Effects of intrauterine retention and postmortem interval other on bodyweight following intrauterine fetal death: implications for assessment of fetal growth restriction at autopsy

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Short title: Bodyweight changes following intrauterine fetal death

+A: Abstract

Introduction Depending on which classification system is used, 15–60% of stillbirths remain unexplained, despite undergoing recommended autopsy examination, with variable attribution of fetal growth restriction (FGR) as a cause of death. Distinguishing small-for-gestational age (SGA) from pathological FGR is a challenge at postmortem examination. This study uses data from a large, well-characterized series of intrauterine death autopsies to investigate the effects of secondary changes such as fetal maceration, intrauterine interval and postmortem interval on interpretation of body weight.

Methods Autopsy findings from intrauterine death investigations (2005–2013 inclusive, from Great Ormond Street Hospital and St George's Hospitals, London), were collated into a research database. Growth charts published by the World Health Organization were used to determine normal expected weight centiles for fetuses born after 23 weeks' gestation and the effects of intrauterine retention (maceration) and postmortem interval calculated.

Results There were 1064 intrauterine deaths, including 533 stillbirths ≥ 24 weeks' gestation) with a recorded birth weight. Of these, 192 (36%) had unadjusted birth weight below the 10^{th} centile and were defined as SGA. The majority (86%) of stillborn SGA fetuses demonstrated some degree of maceration, indicating a significant period of intrauterine retention after death. A significantly greater proportion of macerated fetuses were present in the SGA population compared with the non-SGA population ($P = 0.01$). There was a significant relationship between increasing intrauterine retention interval (IUI) and both more severe maceration and reduction in birth weight ($P < 0.0001$ for both), with an average artefactual reduction in birth weight of around –0.8SD of expected weight. There was an average 12%

reduction in fetal weight between delivery and autopsy and, as postmortem interval (PMI) increased, fetal weight loss increased ($P = 0.0001$).

Conclusion Based on birth weight alone, 36% of stillbirths are classified as SGA. However, fetuses lose weight *in utero* with increasing IUI and continue to lose weight between delivery and autopsy, resulting in erroneous overestimation of FGR.

+A: Introduction

Depending on which classification system is used, between 15% and 60% of stillbirths remain unexplained, despite undergoing recommended autopsy examination[1,2]. Much of this difference is related to the variable attribution of fetal growth restriction (FGR) as a cause of death. FGR implies pathological restriction of growth potential, whereas small-for-gestational age (SGA) simply describes an infant whose biometry falls below an arbitrary centile, usually the 5th or 10th, of an expected normal range for gestational age[3,4]. Not all FGR fetuses are SGA and not all SGA fetuses are FGR; the likelihood of FGR increases with the magnitude of SGA, such that, for example, 30% of fetuses < 10th centile are estimated to be FGR, compared with 70% of foetuses < 3rd centile. Fetal birth weight is affected not aonly by pathological abnormalities but also by physiological differences, including parental ethnicity, height and parity[3]. Customized growth charts have been suggested to reduce such effects[3,5]. Despite this, distinguishing incidental SGA from pathological FGR is challenging, especially at postmortem examination following intrauterine death.

FGR/SGA has been suggested as the single largest population-attributable risk for stillbirth, most studies being based on unadjusted weight at birth, with around 40% of stillborn infants being below the 10th birth weight centile for liveborn infants[6]. However, antenatal detection rates for fetuses delivered SGA are generally poor (15–25%)[7,8] and it has been suggested that increased surveillance for SGA may improve its detection and reduce stillbirth rates[6,9]. Despite these approaches, there are remarkably few published data attempting to quantify the possible effects on body weight of changes occurring following intrauterine death.

This study, therefore, aimed to use data from a large, well-characterized series of autopsies following stillbirth death to investigate the frequency of SGA, and to

address the role that secondary changes such as fetal maceration, intrauterine interval and postmortem interval may have on the interpretation of birth weight.

+A: Methods

As part of a larger study examining autopsy findings in the investigation of intrauterine death, a dedicated Microsoft Access Autopsy Database (Microsoft Corp., Redmond, WA, USA) was used to collate detailed autopsy and antenatal details from intrauterine deaths examined between 2005 and 2013, inclusive, at Great Ormond Street Hospital and St George's Hospitals, London. Every case was reviewed manually and data extracted according to strict, predefined, objective criteria, separating objective autopsy findings from pathologist interpretations. Data were then analyzed through queries and statistical tests using Microsoft Access and Microsoft Excel (Microsoft Corp.), GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) and Stats Direct (StatsDirect Ltd., Altrincham, UK) software packages. Statistical tests included chi-square, *t*-test and Mann–Whitney *U*-test and regression analysis, as appropriate. $P < 0.05$ was considered statistically significant.

Neonatal growth charts published by the World Health Organization (WHO) were used to determine normal expected weight centiles for fetuses born ≥ 24 weeks' gestation, with males and females being evaluated separately since infant sex has a marked effect on birth weight[11]. Customized growth charts were not used due to the relative lack of detailed maternal demographic information available from archival postmortem reports. Nevertheless, since all comparisons between groups were based on the same reference range dataset, all intergroup differences and trends remain valid. These normal range data were used to determine the gestational-age-corrected expected birth weights for each case, from which the birth-weight delta value was

calculated (defined as the number of SDs by which the observed birth weight differed from the expected (50th centile) birth weight from the normal range adjusted for fetal sex). Delta birth weights therefore allowed comparison between cases independent of fetal sex and gestational age. A negative delta birth-weight value indicated that the observed weight was less than expected based on the normal (livebirth) ranges. For the purposes of this study, cases with a delta value below –1.375, representing the 10th centile of the normal range, were denoted as SGA. Gestational age was calculated based on clinical and sonographic information provided.

+A: Results

Among a total of 1064 intrauterine death autopsies, there were 533 stillbirths with a recorded birth weight delivered at ≥ 24 weeks of gestation, which comprise the dataset for this study. Of these, 192 (36%) had unadjusted birth weight < 10th centile and were thus classified as SGA based on the WHO[11] charts for normal livebirths (38% of male infants and 34% of female infants; $z = 0.94$, $P = 0.35$). Around half (47%) of the cases of SGA had no other specific identifiable cause of death at autopsy, whilst the most common specific category of death was placental abnormality (19%), typical of pathological FGR and maternovascular malperfusion[12] (Figure 1). Ninety of the 335 (27%) cases of stillbirth with an otherwise unexplained cause of death and no pathological findings at autopsy were SGA.

The majority of mothers had a normal BMI but the SGA group had a higher proportion of underweight mothers than did the non-SGA group SGA ($z = 2.29$, $P = 0.02$). Twenty-four (12.5%) of SGA fetuses had mothers with some form of hypertension, of whom 42% had pre-eclampsia.

The majority (86%) of SGA stillborn fetuses had some degree of maceration noted at autopsy, indicating a significant period of intrauterine retention following intrauterine death; 76% of non-SGA stillborn fetuses showed maceration (z=2.74, P=0.01). Of the overall 424 cases with maceration present in whom grading was possible, 28 (7%) were SGA with mild maceration and 100 (24%) were SGA with moderate or severe maceration. A significantly greater proportion of macerated fetuses were present in the SGA population compared with the non-SGA population ($z = 2.57$, $P = 0.01$). Since cases of ascending infection are more likely to be fresh stillbirths and hence non-macerated, analysis was performed with these cases excluded, but the significantly greater proportion of maceration in the SGA population persisted ($z = 2.02$, $P = 0.04$), indicating that maceration itself is associated with reduced body weight for gestational age.

To explore this relationship further, the intrauterine retention interval (IUI) was calculated, as the minimum time that the fetus had been dead *in utero* before delivery, according to either serial ultrasound scan findings or clinical history; intrapartum deaths were recorded as having 0 days of IUI. There were 308 cases of stillbirth $\geq$ 24 weeks of gestation with recorded birth weight, bodyweight at autopsy and well-documented IUI. They demonstrated a significant relationship between increasing IUI and increasing severity of maceration ($z = 15.45$, $P < 0.0001$). However, severity of maceration was not related closely to exact IUI; there was moderate or severe maceration in all cases with prolonged (> 4 days) IUI, along with some cases with IUI of only 2 days. Presence of significant maceration therefore appears to be a reliable indicator that an intrauterine death occurred > 48 h prior to delivery, but cannot be used further to determine reliably the period since death and delivery.

Based on the 308 cases delivering ≥ 24 weeks with known IUI in whom delta birth weight could be calculated, there was a significant linear relationship between IUI and delta birth weight ($P < 0.0001$; Figure 2); as IUI increased, delta birth weight decreased, indicating that, with increasing IUI, fetuses lose increasing body weight *in utero*. Following an average interval between intrauterine death and delivery of around 3–4 days in this study, there was an artefactual reduction in birth weight of around –0.8SDs of expected weight. The proportion of SGA cases defined as birthweight <10th centile in stillbirths ≥ 24 weeks was 21% for those with 0 days IUI, 30% for those with 1–2 days IUI and 45% for those with > 2 days IUI. There were significantly more fetuses with an IUI > 2 days in the SGA group ($z = 3.01$, $P = 0.003$; Figure 3).

Finally, among the overall 1064 intrauterine deaths with autopsy results, there were 615 cases across all gestational ages with reliably recorded birth weight and bodyweight at autopsy, and these were used to calculate the percentage fetal weight change during the period between birth and autopsy (postmortem interval (PMI)). There was an average 12% reduction in fetal weight during the period of fetal refrigeration pending autopsy, with a median PMI of 7 days. As PMI increased, the proportion of fetal weight loss increased ($P = 0.0001$; Figure 4). Using body weight at autopsy rather than birth weight resulted in a 12% increase in the proportion of cases classified as SGA.

+A: Discussion

We found that, based on unadjusted birth weight using standard infant weight charts[11], 36% of stillbirths ≥ 24 weeks of gestation are classified as SGA. However, this large dataset allowed investigation of factors which occur following intrauterine

death, demonstrating that, following intrauterine death, fetuses lose weight *in utero* in relation to increasing IUI as the process of maceration occurs, such that after a delay between death and delivery of 3–4 days, there has been an artefactual reduction in birthweight of around –0.8SDs. Furthermore, fetuses continue to lose weight, even when refrigerated, between delivery and autopsy; by 7 days post-delivery there has been an additional 12% loss of bodyweight. These data indicate that, using unadjusted birth weight (and even more so using autopsy bodyweight), there is erroneous overestimation of the proportion of fetuses which are SGA, due to changes that occur after death, and the likely true proportion of pathological FGR with SGA in stillborn infants is likely to be around 20–25% rather than 35–40%.

Few previous studies have attempted to address this issue in a systematic or quantitative manner, although practising pathologists are well aware that maceration after death can result in significant changes in fetal appearance. It is established that stillborn birth weights are lower compared with gestational-age-matched livebirths, as evidenced by 'normal range' data provided in standard perinatal pathology texts[13,14]. However, only by using large well-defined datasets can temporal relationships between changes in bodyweight after death and intrauterine retention be examined. A previous study compared macerated with non-macerated fetuses and reported that organ weights of liver, thymus, and spleen decreased markedly with increasing maceration, consistent with the current data[15]. There is one previous study of fetal weight changes between delivery and autopsy, including 212 cases, which reported an average 7% decrease in fetal bodyweight from birth to autopsy (with a shorter average PMI than our present study), consistent with current data and further highlighting discordancy between birth and postmortem body weights[16].

Whilst those with experience of delivering a fetus which has been retained *in utero* for some time are aware that many changes occur following death, with shrinkage in fetal mass associated with maceration, this is the first study to provide quantitative data regarding the extent of this effect, and demonstrates that significant changes occur within a few days of death. The mechanisms of such changes of intrauterine maceration in humans are uncertain, and are difficult to study directly. Early weight changes are likely to be due to shifts in fluid distribution in association with loss of normal homeostasis, with cellular autolysis and enzymatic degradation particularly affecting metabolically and hormonally active organs such as liver and pancreas. This is supported by data derived from the food industry indicating ongoing metabolic alterations for several hours after death followed by marked changes in tissue proteome profiles by 48 h[17,18]. Evidence of ongoing change is provided by the finding that the degree of maceration increases with IUI[19].

This is the largest dataset available presenting detailed and granular stillbirth autopsy data. Furthermore, the dataset is unique in that variables were collected and recorded into a dedicated research database according to objective and predefined criteria and cases classified objectively according to autopsy and clinical findings to ensure consistency. Nevertheless, this remains a retrospective dataset, an issue which is intrinsic to all studies of stillbirth, since this represents a rare event and hence is generally unsuitable for prospective studies to address reasonably. However, the large size and objective criteria of the current dataset allow analyses to be performed which have not been possible previously and provide the best available evidence for changes after death in stillbirth.

These data support the concept that impaired fetal growth likely contributes to stillbirth; even using adjusted estimates, it confirms that the proportion of SGA in

stillbirths is greater than expected in an unselected population. Nevertheless, the contribution of FGR to otherwise clinically unexpected third-trimester stillbirth is likely to be less than suggested previously based on unadjusted birth weight or autopsy weights, since changes during *in-utero* retention and following delivery had not been taken into account. Current studies to improve detection of FGR are likely to have an important effect on reducing stillbirth rates, but the magnitude of reduction may be less than initially hoped[20]. Our findings, therefore, have potential implications for programmes to increase the detection of FGR and reduce stillbirth rates. To further assess the relationship between stillbirth and FGR, ongoing research is required into the biological and pathological mechanisms of death in FGR, and must also account for postmortem changes. It is essential that birth weight is measured as soon after delivery as is practical, and that a detailed clinical history is obtained to estimate more accurately the duration of *in-utero* retention in all stillbirths.

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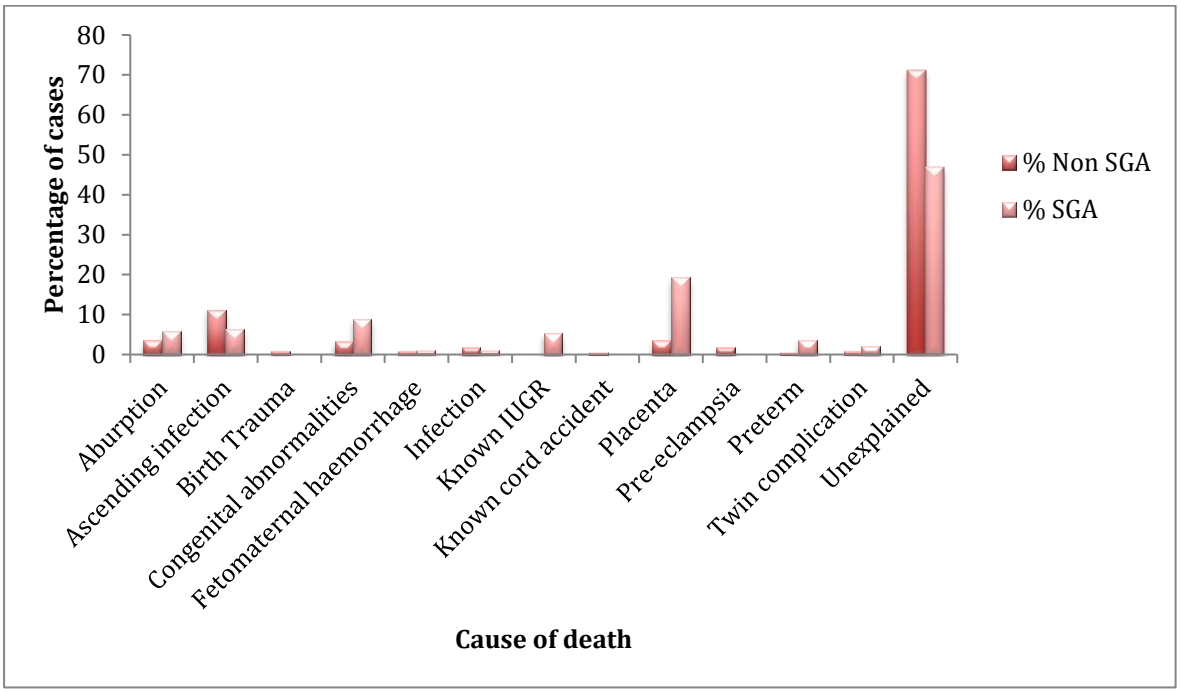
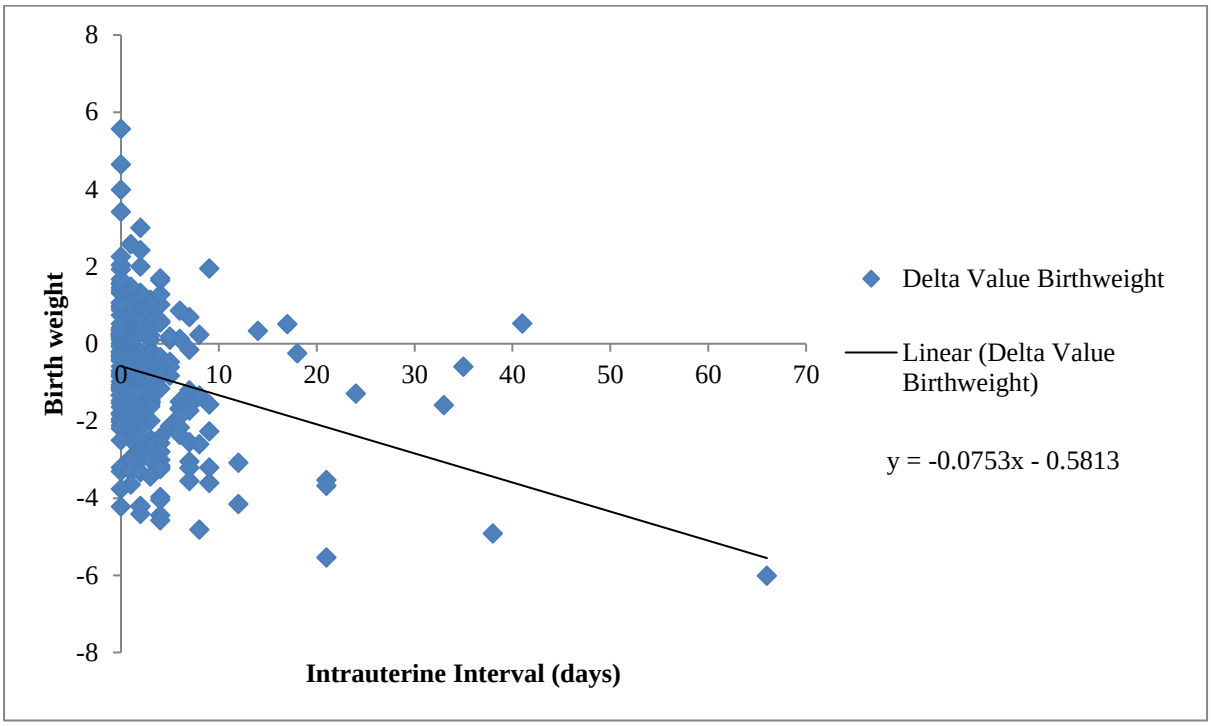


Figure 1 Cause of death in 533 stillbirths with a recorded birth weight, according to whether they were small-for-gestational age (SGA, □) or non-SGA (□). Congenital abnormalities and placental causes of death were more frequent in SGA cases ($z = 2.8$, $P = 0.005$ and $z = 6.04$, $P < 0.0001$, respectively); unexplained deaths were more frequent in non-SGA cases ($z = 5.58$, $P < 0.0001$).



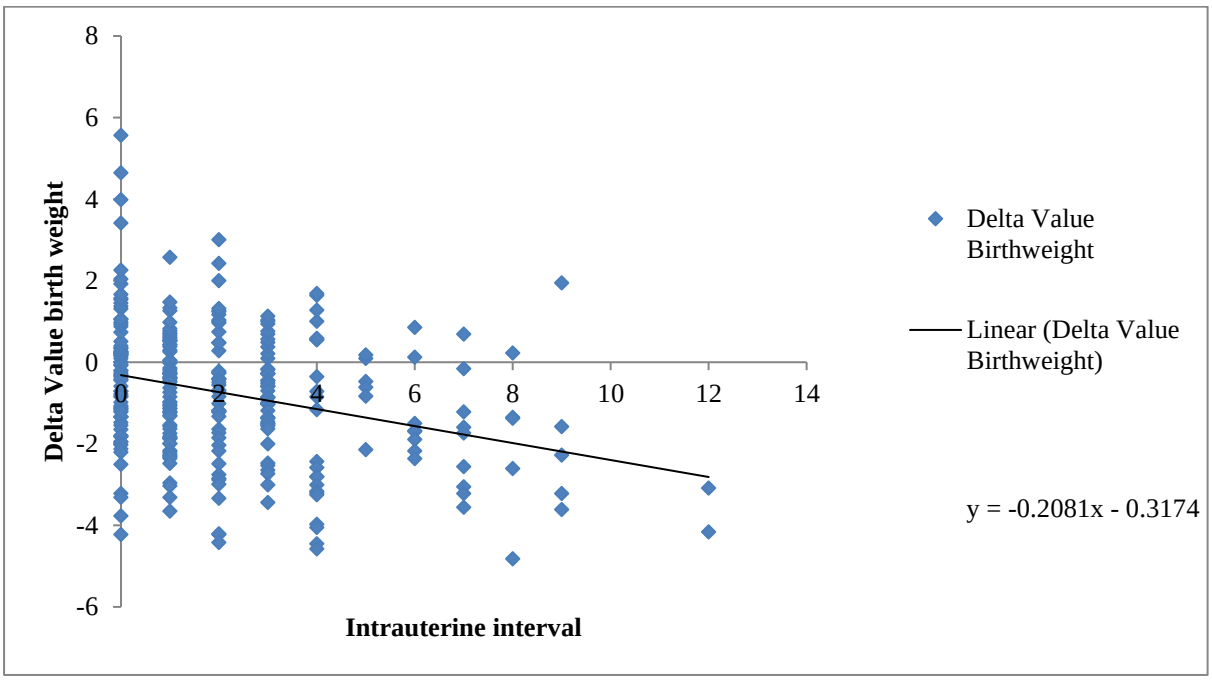


Figure 2 Relationship between interval from intrauterine death to delivery (IUI) and adjusted birthweight in 308 stillbirths ≥24 weeks' for whom well-documented IUI were provided.

(Inset; only those cases with IUI < 12 days (N=296). There was a significant linear relationship between increasing IUI and reducing delta birth weight ($P < 0.0001$); this relationship persisted even when only data with IUI < 12 days were evaluated.

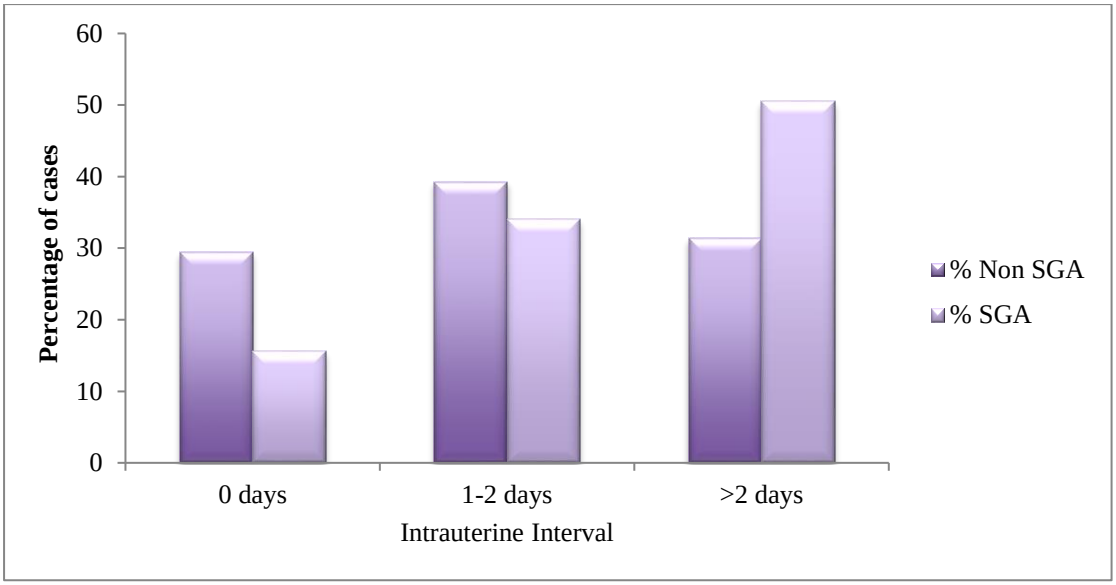


Figure 3 Interval between intrauterine death and delivery (IUI) in 533 stillbirths ≥ 24 weeks with a recorded birth weight, according to whether they were small-for-gestational age (SGA, □) or non-SGA (□). There were significantly more fetuses with a prolonged IUI (> 2 days) in the SGA group ($z = 3.01$, $P = 0.003$) and the proportion of SGA cases increased with increasing IUI.

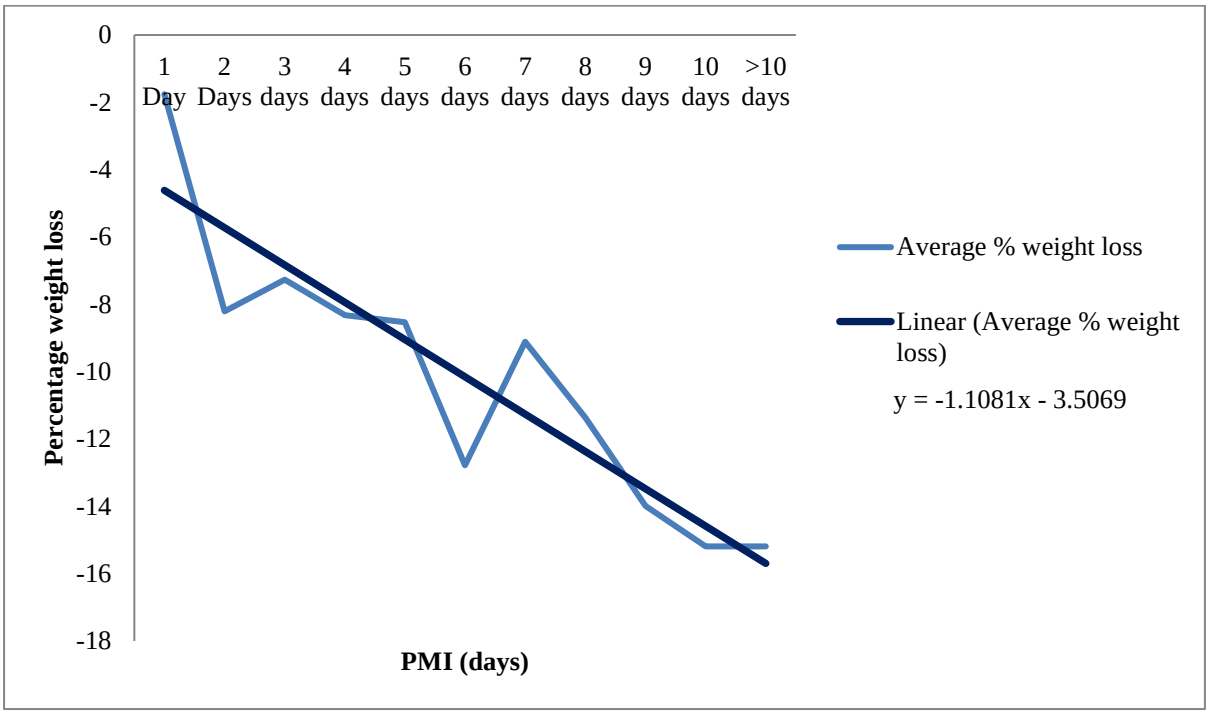


Figure 4 Relationship between average percentage weight loss and postmortem interval (PMI) in 533 stillbirths ≥ 24 weeks with a recorded birth weight; as PMI increased, percentage weight loss increased ($P < 0.001$).