-2+ group, this correlation was reduced (r=0·4322, p=0·0945), and heterogeneity grades were increased when compared to the control group (p=0·0475).
When investigating the entire SARS-CoV-2+ study group (n=34), we found a significant association of GA at MRI and placental heterogeneity (p=0·0472). When approximating the potential effect of placental heterogeneity on fetal LV, we therefore took the impact of GA into account when investigating the potential association with fetal LV and applied a multiparametric GLM including GA at MRI, placental heterogeneity and the interaction term of GA at MRI * placental heterogeneity. By the use of this model, neither placental heterogeneity, nor the interaction term of GA at MRI * placental heterogeneity demonstrated a significant association with normalized fetal LV (p=0·4618 and p=0·4508, respectively). Likewise, when applying an analogous GLM including GA at MRI, placental thrombotic changes and the interaction of term GA at MRI * placental thrombotic changes, neither placental thrombotic changes, nor the interaction term GA at scan * placental thrombotic changes revealed a significant association with normalized fetal LV (p=0·3520 and p=0·4004, respectively).

Limitations
Reference MRI data on fetal LV post 38 weeks of gestation are inherently sparse, as fetal MRI is usually not performed for clinical indications at these late pregnancy stages. Therefore, reference values are not entirely based on absolute measurements but have to partially rely on fitted models. Numerous studies including post-mortem[5]

(n=42, approximately 25 % of the cases >=36 weeks of gestation) and ultrasound studies[6,7] (n=1022, 44,6 % of the cases >=36 weeks of gestation; n=108; approx. 14 % of the cases >=36 weeks of gestation) have demonstrated a continuous increase in fetal LV, i.e. growth until term, best described by power or exponential fits. Analogous fitting models have been shown to best describe fetal lung growth in MRI studies. Supplemental Figure 1 depicts published MRI data on fetal LV, including studies by Duncan at al.[8] (n=56), Rypens et al.[9] (n=214, 88 % >=36 weeks of gestation), Mahieu-Caputo et al.[10] (n=74, 5 % >=36 weeks of gestation), Kasprian et. al.[11] (n=111, 11 % >=36 weeks of gestation), and Meyers et al.[4] (n=665, 4 % >=36 weeks of gestation). These publications demonstrated that the fitted models describe a continuous increase of LV until term age (power or exponential fits; for an overview of the exact fitting functions see review by Deshmukh et al.[12]). From these studies, we decided to use the reference values published by Meyers et al., as they are derived from the largest MRI data set currently available.

In our study, fetal lung volumetry was performed on 5 mm axial TRUFI images. While fetal lung volumetry would ideally be performed in 3-4 mm turbo-spin echo (TSE) T2-weighted images, imaging of the fetal body was restricted to 5 mm TRUFI sequences in our study due to the extensive study protocol that included imaging of the fetal brain and body, the placenta as well as the maternal brain and lungs. We do, however, not expect relevant bias by the use of 5 mm sequences as partial volume effects caused by higher slice thickness would rather result in an over-estimation of lung volumes and therefore ameliorate the effects observed, i.e. the decrease in lung volumes.

Another limitation of our study pertains to the assessment of the genetic background. An impact of ethnicity has been identified for both, the course of COVID infections[13] as well as the development of chronic lung disease[14]. These considerations were the reason for including the available information on ethnicity of the mother in the study groups. In the control group, all women were Caucasian as reported in the pregnancy records, whereas paternal genetic background was not available. In the SARS-CoV-2+ group, 50 % of fetuses had a reported Caucasian background for both parents, whereas for 50 % only data for the maternal background were available.

Furthermore, despite our observation that placental changes were not statistically associated with changes in fetal LV in our study, placental malfunction could significantly affect lung growth and can thus – with the data provided – not be ruled out entirely.

Supplemental Figures

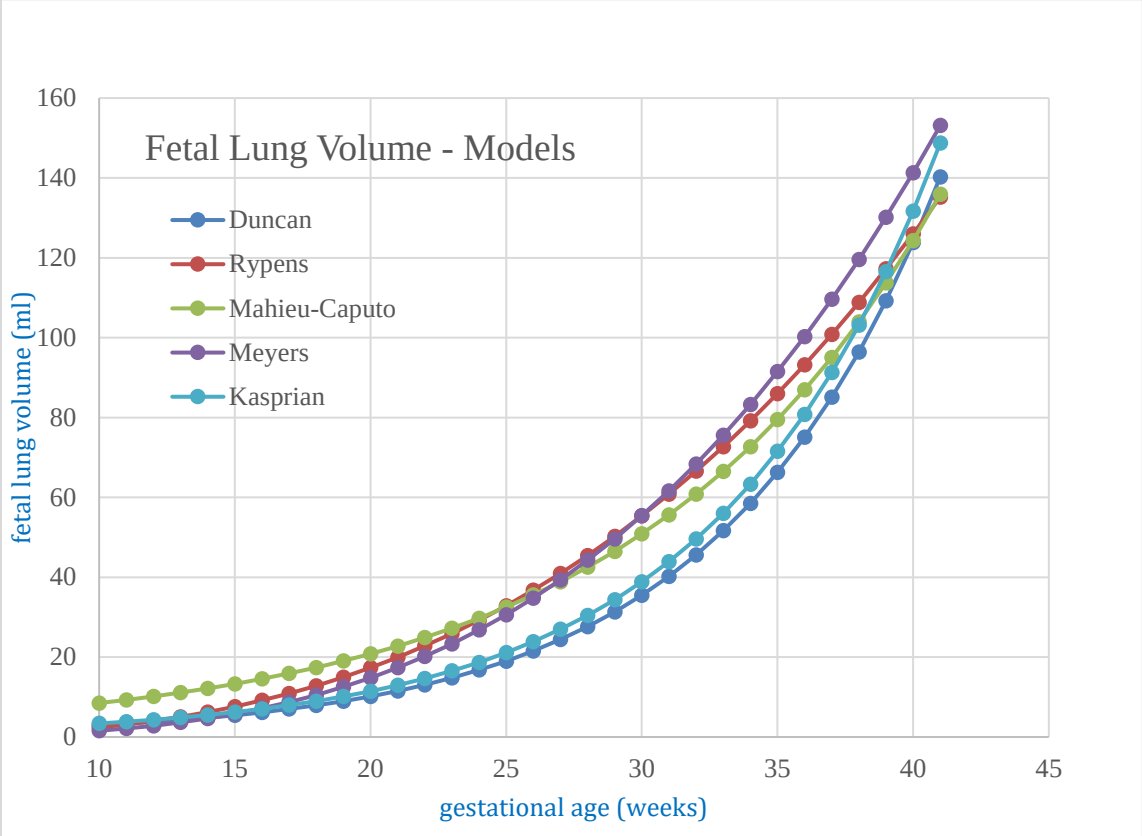

Figure S1: Published MRI data on fetal lung volume including studies by Duncan et al.[8] (n=56), Rypens et al.[9] (n=214, 8 % >=36 weeks of gestation), Mahieu-Caputo et al.[10] (n=74, 5 % >=36 weeks of gestation), Kasprian et. al.[11] (n=111, 1 % >=36 weeks of gestation), and Meyers et al.[4] (n=665, 4 % >=36 weeks of gestation).

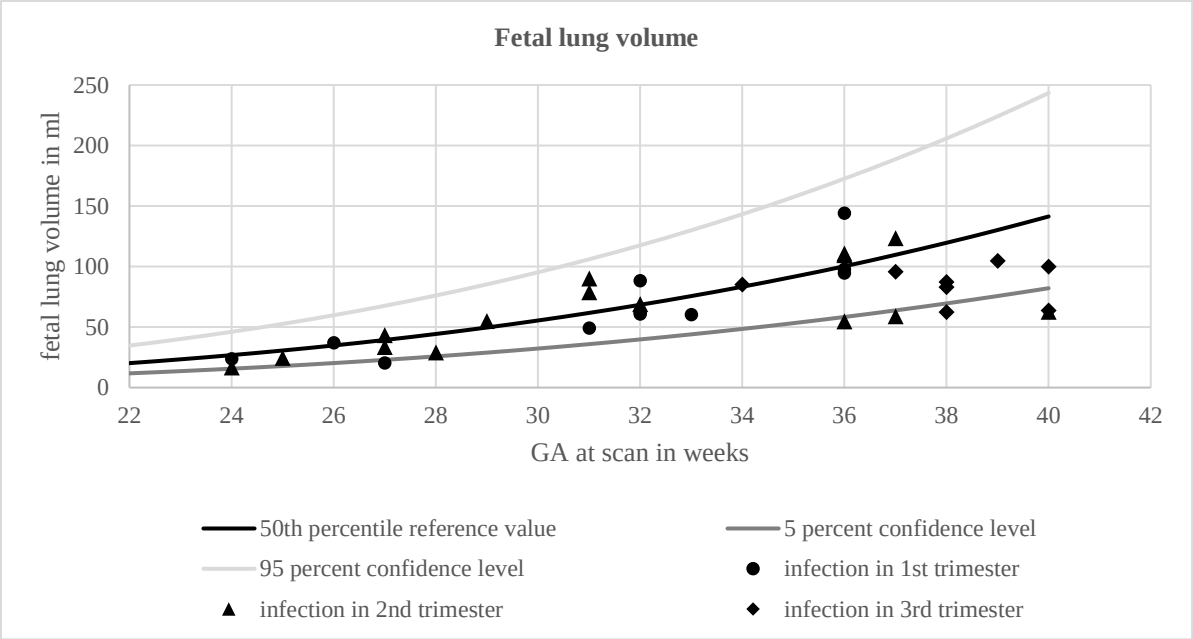

Figure S2: Significant reduction of total fetal lung volume (LV, ml; over gestational age (GA), weeks at MRI) in SARS-CoV-2+ pregnancies (50th percentile, black line). Circles indicate infection in the first trimester, triangles indicate cases of infection in the 2nd trimester, and rhombs indicate cases of infection in the 3rd trimester.

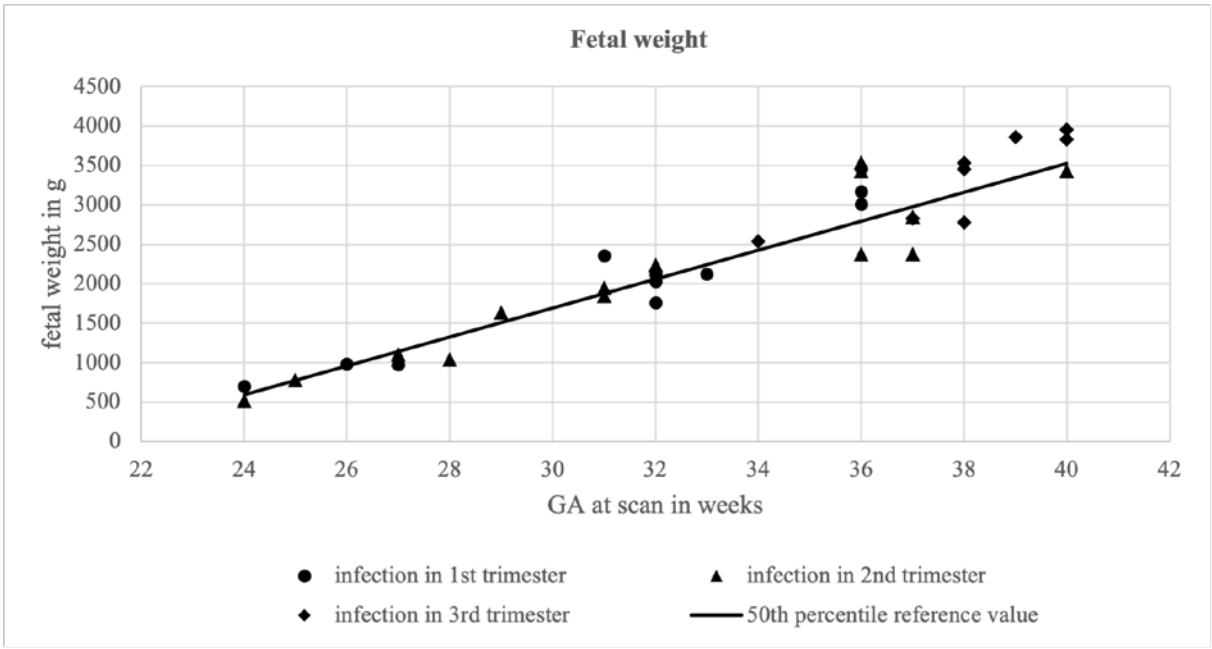

Figure S3: Distribution of estimated fetal body weight (gram; over GA, weeks at MRI) in SARS-CoV-2+ pregnancies (50^{th} percentile, black line). Circles indicate infection in the first trimester, triangles indicate cases of infection in the 2^{nd} trimester, and rhombs indicate cases of infection in the 3^{rd} trimester.

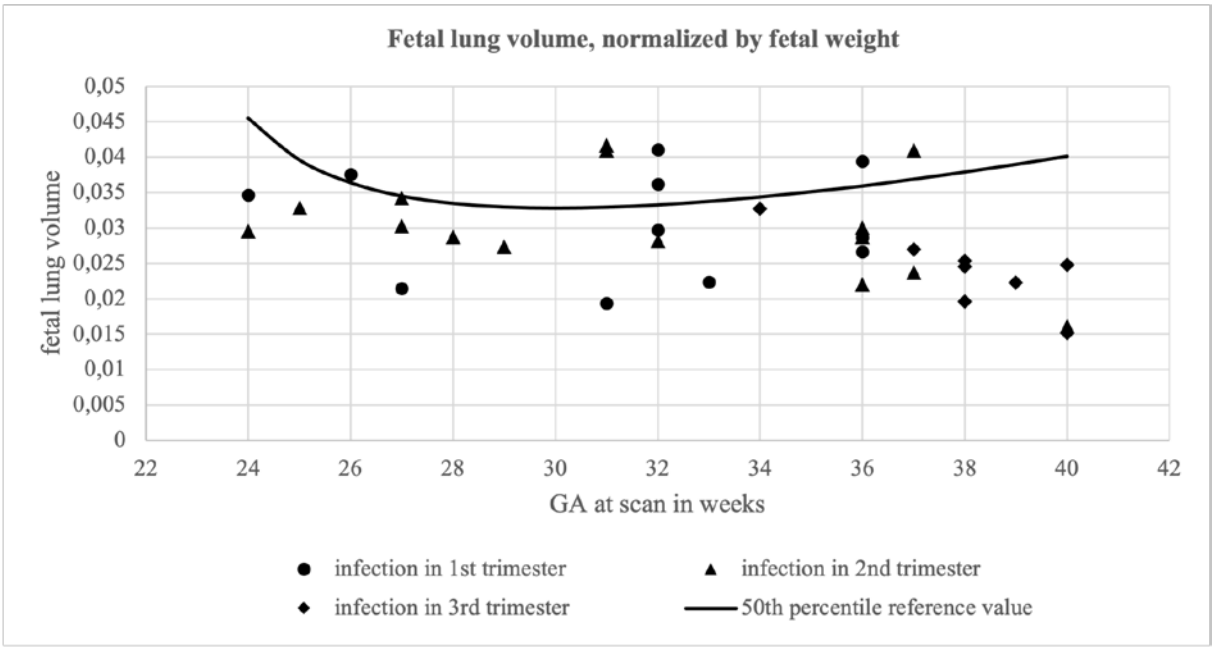

Figure S4: Significantly reduced fetal LV-to-weight-ratio (over GA, weeks at MRI) in the 3rd trimester in SARS-CoV-2+ pregnancies. Circles indicate infection in the first trimester, triangles indicate cases of infection in the 2nd trimester, and rhombs indicate cases of infection in the 3rd trimester.

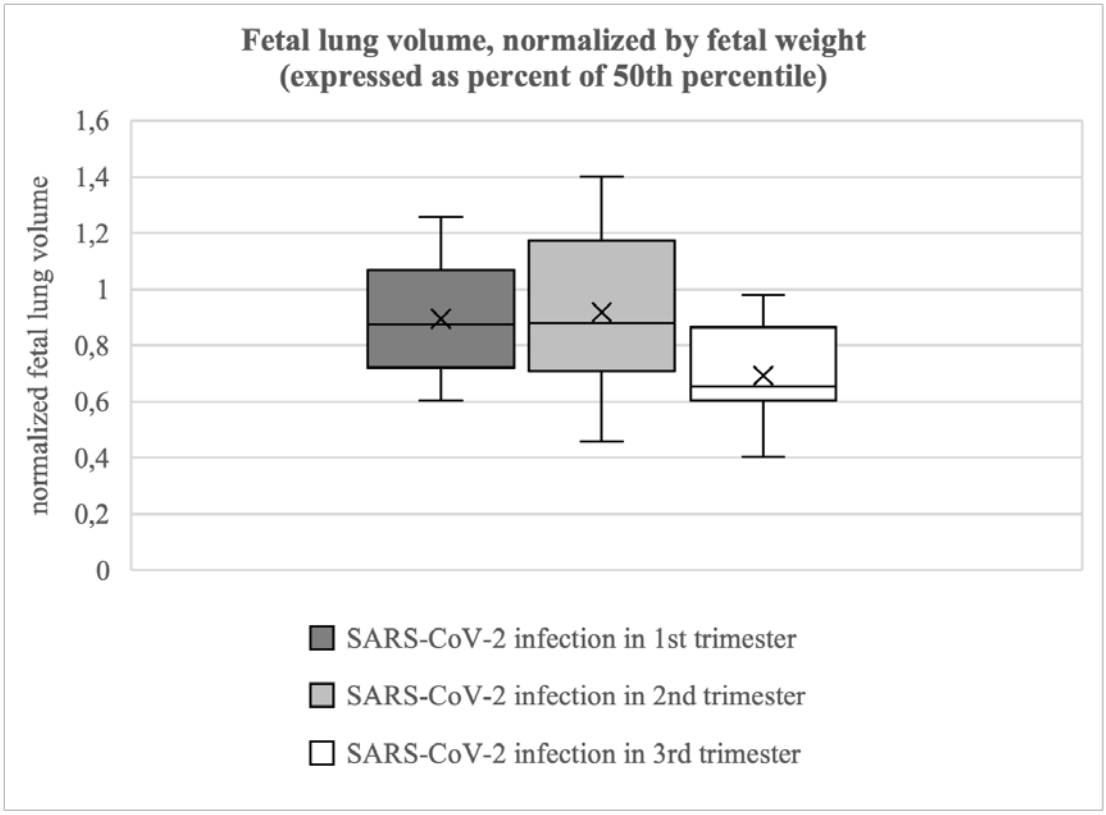

Figure S5: Reduced normalized fetal LV (percent of 50th percentile) in pregnancies with an infection time-point in the 3rd trimester (white) vs. the 1st (dark grey) or vs. the 2nd (light grey) trimester.

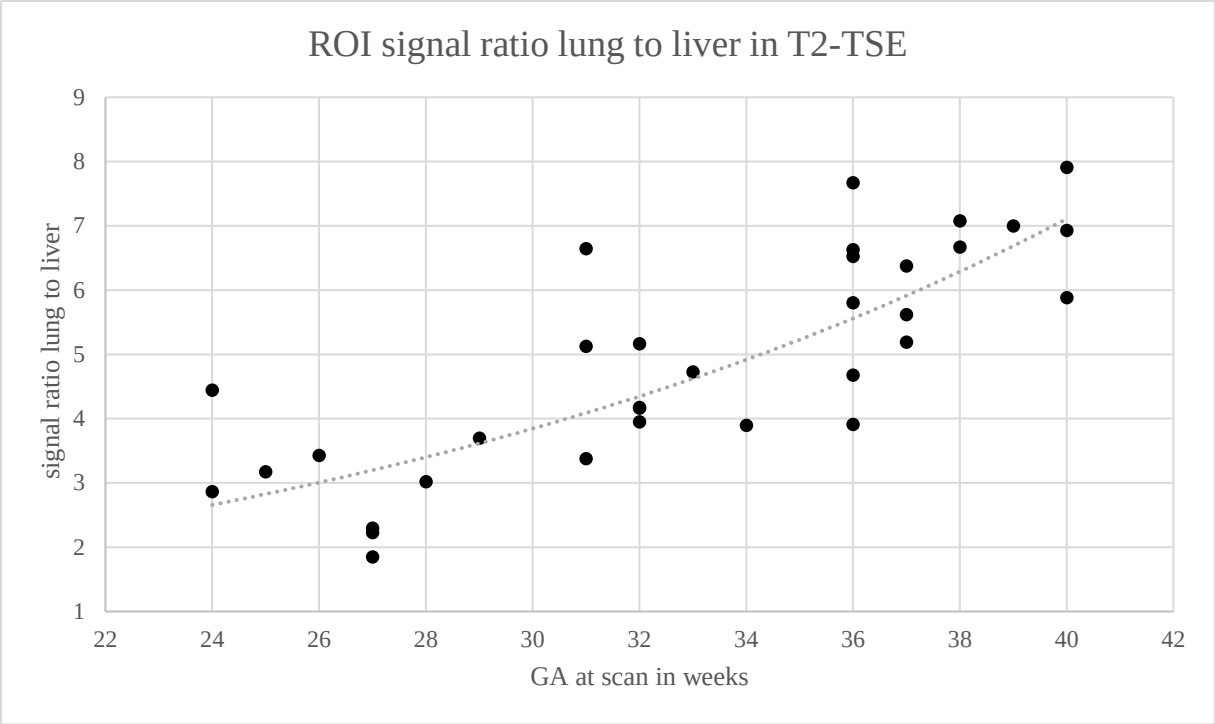

Figure S6: Mean T2-weighted signal intensity of a region of interest (ROI) in the fetal lung, normalized to the mean T2-weighted signal intensity within a ROI in the liver, plotted over GA (gestational age (GA) in weeks at scan), indicating increased an increase in ration, i.e. lung parenchymal development.

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