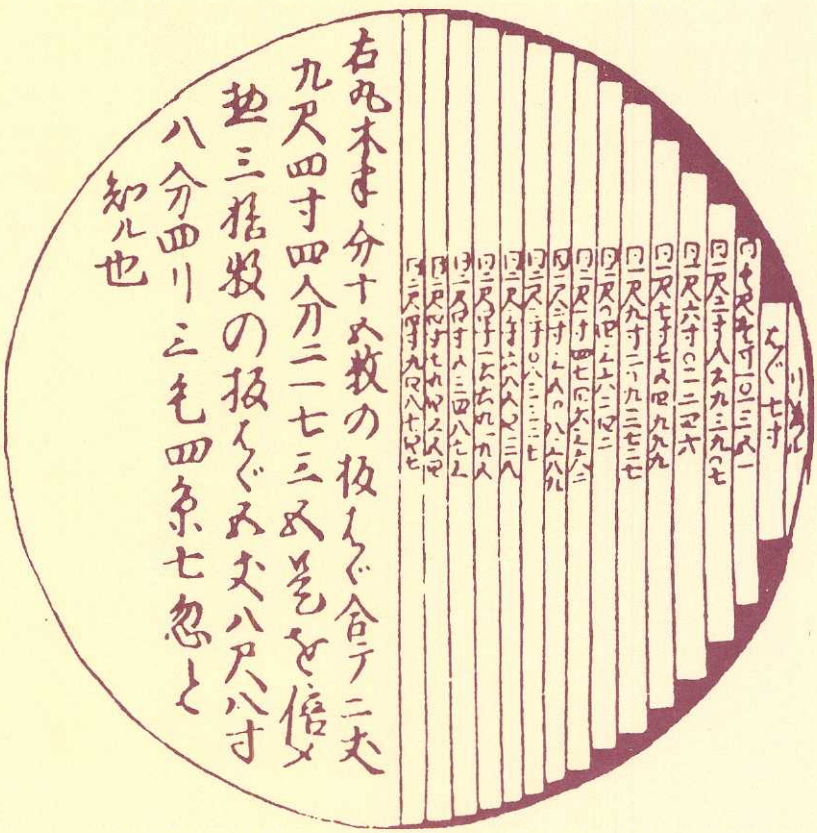
A modification of the method for estimating the urine production rate of the human fetus using 2 D ultrasound

Mats Fägerquist



A MODIFICATION OF THE METHOD FOR ESTIMATING THE URINE PRODUCTION RATE OF THE HUMAN FETUS USING 2D ULTRASOUND

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av
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Professor Karel Marsal
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II. Fägerquist, M. Fägerquist, U. Steyskal, H. Odén, A. and Blomberg, S.G. (2002). Accuracy in estimating fetal urinary bladder volume using a modified ultrasound technique. *Ultrasound Obstet Gynecol. 19, 371-379.*

III. Fägerquist, M. Fägerquist, U. Odén, A. and Blomberg, S.G. (2003). Estimation of fetal urinary bladder volume using the sum-of-cylinders method vs. the ellipsoid formula. *Ultrasound Obstet Gynecol. 22, 67-73.*

IV. Fägerquist, M. Fägerquist, U. Odén, A. Blomberg, S.G. and Mattsson, L-Å. The accuracy of measurements of fetal urine production rate depends on the choice of method and the number of documented images. *Submitted for publication*

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Abstract

Using 2D ultrasound, the fetal anatomy, body motions and pattern of breathing, as well as growth and blood circulation, can be studied. The fetal urinary bladder has filling and emptying phases and the increasing volumes during filling phase reflect fetal urine production.

Urine production increases during pregnancy and, at term, about 50 mL/hour is produced. Conventionally, the bladder volume has been estimated using the volume formula for ellipsoids, based on three orthogonal bladder diameters, the long diameter in a longitudinal image and two transverse diameters. Obvious divergent results have been presented in studies of the normal fetal urine production rate and they could be due to methodological deficiencies. Knowledge of fetal urine production will probably add to the science of patho-physiological changes during complicated pregnancies. However, to enable the detection of minimal changes in urine production as well, it is necessary to use accurate measurement methods. The aims of our study were to examine measurement errors when estimating the fetal urine production rate and to see whether modifications of the method would reduce measurement errors.

Questions at issue

What procedures are involved when estimating the fetal urine production rate using 2D ultrasound and to what extent do these different procedures cause measurement errors?
Will modifications of the method reduce these measurement errors?
What part of the total variation in urine production rate is caused by measurement errors?

Methods

The standard deviation (SD) was used to characterise the measurement error when selecting bladder image and estimating bladder diameters and volumes. The conventional method was compared with a new method, where 1) the image selection was systematically controlled by a special movement of the ultrasound transducer ("rocking" motion), 2) the image was digitised and 3) the volume was calculated using the "sum-of-cylinders" method instead of the ellipsoid formula. To illustrate the clinical impact of measurement errors, some examples were given.

Results

When the urine production rate was estimated conventionally, in a group of fetuses aged 24 to 40 weeks, the biological variation accounted for 51-86%, while the measurement errors accounted for the remaining part 49-14% of the total variation (SD). The selection of non-appropriate bladder images accounted for the dominant part of the measurement errors. However, by using the new method, the SD was reduced from 17.3-10.9% to 8.8-3.5% for volumes of 5-40 ml. The reduction in SD consequently also led to the detection of small changes in the urine production rate. For a fetus producing about 10 mL/hour of urine, the difference in urine production, which could be caused exclusively by measurement errors, was reduced from 5 to 3 mL/hour, when using the new method.

Conclusions

When estimating bladder volumes conventionally, the dominant part of the measurement errors was caused by the selection of bladder image. The measurement error was significantly reduced by appropriately controlling image selections, using a special movement of the transducer ("rocking motion"), and by digitising bladder images, the modified method. Moreover, using the "sum-of-cylinders" method for volume calculation tended to reduce the measurement error still further. This new method is therefore preferable, when estimating the fetal urine production rate.

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Area estimation according to integral calculus, which was created by the Japaneese mathematician Seki Shinsuke Kowa (1642-1708), known as the Isaac Newton of Japan.
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List of Publications

Fagerquist, M. Fagerquist, U. Odén, A. Blomberg, SG: Fetal urine production and accuracy when estimating fetal urinary bladder volume. Ultrasound Obstet. Gynecol. 17(2):132-39, 2001

Fagerquist, M. Fagerquist, U. Steyskal, H. Odén, A. Blomberg, S G: Accuracy in estimating fetal urinary bladder volume using a modified ultrasound technique. Ultrasound Obstet. Gynecol. 19: 371-79, 2002

Fagerquist, M. Fagerquist, U. Odén, A. Blomberg, SG: Estimation of fetal urinary bladder volume using the "sum of cylinders method" vs. the ellipsoid formula. Ultrasound Obstet. Gynecol. 17(2):132-39, 2003

Fagerquist, M, Fagerquist, U, Odén, A, Blomberg, S.G, Mattsson, L-A: The accuracy of measurements of fetal urine production rate depends on the choice of method and the number of documented images. Submitted for publication, 2004.

To my wife Rosa, my children and grandchildren

Contents

Introduction

The first ultrasound examination of human fetal anatomy was published in 1958[1]. Since then, examinations of the human fetus using the ultrasound technique have expanded enormously and include not only gross fetal anatomy but also fetal dynamics. Information on fetal structural abnormalities, growth, movement, breathing and cardiovascular function continues to accumulate[2, 3, 4, 5]. Amniotic fluid characteristics and blood flows in different fetal vessels and maternal uterine arteries have been the focus of investigations for many years[6, 7].

Urine production probably starts in the first trimester. Autopsy findings of filled urinary bladders in human fetuses have been reported from 11 gestational weeks[8]. In 1970, Garrett et al. published the first report on ultrasound investigations of the fetal urinary bladder[9]. Three years later, a method for estimating fetal urine production was introduced[10].

The assessment of urine production may be important in order to detect disturbances in fetal kidney function as well as water balance. As a result, the estimation of urine production in adults and children plays an important role in the intensive care setting and on ordinary wards. Assessing the urine production in fetuses may lead to important advances in our understanding of fetal physiology in normal and complicated pregnancies.

Amniotic fluid

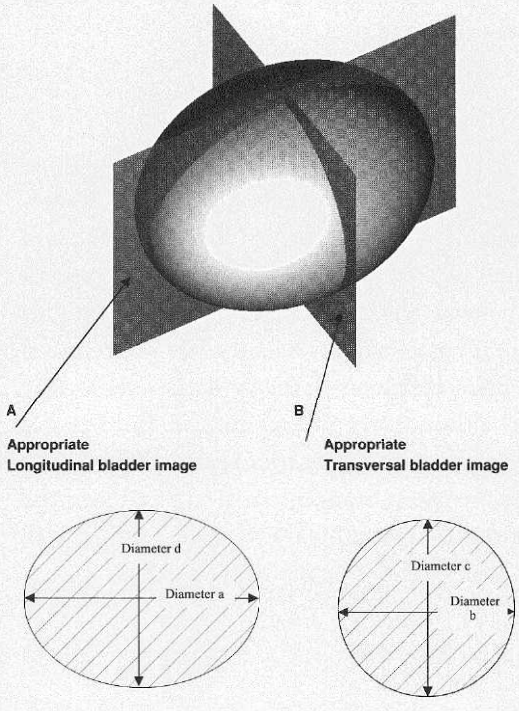
The volume of amniotic fluid increases during pregnancy[11]. Abnormal amounts of amniotic fluid may be caused by fetal malformations. Oligohydramniosis (reduced volume) is often caused by kidney abnormalities and polyhydramniosis (increased volume) can be the result of oesophageal atresia and defects in the fetal CNS[2]. In general, the secretion of liquid by the kidneys and from the fetal lungs and oral-nasal cavity is balanced by the removal of equal amounts of liquid[6]. The main clearance pathway is the swallowing of fluid by the fetus. Additionally, although to a lower degree, fluid passes from the amniotic lumen via the surfaces of the placenta and umbilical cord into the fetal blood circulation (the intramembranous pathway) and into the mother's circulation (the transmembranous pathway) via the uterine wall through the surface of the amniotic sac outside the placental border.

Table 1 The amniotic fluid volume does not change from day to day, but the amniotic fluid itself is completely replaced. In the third trimester 1000 mL of fluid flows into and out of the amniotic cavity daily so that even small changes in one of the paths of fluid migration could rapidly effect the amniotic fluid volume.

Inflow	*Volume (mL/24 hours)*	*Outflow*	*Volume (mL//24 hours)*
Fetal urine production	800-1200	Fetal swallowing	500-1000
Fetal lung liquid secretion	170	Intramembranous flow	200-400
Oral-nasal secretions	25	Transmembranous flow	10

Measurement of fetal urine production

During the filling phase, the increasing volume of the urinary bladder can be observed, documented and assessed by 2D ultrasound scans. Conventionally, three orthogonal bladder diameters on longitudinal and transverse bladder images and the formula for an ellipsoid are utilised for volume calculation[10].



Figur 1 The largest longitudinal and transverse sections of the bladder were regarded as the appropriate bladder images. According to the conventional method the longitudinal bladder diameter (a) and the transverse diameters (b) and (c) were used for volume calculation.

The hourly fetal urine production rate (HFUPR) can be estimated by regression analysis of calculated bladder volumes documented at different time points within one filling phase[10, 12, 13, 14, 15, 16], or by the difference between the maximum and minimum volumes divided by the time interval[17, 18, 19, 20].

Studies of the fetal urine production rate have produced divergent results, ranging at term from 24 mL/h[13] to 95 mL/h[16]. One possible reason for the low urine production rate values may have been episodes of partial emptying of the urinary bladder during the filling phase, which were unnoticed by the operator[14]. In such a case, the urine production rate will be underestimated. The value of 95 mL/h in Groome's study is more difficult to hypothesise about[16]. These varying results may reflect methodological problems.

The filling and emptying dynamics of the fetal urinary bladder have been investigated in detail[14]. The HFUPR for fetuses with a gestational age of 20, 24, 28, 32, 36 and 40 weeks was estimated to 5, 9, 14, 22, 33 and 51 mL/hour respectively. The maximum bladder volumes at different gestational ages before emptying were presented. The mean time for bladder filling phase was 25 minutes (range 7-43 minutes) and it was not significantly influenced by gestational age. The emptying of the bladder was visualised on 101 occasions and the duration was less than 30 seconds. The emptying was complete on 28 occasions but incomplete on the remaining 73 occasions. On 55 of these, the residual bladder volume was less than 25% of the maximum bladder volume, in 16 between 26% and 50% and on two occasions between 60% and 65%.

Urine production in growth-retarded fetuses

When comparing Intra Uterine Growth Restricted (IUGR) fetuses with Appropriate weight for Gestational Age (AGA) fetuses at the same gestational age, the HFUPR was significantly lower for IUGR fetuses[12, 13, 15, 17, 20]. However, there were no significant differences when the IUGR fetuses were compared with controls of corresponding body weights, but with lower gestational ages[12]. It was assumed that the reduced urine production rate for IUGR fetuses reflected renal hypoplasia, due to growth retardation. In three cases, a much lower urine production rate was also demonstrated when compared with control fetuses with corresponding body weights. It was suggested that the reason for this was increased tubular re-absorption, i.e. a different etiological mechanism[12]. Although different investigations have presented various normal values, the HFUPR in IUGR fetuses compared with fetuses of normal size (AGA) has generally been reported to be lower[13, 17, 15, 20].

Fetal kidneys

In a human 2D ultrasound study comprising IUGR and AGA fetuses, the volume of fetal kidneys as well as the urine production rate was estimated[18]. In IUGR fetuses, both the volume of the kidneys and the HFUPR were significantly reduced when compared with the AGA fetuses. In concordance to this study, the growth of fetal kidneys was significantly slower in Small for Gestational Age (SGA) vs. AGA fetuses when it came to the anterio-posterior diameter and transverse circumference of the kidneys[21]. This divergence was most marked after 26 weeks of gestation.

The fetal kidneys gradually increase in volume with gestational age[2]. Renal weight as an autopsy finding is, however, often compromised and associated with a coefficient of variation as large as 50%, due to oedema and passive venous engorgement[22]. Renal functional capacity depends on the number of nephrons, but no known relationship exists between renal weight and the number of glomeruli[22]. For many years, estimates of glomerular numbers have therefore been derived from a variety of methods[23]. Unfortunately, these methods have been shown to have some degrees of bias. However, a new stereological dissector technique permits a direct, unbiased estimation of glomerular numbers[22]. This new dissector method was used to estimate the number of nephrons (both kidneys) in fetuses. The total number was 15,000 in human fetuses at 15 gestational weeks and between 740,000 and 1,060,000 at term[22].

The total number of nephrons was also estimated in a comparative investigation of six IUGR stillbirths of known gestational age with controls comprising eleven stillbirths with a birth weight greater than the 10th percentile (prenatal period) and eight liveborn IUGR infants who died within a year of birth with a control group of seven appropriately grown infants who died within a year of birth (postnatal period). The number of nephrons for five of the six IUGR stillborn children and all the growth-retarded children who died within one year was significantly reduced compared with the controls[24].

Moreover, in animal models, growth restriction has been associated with a reduced number of nephrons[25, 26].

It has been suggested that the mechanism underlying the reduced number of nephrons in IUGR fetuses is increased apoptosis due to changes in the levels of apoptosis-related proteins[27].

The compromised fetus

The hourly fetal urine production rate (HFUPR) was determined by 2D ul-

trasound immediately before cordocentesis for blood gas analysis in 27 SGA and 101 AGA fetuses[15]. The HFUPR was reduced for the group of SGA fetuses in comparison with AGA fetuses. Furthermore, the reduction in urine production for the SGA fetuses was correlated with the degree of fetal hypoxemia, while the degree of fetal hypoxemia did not correlate with the degree of fetal smallness.

Several studies demonstrate associations between increased impedance in the fetal renal arteries and factors suggestive of compromised fetal conditions and, in some studies, also reduced urine production rates[28, 29, 30, 31].

In a study by Vyas et al.[28], the renal artery flow-velocity wave forms were examined in normal and hypoxemic human fetuses[28]. The Pulsatility Index (PI), which is peak systolic velocity minus end diastolic velocity over mean velocity, was higher in SGA than in AGA fetuses. Furthermore, using cordocentesis in the SGA fetuses, a significant, direct correlation was found between blood oxygen deficit and increased renal artery PI. Moreover, in a recent study of 35 IUGR fetuses, the PI in the fetal renal arteries was significant increased[31]. In studies of fetal urine production, it was demonstrated that the PI in the renal artery was higher in IUGR than in AGA fetuses and that it displayed a negative correlation to the urine production rate and the amniotic fluid volume[29].

However, in a recent study[32], in which IUGR fetuses were compared with controls, no difference in the PI in fetal renal arteries was reported. Furthermore, in a longitudinal study by Stigter et al.[30] of sixteen severely IUGR fetuses (all with a weight below the 10th percentile), the PI values of the renal artery were within normal limits. However, the peak systolic velocities in the renal artery displayed a significant reduction with time. These findings did not support the concept of increased renal vascular resistance in IUGR fetuses. According to the authors, however, in connection with the progressive deterioration of the fetal condition, there may be a reduction in renal artery flow due to impaired cardiac function or as a result of redistribution and reduced aortic volume flow.

In spite of varying results regarding the PI in fetal renal arteries, these data suggest that, in fetal hypoxemia, there is a redistribution of blood flow with a decrease in renal perfusion and a decrease in HFUPR. The findings may be important, as it would be of great clinical interest to detect whether or not a particular fetus with growth restriction is further compromised.

Biochemical findings associated with damage to the proximal tubules

A biochemical investigation supported the presumption of a reduction in blood flow to the kidneys in compromised fetuses[33]. It was postulated that a reduction in blood flow through the fetal kidney would induce hypoxia, which would damage the cells of the proximal tubules. When damaged, these cells leak N-acetyl-beta-D-glucosaminidase (NAG), which can be detected in the amniotic fluid. When AGA fetuses were compared with IUGR fetuses, the findings were suggestive of a redistribution of the fetal circulation in the IUGR group. Consequently, the level of NAG in amniotic fluid was significantly higher for IUGR fetuses compared with AGA fetuses.

Diurnal variation – a confounding factor

The fetal urine production rate did not display any significant diurnal variations in human fetal studies by Campbell et al.[10] and Wladimiroff et al.[12] However, a highly significant diurnal rhythm was observed in an on-line, computerised study of sheep by Brace et al.[34] In that study, the urine flow rate was measured continuously over a period of days. In 8/9 animals, the peak flow rate occurred at around 9 pm and at 9.30 am in the remaining sheep fetus. The maximum urine flow was 28 ± 5% above the 24-hour mean.

The studies by Campbell and Wladimiroff were performed in the daytime and a significant diurnal variation in human fetal urine flow around 9 pm could have escaped observation.

Although this significant diurnal variation was demonstrated in an animal model, it is important not to disregard a possible diurnal variation in human fetal urine production as well.

Fetal behavioural states

There are some reports on fetal behavioural states and urine production rate (HFUPR). Fetal behavioural states were investigated by Nijhuis et al.[35] and were characterised according to the association between the heart rate and body and eye movements of the fetus. Three states are undisputed: 1F (=quiet sleep), 2F (=active sleep) and 4F (being awake). The HFUPR was shown to be significantly higher during 1F vs. 2F[36, 37]. This observation was supported by the finding of increased fetal blood pressure during 1F, which may lead to higher glomerular filtration rate and urine production[38].

Moreover, micturition was reported to be behavioural-state dependent[39, 40]. In the study by Ohel et al.[39], voiding was observed almost exclusively during 2F periods and, in the study by Stigter et al.[40], fetal voiding occurred in association with 22 of 36 transitions from 1F to 2F and in only one of 13 transitions from 2F to 1F. Furthermore, in that last study[40], the urine production rates were calculated in 43 of 123 fetuses, based on complete bladder filling phases during both behavioural states 1F and 2F (identical fetuses).

The maximum bladder volumes (before emptying) were greater during 1F vs. 2F, mean 36.1 mL (7.5–73.0 mL) and mean 25.0 mL (5.9–67.7 mL) respectively. In 24 of the 43 cases, the maximum bladder volume before emptying was > 10 mL higher during 1F vs. 2F.

Exclusively in these cases, the HFUPR was significantly increased and, according to the authors, this was related to an overestimation of volumes of the large bladders. We agree, the bladder volumes will be overestimated, when using the conventional method of bladder volume estimation, based on longitudinal and transverse bladder scans (page 36).

Maternal food and water ingestion

The urine production rate was estimated two hours before and two hours after maternal breakfast in 25 AGA and 15 IUGR fetuses. After breakfast, the HFUPR increased in AGA fetuses but did not change in IUGR fetuses[41]. The PI of the fetal renal artery was significantly reduced after maternal food ingestion in uncomplicated pregnancies.

The influence of maternal water ingestion on fetal urine production was investigated in twenty-one healthy, pregnant women with fetuses of 37-40 weeks. There was a significant increase in the fetal urine production rate during quiet sleep (F1) after the maternal ingestion of one liter of water after four hours of fluid deprivation[42]. The limitation of that study was, however, the exclusion of 11/21 subjects.

Conflicting results were observed in a previous study of women with third-trimester oligohydramniosis[43], in which the women ingested 2 L water in 2 hours. Although the osmolality was significantly reduced in maternal plasma and the amniotic fluid volume was increased in cases with oligohydramniosis[42], no significant change in the fetal urine production rate was observed compared with controls.

Maternal water ingestion thus appears not to influence the fetal urine production rate with any certainty. However, to avoid the confounding variation

due to diurnal variation and maternal meal ingestion, it is recommended that the estimation of fetal urine production should be performed under standardized conditions.

Fluid load in fetal sheep model

In a fetal sheep model, it was demonstrated that fluid load enters the interstitial space[44]. Similar mechanisms may also be relevant to human fetuses and could explain why no change in urine production rate took place during a period of two hours of hydration[43]. The arterial and venous pressure, the hormone concentrations and the urine production rate were studied after an intravascular isotonic fluid load equal to 2% of the body weight in non-pregnant adult and near-term fetal sheep[44]. The concentrations of vasopressin and renin decreased, whereas venous and arterial pressures and the concentration of atrial natriuretic factor (ANF), as well as the urine production rate, increased for both fetuses and adult animals. Interestingly, while they were maintained in the adults, both the ANF and urine production rate returned to normal values in the fetuses within 20 minutes. It could be estimated that fetal urine flow returned to control values before < 10% of the volume load had been excreted. The remaining fluid might have entered the interstitial space.

Maternal water ingestion in relevant volumes is therefore not assumed to influence the fetal urine production rate.

Reliability of the measurements of the fetal urinary bladder

Calculated bladder volumes cannot be validated in living human fetuses, as the true fetal urinary bladder volumes are not known. The reliability of the measurement procedures on human fetal bladder dimensions, on the other hand, can be evaluated, while systematic errors will escape observation. The measurement error when estimating the length of the fetal urinary bladder diameter and volume have, however, been the topic for investigation by our group.

Several authors have attempted to validate the ultrasound accuracy of bladder volumes on balloons and on stillborn babies. Two studies used balloons of different sizes in water baths to validate 2D vs. 3D ultrasound[45, 46].

In another study, the bladder of one stillborn baby in a water bath was filled with a known amount of water in consecutive steps[10]. A comparison was then performed between the known volumes and the mean and range of three ultrasound estimations of the bladder regarding this volume.

Yet another study[47] investigated the ultrasound accuracy of bladder volumes (3-30 mL in 2 mL incremental steps) in three neonates, who died within 24 hours, and eight stillborn infants with gestational ages ranging from 22-40 weeks. The traditional ellipsoid formula was found to overestimate the bladder volumes. The authors presented two formulas for volume calculation, which were based on the sagittal and coronal area respectively. Although the accuracy of the measurement of these bladder volumes can be improved by modifying the calculating formulas according to the known bladder volumes[47], they still lack credibility due to the artificial setting. Bladders from stillborn babies and balloons are probably not representative of bladders in living fetuses.

In a study by Stigter et al. of 115 normal, term pregnancies (36-42 weeks), the possible measurement error was estimated when using the transverse bladder scan for volume calculation[48]. The authors also discussed the problem of disturbing shadows; in practice, when the fetus tends to lie with its back directed towards the maternal abdominal wall, so that the fetal spine varies from directly anterior to left or right laterally. As a result, only a more or less oblique sagittal section of the bladder can usually be viewed and, for these ultrasound bladder scans, the authors introduced a new formula for bladder volume calculation. The calculated bladder volumes of the included fetuses were referred to the corresponding results based on the formulas, which were presented by Hedriana et al.[47]

Some studies address the issue of the reliability of 2D ultrasound measurements of the fetal urine production rate[49].

Although some publications touch on the aspects of reliability[14, 30], there are no previous, detailed studies assessing the reliability of the urinary bladder dimensions of living fetuses. A knowledge of this is, however, fundamental in order to evaluate the reliability of measurements of the fetal urine production rate.

Aims of the Study

1. To reassess the urine production rate for fetuses of different gestational ages and to investigate the variability of measurement errors, when estimating fetal bladder diameters and volumes.

2. To evaluate the errors when selecting the proper image and calculating the volume of that image ("freezing" and the "frozen error"), when using a modified manual ultrasound technique (adding a "rocking" transducer movement to the conventional method) and digitising the selected ultrasound images.

3. To evaluate the sum-of-cylinders method for calculating the fetal urinary bladder volume.

4. To estimate SD when calculating urine production and to evaluate the magnitude of the changes in the hourly fetal urine production rate (HFUPR) that might be caused exclusively by measurement errors. Furthermore, to elucidate the probability of false HFUPR readings due to measurement errors and, finally, to assess the part of the total variation around the mean of the HFUPR due to biological variation for fetuses with a gestational age of 22 to 40 weeks.

Material and Methods

Ultrasound equipment, computers and computer programs

The ultrasound investigations were performed using an Acuson 128 with an L 312 (3.5 Mhz frequency) linear transducer and either an Acuson 128 XP 10 or an Acuson 128 XP ART (Mountain View, CA, USA), both attached to an L 582 (5.0 Mhz frequency) linear transducer. The results were documented using a Panasonic VHS AG 6200 video-cassette recorder + a Mitsubishi P 71E video-graphic printer (Acuson 128) and a Sony SVO-9500 MDP video-cassette recorder + a Sony UP-890 CE video-graphic printer (Acuson 128 XP 10 and Acuson 128 XP ART) respectively.

The accuracy of the ultrasound measurement program was evaluated by a measurement phantom RMI 403GS. After scanning the video-printer copies, (architect software) Computer Aided Design (CAD) was used to test the measured distances (Paper I).

Using an interface (Dazzle, SCM Microsystem Inc, Fremont, CA, USA), which was connected to the video-cassette recorder and a laptop PC (Multimedia Portable Computer, model 98 M830 NTA 0-0357, INET Data, Gothenburg, Sweden), the recorded images were digitised. To assess bladder diameters, a computer program (Microsoft-Paint, Microsoft Corporation, Redmond, WA, USA) displaying co-ordinates was used (Paper II).

The volumes of the digitised bladder images in Paper II were re-calculated in Paper III, using a PC (Dell Latitude CPx J 750 portable computer). Microsoft Paint, Corel Photo-Paint (Adobe Systems Incorporated, San Jose, California, USA) and MathCad Professional (Math Soft Incorporated, Cambridge, MA, USA) were used for image processing and volume calculation.

Paper I

Estimation of fetal urine production rate

Women who visited the ultrasound laboratory for sonographic fetal weight estimation, placenta localisation or the recognition of fetal position in the uterus were invited to participate in extended examinations of fetal urinary bladders. Eighty-four women consented to participate. Twenty-two cases were excluded due to the absence of an obvious filling phase (n=7), videotape recording lasting < 5 minutes (n=4), fewer than three bladder volume calculations (n=9) and anomaly of the urinary system (n=2). Documented (on videotapes) sequences of bladder images of the remaining 62 fetuses (16-40

weeks) were utilised for this study. This was a mixed material according to birth weight.

The hourly fetal urine production rate (HFUPR) was estimated according to the conventional method, demonstrated in 1973 (Campbell 1973). A number of bladder images were documented by 2D ultrasound during a filling phase. The volumes were calculated according to the ellipsoid formula, which was based on the three orthogonal bladder diameters: the longest diameter of an appropriate longitudinal bladder image (a) and two perpendicular diameters (b) and (c) in an appropriate transverse bladder image.

An appropriate longitudinal image is obtained, when the ultrasound beam passes through (and not only parallel to) the entire long axis of the longitudinal bladder images. The appropriate transverse bladder image is selected, when the ultrasound scan runs perpendicular to the longitudinal axis and the image displays the largest transverse appearance of the bladder (Figure 1).

The material that was used consisted of documented image sequences with at least three pairs of longitudinal and transverse bladder images. The delay between the longitudinal and transverse bladder images in each pair was at most one minute. One sequence from each fetus was randomly selected. Each diameter was measured three times by the callipers on the ultrasound screen to calculate the mean. The operator was blinded to the estimated distances, which were documented on video-printer copies. The increasing volume of the urinary bladder was calculated by regression analysis.

Bladder diameter measurement errors

A set of material consisting of 2D ultrasound examinations of fetuses with a gestational age of 21-40 weeks (n=21), which was documented on videotape, was used to estimate the diameter measurement error. The results of several examinations on each fetus were included in the material, because the position of the fetuses, as well as the bladder volume, varied. The measurement error was characterised by the variability (SD), when repeating the diameter measurement 10 or 30 times in 101 appropriate longitudinal and 90 appropriate transverse images. Each image was displayed on the ultrasound screen and the operator defined the suitable bladder diameters and placed the two callipers on the end points of these diameters: the longitudinal diameters (a) and (d) and the transverse bladder diameters (b) and (c) (Figure 1). The operator was blinded to the results and video-printer copies were used for subsequent statistical calculations.

Relationship between the bladder diameters

The relationship between the diameters in the longitudinal and transverse bladder images characterised the bladder shape. A first-order regression analysis was used to estimate linear functions for the relationships between diameters (a) and (d) in the longitudinal bladder images and between the two transverse diameters (b) and (c).

The relationship between the transverse diameters (b) and (c) was further analysed using the regression and correlation coefficients of this linear function. Using mathematical statistics, it was demonstrated that the ratio between these regression and correlation coefficients displays the relationship between the two transverse diameters without random measurement errors (Addendum). A ratio deviating from 1.0 will then demonstrate: 1) a bladder shape deviating from circular form in the transverse section or 2) an inappropriately selected ultrasound scan of a circularly-shaped transverse bladder.

Bladder shape in transverse scans

It was assumed that the fetal urinary bladder has a circular transverse shape, based on the findings in the current study. The filled bladder could then be regarded as a rotational body with the long axis of the longitudinal bladder image as a centre.

To evaluate the consequences, the material for HFUPR calculation in this study was used. The differences were estimated for 484 pairs of bladder images, when calculating bladder volumes conventionally by the ellipsoid formula based on three orthogonal diameters (a), (b) and (c) vs. the diameters (a) and (d) and the simplified ellipsoid formula (Statistics).

Bladder volume measurement errors

Estimation of the fetal urinary bladder volume includes several procedures characterised by uncertainty. They include the operator's 1) selection of an appropriate bladder image (designated the "freezing error" by us), 2) identification of bladder borders and 3) ability to place the callipers in the correct positions on the ultrasound screen, plus 4) the technical resolution power of the image. The measurement errors 2), 3) and 4) were denoted "frozen errors" (errors when measuring the "frozen" images on the ultrasound screen). Furthermore, there is a moment of uncertainty when the bladder volume is calculated according to the ellipsoid formula. During the filling phase, the urinary bladder changes its longitudinal shape and this may result in volume calculation errors. However, in Papers I and II, the ellipsoid formula was used and the bladder shape was the subject of the study in Paper III.

Volume measurement error when excluding image selection

The volume measurement error (SD) excluding the image selection procedure was due to the "frozen errors", i.e. the volume measurement error of selected bladder image. In just the same way that the bladder volumes can be calculated for different diameter lengths, the volume measurement error can be estimated, based on the corresponding diameter measurement error (SD), according to a mathematical method, which is called convolution (Addendum). The urinary bladder was assumed to be a rotational body and, as a result, only the lengths of the longitudinal bladder diameters were needed for volume calculation according to the simplified ellipsoid volume formula.

The relationship between bladder volume and volume measurement error (SD) was given as a linear function, based on a number of arbitrary longitudinal diameter lengths, when taking the relationship between the two longitudinal diameters into consideration.

Volume measurement error when including image selection

The variation when estimating the total volume measurement error depends on all four presented procedures of measurement uncertainty. As a result, this SD characterised the total volume measurement error including the image selection procedure of "freezing" and the volume measurement error of the "frozen" image.

Documented appropriate longitudinal bladder images of fetuses with a gestational age of 17-38 weeks were used for the investigation (n=46). Assuming the bladder to be a rotational body, at least two longitudinal bladder images, which were selected within one minute were needed. The diameters (a) and (d) were measured three times, giving two mean values for volume calculation. The SD for the calculated bladder volumes characterised the volume measurement error. A linear function for the relationship between bladder volumes and this SD (volume measurement error) was estimated for the entire material. This SD was referred to as the measurement error when bladder volumes were estimated according to the conventional or traditional method.

Paper II

A computer model mimicking the filled fetal urinary bladder

Two of the authors (S. H. and F. U.) created a computer model of a three-dimensional (3D) ellipsoid configuration on the screen (mimicking the filled urinary bladder) and an ultrasound plane, based on Fortran code calculations

(www.ptech.se-mf). The filled urinary bladder was assumed to be a rotational body around the long axis. Identical co-ordinates for these two geometrical objects built up images of the intersection planes.

The two geometrical objects (the ellipsoid and the intersection plane) were parameterised, which means that it was possible to create a series of images which display the resulting appearance of the ultrasound plane from different diameters of the ellipsoid and transducer positions. It was thus possible to simulate the filling phase and different positions and angles of the transducer in relation to diameter (a) in the fetal urinary bladder (Figure 1). The area on the screen and the corresponding bladder volume were automatically displayed on the screen (www.ptech.se-mf).

The "rocking" motion

The appropriate longitudinal 2D scan displays the largest area and is obtained when the ultrasound beam passes through the entire long axis (Figure 1). By inducing a gradual "rocking" motion of 5-10° in both directions from the appropriate transducer orientation, the image will be reduced equally on both ends of the long axis with a fixed position for the centre. However, when a "rocking" motion is added to the transducer, which is not appropriately positioned ("twisted" or "slid" or a combination of these), the entire bladder image moves to the left or right of the screen (when "rocking"), or the image is alternately enlarged and diminished (when "slid"). This use of this "rocking" motion is a way of checking whether or not the long axis is sectioned appropriately. The crucial issue is to observe the bladder image movements, when performing this motion (www.ptech.se-mf).

Bladder volume estimation by the modified method

The volume measurement error (SD) was investigated on a set of material comprising fetuses with a gestational age of 24-40 weeks (n=34). The urinary bladder of each fetus was examined by ultrasound and documented three times within one minute (images 1, 2 and 3), to prevent urine production influencing the results. The current study was based on longitudinal bladder images (assuming the bladder to be a rotational body) and the moment of "rocking" motion to control appropriate image selection.

All the examinations were documented on videotape. The image, which the operator regarded as "optimal" was made obvious during documentation, the transducer was then held in a fixed position for a few seconds. The images were subsequently digitised and analysed by a computer program, which displayed x and y co-ordinates on screen. The operator placed the pointers

on the borders of the bladder for diameter assessments, but the operator was blinded to the actual co-ordinates , whereas another investigator performed the reading of co-ordinates. Two diameters, (a) and (d) in the longitudinal section of the fetal urinary bladder (Figure 1), were assessed and the volume was calculated according to the simplified ellipsoid formula (Statistics).

The "total error$_{\text{straight sequence}}$"

When analysing the documented images, the delay between various image observations could influence the operator's opinion of how to define the bladder borders. This part of the measurement error will probably be small, if the volumes of images 1, 2 and 3 are calculated in a straight sequence. This volume measurement error was characterised by SD and by us called the "total error$_{\text{straight sequence}}$".

The research design matrix

The volumes of the documented bladder images (1, 2 and 3) for each fetus were calculated on occasions I, II and III, with 24 hours in between. These volumes were illustrated in a research design matrix.

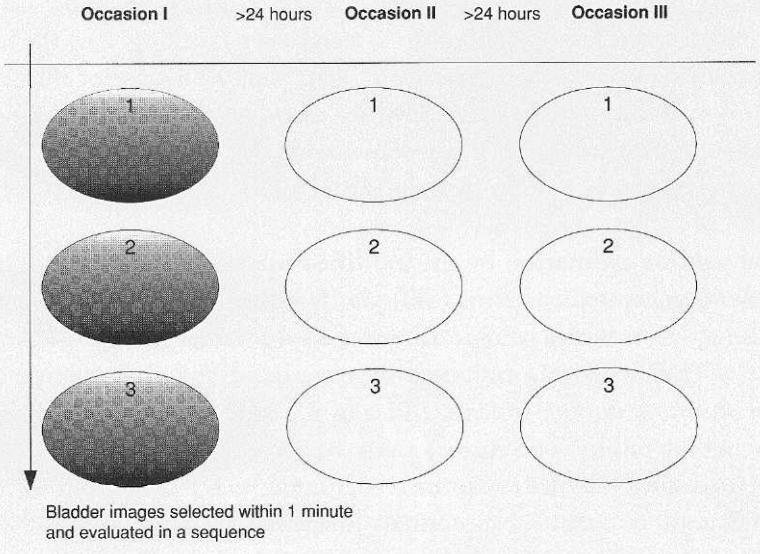


Figure 2 The bladder volumes of the images (1, 2 and 3) for each fetus, which were calculated on occasion I, were placed in the first column. The repeated volume estimations of the images (1, 2 and 3) on occasions II and III were placed in the second and third columns respectively.

In the design matrix, the calculation of SD "total $error_{straight\ sequence}$" was performed "vertically" in the first column (Figure 2). The variation (SD) in volume between images 1, 2 and 3 for each fetus was to some degree dependent on all the measurement errors presented on page 21. By also utilising the second and third column of estimated bladder volumes for each fetus, the material for the estimation of SD " total $error_{straight\ sequence}$ ", was extended. All the fetuses in the material were included in the study (given 3x34 results).

The influence of urine production

The production of urine by the fetus continues throughout the examination. Although the duration of the examination is at most one minute, the volume of urine that is produced could affect the estimated SD "total $error_{straight\ sequence}$". The measurement error, when taking account of the fetal urine production, was denoted "total $error_{straight\ sequence\ corrected}$". This volume measurement error (SD) was calculated on the basis of the differences between each estimated volume and approximated reference volumes, which were calculated in the following way.

The mean volume of the bladder in images 1, 2 and 3 on occasion I was assessed as the "true" volume at the "mid-point" of the examinations.

In an example, the estimated volumes were 10.5, 12.3 and 11.9 mL at the time points of 09.12.10, 09.12.26 and 09.13.04. The total examination time was 54 seconds, the mid-point was 09.12.37 and the mean volume was 11.6 mL.

When it came to this time and volume information, the reference bladder volumes for images 1, 2 and 3 could be calculated on the basis of the normal urine production rate for fetuses of corresponding gestational ages (Rabinowitz 1989).

To extend the base for the calculated SD "total $error_{straight\ sequence\ corrected}$", all the columns (I, II and III) for each fetus and all the subjects in the study were utilised (given 3 x 34 SD values).

The "frozen error"

When the bladder volumes on each row of the research matrix are used for SD calculation, the variation depends on the operator's identification of the bladder borders, the technical ability to place the callipers in the correct positions on the ultrasound screen and the technical resolution power of the image. These volume measurement errors were added up to produce the SD "frozen error". No moment of image selection was involved between the bladder volumes in an identical row (identical bladder image). The total amount

of SD "frozen error" was included because of the delay of 24 hours between the measurement occasions. All three rows for each fetus, as well as the total material, were used to calculate this error (3x34 values of SD).

The "total error$_{\text{random sequence}}$"

To estimate the total volume measurement error, the error due to the selection procedures must be added to the "frozen error". This was obtained by combining the "directions" of calculations in the research matrix. The SD was calculated on the basis of one randomly-selected volume in the first column, which was then combined with one random volume in the second column and one volume in the third column, but not in the same row. The remaining bladder volumes were utilised for the corresponding random combination and the estimation of the "total error$_{\text{random sequence}}$" was based on the total material (3x34 SDs).

The "frozen error$_{\text{one image}}$"

The "frozen error" can be subdivided into the observer's part and the technical parts of the measurement error. The technical part includes the ability to place the callipers on the correct positions on the ultrasound screen and the technical resolution power of the image (designated "frozen error$_{\text{one image}}$" by us). This volume measurement error (SD) was estimated by repeating the volume estimation three times on bladder image 2 in one sequence for each fetus. We assumed that the observer's part of the measurement error was reduced to a minimum because a very short time elapsed between the repeated measurements on the identical bladder image. The base for calculation was extended to the entire material (given one SD for each fetus, 1 x 34).

The "freezing error" and "frozen error$_{\text{straight sequence}}$"

The word "total" in the terms "total error$_{\text{random sequence}}$" and "total error$_{\text{straight sequence}}$" was used to indicate that these errors included amounts of "freezing" as well as "frozen" errors.

The "total error$_{\text{random sequence}}$" includes the total amount of the "freezing error" and "frozen error". The exclusive SD "freezing error" could be estimated because the variance in the "freezing error" equals the difference between the variances in the "total error$_{\text{random sequence}}$" and "frozen error".

The "total error$_{\text{straight sequence}}$" includes the total amount of the "freezing error" as well as some of the "frozen error", which is denoted "frozen error$_{\text{straight sequence}}$". The difference between the variance in "total error$_{\text{straight sequence}}$" and the "freezing error" equals the variance in "frozen error$_{\text{straight sequence}}$".

SD "freezing error" illustrated in the computer model

The magnitude of the SD "freezing error" was illustrated by utilising the computer model for simulation. The SD and the corresponding bladder volumes were plotted in a diagram. In addition, the amounts of volume reduction were plotted, when applying the "rocking" motion 1, 2, 3, and 4° degrees from the "true" appropriate transducer position in this computer model for bladder volumes of 1 to 40 mL (www.ptech.sc-mf).

Paper III

The shape of the fetal urinary bladder

The shape of the longitudinal fetal urinary bladder changes during the filling phase.

At the beginning, the shape is circular (spherical) or pear-shaped (Figure 3), with the largest appearance in the region of the bladder neck, then elliptical (ellipsoid) and finally almost rectangular (super-ellipsoid) (Figure 4). The calculated bladder volumes may therefore be miscalculated at the beginning and end of the filling phase (volume calculation error), when using the ellipsoid formula. Furthermore, this may cause false estimations of fetal urine production rates.

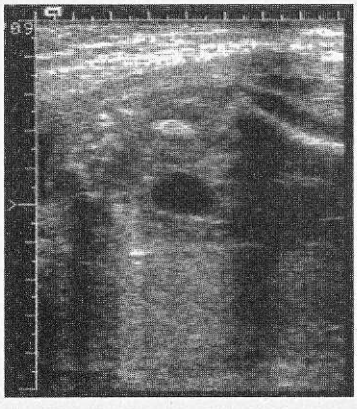


Figure 3 The shape of the longitudinal fetal urinary bladder changes during the filling phase and at the beginning, the shape is circular (spherical) or pear-shaped, with the largest appearance in the region of the bladder neck.

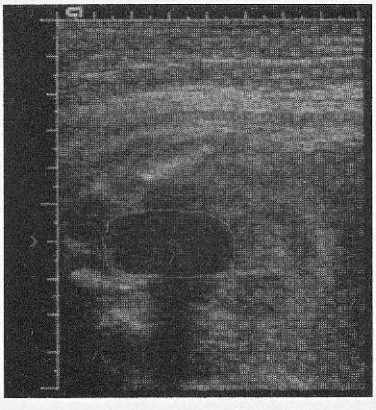


Figure 4 During the filling phase the shape of the longitudinal fetal urinary bladder changes from circular (spherical) or pear-shaped to elliptical (ellipsoid) and finally almost rectangular (super-ellipsoid).

Volume calculation according to the sum-of-cylinders method

The material for examination was the identical longitudinal bladder images, which were utilised in the previous study, Paper II, and were taken from thirty-four fetuses with a gestational age of 24-40 weeks. The volumes of bladder images 1, 2 and 3 for each fetus were calculated on one occasion using the sum-of-cylinders method (instead of the ellipsoid formula, as in Paper II).

The fetal urinary bladder was assumed to be a rotational body and, as a result, only longitudinal bladder images 1, 2 and 3 were used. The volume calculation using the sum-of-cylinders method is independent of the longitudinal bladder shape. It was necessary to process the digital bladder image before the volume could be estimated. A computer program was developed for this image processing and volume calculation (www.ptech.se/hem-mf).

The volume measurement errors SD "total $\text{error}_{\text{straight sequence}}$" and SD "frozen $\text{error}_{\text{one image}}$" were estimated for each fetus. In addition, the volume differences when performing the calculation with the two different methods were compared with the gestational age, the mean volume, the degree of filling and, finally, the ratio between the shortest and longest diameter of the longitudinal bladder image.

Image processing and volume calculation

Each digitised bladder image is composed of a matrix with elements called pixels. Each pixel has the shape of a cube with a side length of approximately 0.2 mm. The colour of each pixel is determined by the additive combination of a red, a green and a blue component based on an international Red-Green-Blue (RGB) colour model. In the RGB standard, the intensity of each colour is determined by the value of each colour component, which ranges from 0 (lowest intensity) to 255 (highest intensity).

When the sum-of-cylinders method is used to calculate bladder volumes, the bladder images must be prepared in a three-stage process. 1) The operator traced the bladder borders with a red digital marker on the screen (using Microsoft-Paint). The red image component was used for the continued process, as the red marker was easiest to distinguish from the background. For the red component, this colour has an intensity of 255, but values of 0 for the green and blue components. 2) The initial image was divided into three new images based on red, green and blue colour components (using Coral Photo-Paint). The red component image was selected. 3) In the third processing stage, using MathCad computer software, the red component image was used to convert the bladder contour of marked pixels to the 0 value and all the other pixels to a value of 255. This image process resulted in a white background with a black bladder contour ready for volume estimation (Figure 5).

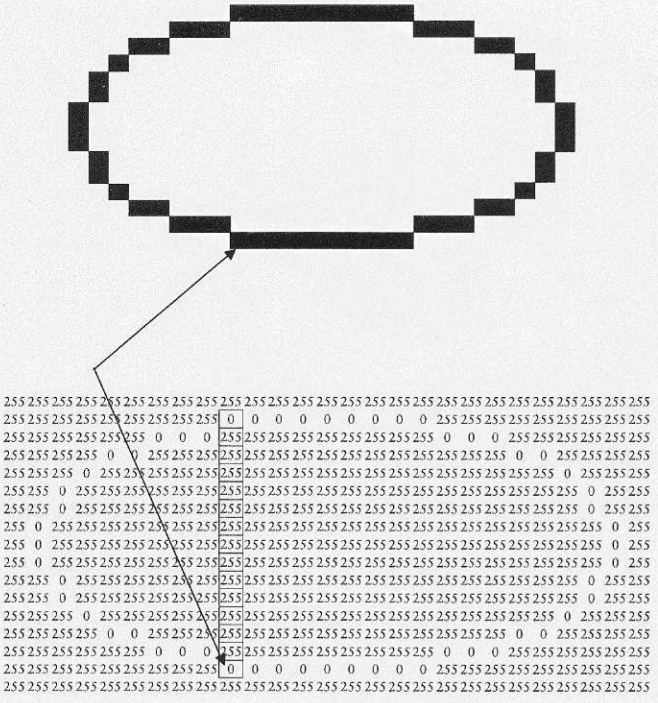


Figure 5 In the final processed image, the software determines the co-ordinates for the bladder boundary pixels (value 0, black). The image was analysed by the software and each column of pixels was scanned.

The process of volume calculation

The final processed image was electronically subdivided into numerous thin cylinders (approximately 0.2 mm). The volume of each cylinder was calculated and added to estimate the total bladder volume www.ptech.se/hem-mf). (Figure 6). If a bladder was obliquely located on the image, the image was adjusted to match a given horizontal position of the bladder.

Theoretical estimations were made of the volume miscalculation, when the ellipsoid formula was used, in spite of "pear-shaped" and super-ellipsoid bladders. The "pear-shaped" bladder was approximated by a half-ellipsoid fused to a semi-sphere and the super-ellipsoid bladder by a cylinder respectively. The volumes of identical structures were then calculated using well-known geometrical formulas and the ellipsoid formula.

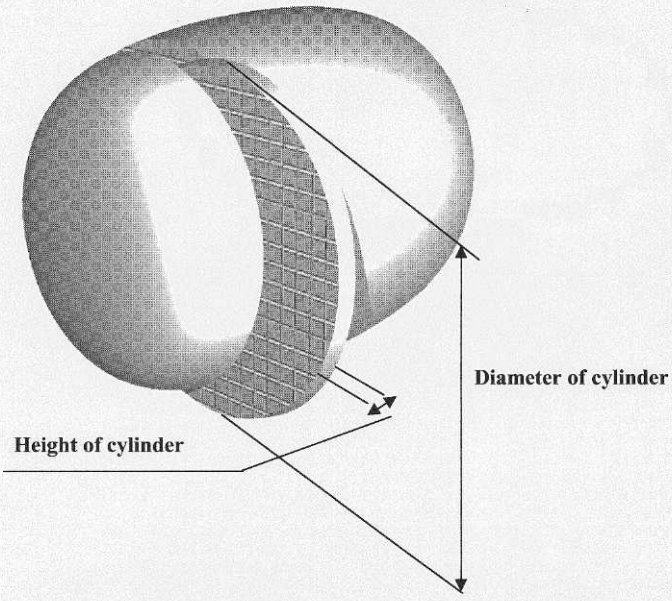


Figure 6 The length of each vertical strip (from top to bottom black pixels in each column) perpendicular to the long axis was computed. This length was regarded as the diameter of a cylinder, one pixel high (approximately 0.2 mm). The volume of each cylinder was calculated and added to estimate the total bladder volume.

Paper IV

The measurement error when the fetal urine production rate is estimated

The hourly fetal urine production rate (HFUPR) can be estimated according to regression analysis, based on a number of estimated bladder volumes during the filling phase.

According to a mathematical derivation, it was obvious that the measurement error for HFUPR (called "SD_{HFUPR}") was dependent on 1) the bladder volume measurement errors SD "total $error_{straight\ sequence}$", 2) the number of volume estimations and 3) the time intervals between these estimations.

The SD_{HFUPR} turned out to be the fundamental feature for the estimation of HFUPR and the significance of SD_{HFUPR} was illustrated by comparing the conventional and the modified method when answering two clinically related questions.

1) What are the maximum changes in the HFUPR that could be caused exclusively by measurement errors?
2) What is the probability that the HFUPR is calculated lower than the 2.5th percentile, although the true HFUPR is at the 10th percentile point?

Pooled material for evaluation of fetal urine production rate

Two studies of the HFUPR were combined to extend the material for evaluations of the HFUPR.

1) The results of the urine production study in Paper I relating to fetuses with a gestational age of more than 20 weeks (56 of 62 cases) were combined with

2) the HFUPR of controls in a previous study by Takeuchi et al. (n=358). In the latter study, the included pregnancies (between 21 and 40 weeks) were subdivided into two-week gestational classes. The bladder volumes were estimated every five minutes during the filling phase and the HFUPR was calculated from the maximum and minimum volumes.

The pooled material comprised fetuses, which were subdivided into two-week of gestation subgroups. The calculation was referred to the latter one of these two weeks of gestational age.

When it came to the tolerance interval of the estimated HFUPR in this pooled material (n=414), the proportions between the biological variation and the variation caused by measurement errors were calculated. It should be noted that the findings were based on the pooled material, in which the bladder volume was calculated in the traditional manner, and consequently the corresponding SD_{HFUPR}.

STATISTICS

Paper I

When assessing the relationship between the hourly urine production rate (HFUPR) and gestational age in the investigation material, a second order regression analysis was performed. The mean ± 2 SD (the 95% tolerance interval, including 95% of the subjects) were calculated.

The repeated measurements of 382 diameters produced 382 means and corresponding standard deviations. The distribution of SD was studied using kurtosis and skewness (kurtosis = 3.0 and skewness = 0.0 for perfect Normal distribution). Since Normal distribution is a prerequisite for regression analysis, the standard deviations were Normalised by logarithm transformation,. The relationship between SD (= dependent variable) and the distance of diameters (= independent variable) was assessed by regression analysis. $Log_e SD = p + q \times Log_e Distance$, in which p and q are constants. In order to turn back exponents of the regression function from Log_e to the original scale, an adjustment with $\frac{s^2}{2}$ was performed; s is the estimated standard deviation around the regression line.

$$SD = \exp.\left(p + q \times Log_e Distance + \frac{s^2}{2}\right).$$

The ratio between the regression and correlation coefficients equals the ratio between Diameters (c) and (b) without measurement error (Addendum).

$$\text{True Diameter (c)} = \frac{\text{regressioncoefficient}}{\text{correlationcoefficient}} \times \text{True Diameter (b)}.$$

The volume of the fetal urinary bladder was calculated by the ellipsoid formula based on the longitudinal bladder Diameter (a) and the transverse Diameters (b) and (c):

$$\text{Volume} = \frac{4}{3} \times \pi \times \frac{\text{Diameter(a)}}{2} \times \frac{\text{Diameter(b)}}{2} \times \frac{\text{Diameter(c)}}{2}.$$

However, if the fetal urinary bladder can be regarded as a rotational body, the ellipsoid formula can be simplified and the volume can be calculated, based exclusively on the longitudinal bladder diameters (a) and (d):

$$\text{Volume} = \frac{4}{3} \times \pi \times \frac{\text{Diameter(a)}}{2} \times \left(\frac{\text{Diameter(d)}}{2}\right)^2$$

In just the same way that the distance measurement error (SD) was estimated for the bladder diameters, the volume measurement error (SD) for the urinary bladder can be calculated, based on the diameter measurement error. This was performed in three steps:

1) The relationship between Diameters (a) and (d) on the longitudinal bladder images was used to obtain an approximation of the urinary bladder shape.

2) According to a method called convolution, the volume measurement error (SD) could be calculated according to the simplified ellipsoid formula, the relationship between Diameters (a) and (d) and, finally, the estimated diameter measurement error (SD).

3) Based on a set of arbitrary lengths of Diameter (a) and corresponding Diameter (d) a relationship was assessed between SD and bladder volumes: $SD = m + k \times Volume$ (m is a constant and k is a factor).

Paper II

When analysing measurement errors, which are composed of different parts of measurement error, it is necessary to use the corresponding variances $(SD)^2$, i.e. when subdividing SD "total $error_{random\ sequence}$" into SD "freezing error" and SD "frozen error". The subsequent use of the square root gives the SD "freezing error" and SD "frozen error".

Paper III

Based on a well-known geometrical formula, the volume of a semi-sphere fused to a semi-ellipsoid (mimicking a pear-shaped urinary bladder) equals the result when using the ellipsoid formula. However, the ellipsoid formula overestimates the volume up to 40%, if the semi-ellipsoid part changes to a conical form. However, the volume will be underestimated by about 50%, if the volume of a cylinder (mimicking the super-ellipsoid bladder) is estimated by the ellipsoid formula, based on the cylinder height as the long ellipsoid axis and the cylinder diameter as the transverse ellipsoid diameter (Addendum).

The estimated volumes of identical bladders were compared, using the sum-of-cylinders method vs. the ellipsoid formula. In the previous study (Paper II), the bladder images (1, 2 and 3) for each fetus were calculated on three different occasions.

The SD were estimated, based on the differences of all images, 1, 2 and 3 respectively. The variances were added, divided by three and the square root

of the result was used as the SD for the total material comprising 102 image pairs.

For comparison, only assessments from one of these calculation occasions on each fetus was randomly chosen and, in the current study, the three bladder images, 1, 2 and 3, were analysed only once. The distribution of the calculated volumes (3 x 34) was positively skewed. A possible systematic difference in estimated volumes was therefore evaluated using Wilcoxon's matched-pairs test.

The differences when calculating the bladder volumes using two different methods were Normally distributed. The mean volume of the estimated 102 pairs of volumes was given on the X-axis. On the Y-axis, the differences between the volume calculated by the sum-of-cylinders method vs. the ellipsoid formula were marked[50].

The relationship between SD and bladder volume for each fetus was approximated by a linear function. A special t-test for significant differences between regression functions was used.

Stepwise multiple regression analysis was performed to identify possible relationships between the volume difference and the gestational age, the mean volume, the degree of filling (the estimated volume vs. maximum bladder volume for the actual gestational age) and, finally, the ratio between the shortest and longest diameter of the longitudinal bladder image.

Paper IV

The results were combined of two previous HFUPR publications. It was assumed that N_i ,M_i and SD_i were the number, mean and SD.

When it came to Study 1 and Study 2, we used N_1, M_1 and SD_1 and N_2, M_2 and SD_2 respectively. The SD is the variability in the HFUPR around the mean and depends on both measurement errors and biological variation and was denoted by us $SD_{INCLUDED}$, which was assumed to be a linear function of the mean. The mean and $SD_{INCLUDED}$ of the pooled material can then be calculated as: $\text{Mean} = (N_1 \cdot M_1 + N_2 \cdot M_2) / (N_1 + N_2)$

$$SD = [(SD_1^2 \cdot (N_1 - 1) + N_1 \cdot (M_1)^2 + SD_2^2 \cdot (N_2 - 1) + N_2 \cdot (M_2)^2 - (N_1 + N_2) \cdot (\text{Mean})^2) / (N_1 + N_2 - 1)]^{1/2}.$$

The relationship between the estimated HFUPR and the gestational age of the pooled HFUPR material was found to follow linear regression functions within three intervals of the gestational age. Although it was positively related to the fetal gestational age, this increase in HFUPR was different for the three intervals (22 to 27, 27 to 33 and 33 to 40 weeks).

To reduce the calculation error and to obtain a continuous function, the formula was divided into three parts. For the interval 22 to 27 weeks, the value is zero for the second and third parts, but, for the interval 27 to 33 weeks, the value of the third part of the function is zero (Addendum). One alternative would be to describe the relationship between the estimated HFUPR and gestational age by exponential or polynomial functions. However, these functions tend to demonstrate a poor approximation for fetuses with low and high gestational ages. Using the table for Normal distribution and a Z-value of 1.96, 1.28 and 0.67, the HFUPR at the 2.5th, 10th and 25th percentile points was estimated.

The measurement error when estimating HFUPR was called SD_{HFUPR}. $SD_{HFUPR} = c + d \times (HFUPR)$ (Addendum). This SD was positively related to the urine production rate and could be calculated on the basis of the time points and method used for bladder volume estimations. The significance of the volume measurement error SD "total $error_{straight\ sequence}$" was therefore obvious.

When answering the clinically relevant questions, the importance of the constants c and d was demonstrated.

If we assume that the true HFUPR = x on two occasions, we can calculate the probability that the difference between two determinations is at least as extreme as a quantity Δ. The probability is $2 \cdot \Phi\left(\frac{-|\Delta|}{(c+d*x)\sqrt{2}}\right)$, where Φ is the distribution function of the standardised Normal distribution. The probability attains a value of 5%,if: $\Delta = 1.96 \times (c + d*x)\sqrt{2}$. If the difference is at least this extreme, it is not likely that it is caused exclusively by measurement errors.

The probability that the HFUPR is calculated lower than the2.5th percentile, although the true HFUPR is at the 10th percentile point, can be calculated according to $\Phi\left(\frac{x_{2.5} - x_{10}}{c + d * x_{10}}\right)$.

The ability of the fetus to produce urine could be calculated as a function of the estimated HFUPR. $SD_{INCLUDED}$ corresponds to the tolerance interval of HFUPR for each gestational age. This variation includes the measurement error SD_{HFUPR} and the variation caused by different abilities of the fetus to produce urine, denoted by us $SD_{EXCLUDED}$. The derivation in $SD_{EXCLUDED}$ is presented in the Addendum.

$$SD_{EXCLUDED} = \sqrt{\frac{\left(HFUPR_{mean}\right)^2 + \left(SD_{INCLUDED}\right)^2 - \left(c^2 + 2 \times c \times d \times HFUPR_{mean}\right)}{1 + d^2} - \left(HFUPR_{mean}\right)^2}$$

Results

Paper I

Estimation of fetal urine production rate

In mixed material comprising SGA, AGA and HGA fetuses (n=62), the hourly fetal urine production rate (HFUPR) in relation to gestational age (GA) was:

$HFUPR = -0.258430 - 0.865381 \times GA + 0.054410 \times GA^2$ and

$SD = 0.41905 \times GA$.

For fetuses with gestational ages of 20, 25, 30, 35 and 40 weeks, the HFUPR was 4.2, 12.1, 22.7, 36.1 and 52.2 ml/hour.

Bladder diameters

The range of 202 longitudinal and 180 transverse bladder diameters was 13.9-57.8 mm and 6.8-55.7 mm respectively. The diameter measurement error (SD) was:

$$SD = \exp(-1.618 + 0.325 \times \mathrm{Log_e Distance}_{mean} + \frac{0.4383^2}{2}).$$

For diameter distances of 5-50 mm, the SD was 0.4-0.8 mm.

The relationship between Diameter (d) and Diameter (a) in the longitudinal images was: Diameter (d) = –4.596 + 0.672 × Diameter (a).

For the transverse diameters, the relationship between Diameter (c) and Diameter (b) was: Diameter (c) = 1.883 + 0.829 × Diameter (b). The regression and correlation coefficients were 0.829 and 0.867 respectively. The ratio between the regression and correlation coefficient equals the ratio between the two transverse bladder diameters excluding measurement errors (Addendum). In this case, the quotient ratio was 0.96 and, because of the finding of near equality between diameters b and c, the transverse bladder shape was assumed to be a circle and the bladder to be a rotational body.

The bladder volumes were calculated according the conventional method, based on three orthogonal diameters (a), (b) and (c) vs. the modified method using only the two longitudinal diameters (a) and (d). The conventional method produced larger volumes of identical bladders compared with the modified method. Considering 484 cases, the mean difference was 1.1 mL (95% CI 0.9-1.3 mL), which according the Sign-test was highly significant, $p<0.001$. (Figure 7, 8).

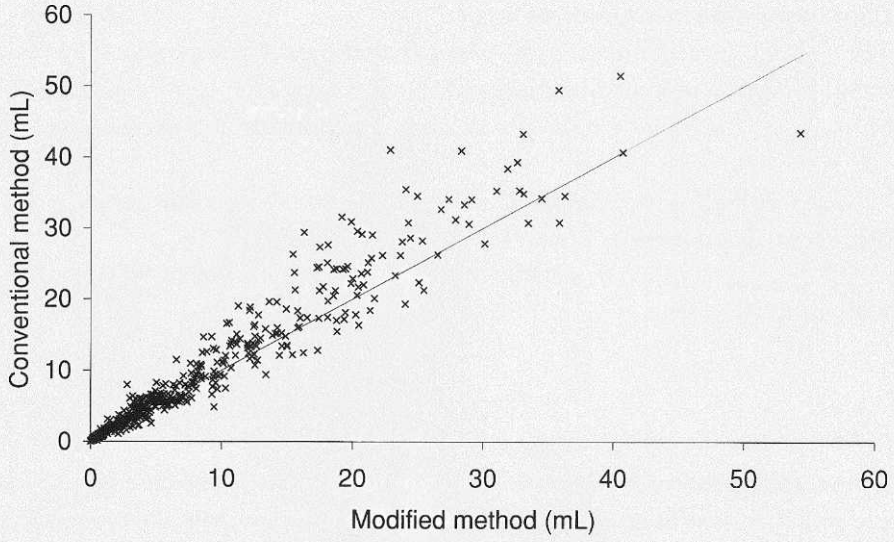


Figure 7 The volume of identical bladder images were estimated using the conventional (three orthogonal diameters) and the modified method (two longitudinal diameters to estimate the urinary bladder volume. The volumes are illustrated in relation to the line of equality.

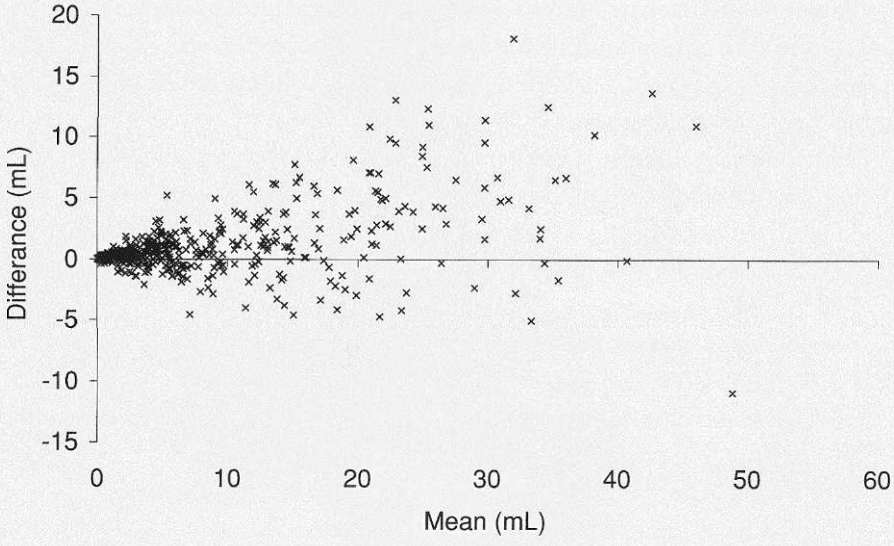


Figure 8 Differences when using the conventional (three orthogonal diameters) and the modified method (two longitudinal diameters to estimate the urinary bladder volume of identical bladder images.

Bladder volume measurement error

The volume measurement error, when excluding the image selection moment, was given as linear functions: SD = a + b x Volume.

SD "$_{\text{frozen error}}$" = 0.097 + 0.0179 x Volume. For volumes of 5-40 mL, the SD was 3.7-2.0%.

When including the procedure of selecting an appropriate longitudinal bladder image, the values of a = 0.36516 and b = 0.09978.

SD "$_{\text{total error}}$" = 0.36516 + 0.09978 x Volume. For volumes of 5-40 mL, the SD was 17.3-10.9%.

Paper II

In this study, the selection of appropriate longitudinal bladder images was verified by the use of the "rocking" motion. The bladder images were digitised and the volume was calculated by using the simplified ellipsoid formula based on the longitudinal bladder diameters (a) and (d).

Compared with "$SD_{\text{total error}}$" (Paper I), the volume measurement error was significantly reduced ($p<0.05$).

SD "total $error_{\text{straight sequence}}$" = 0.42042 + 0.04479 x Volume. For volumes of 5-40 mL, the SD was 12.9-5.5%.

When taking the urine production during the examination time into consideration, the corresponding volume measurement error was the SD "total $error_{\text{straight sequence corrected}}$" = 0.40999 + 0.04543 x Volume, which corresponded to 12.7-5.6% for volumes of 5-40 mL.

The following volume measurement errors (SD) were estimated by calculating the SD based on combinations of bladder volumes:

SD "frozen $error_{\text{one image}}$" = 0.05448 + 0.01882 x Volume

SD "frozen error" = 0.28913 + 0.02259 x Volume

SD "total $error_{\text{random sequence}}$" = 0.47492 + 0.04692 x Volume

For bladder volumes between 5 and 40 mL, these SDs corresponded to 2.8-1.8%, 8.0-3.0% and 14.2-5.9% respectively.

Using the corresponding variances, the following SD were calculated:

SD "freezing error": SD = 0.37892 + 0.04119 x Volume

SD "frozen $error_{\text{straight sequence}}$" = 0.18247 + 0.01760 x Volume

For bladder volumes between 5 and 40 mL, these SDs were 11.7-5.1% and 5.4-2.1% respectively.

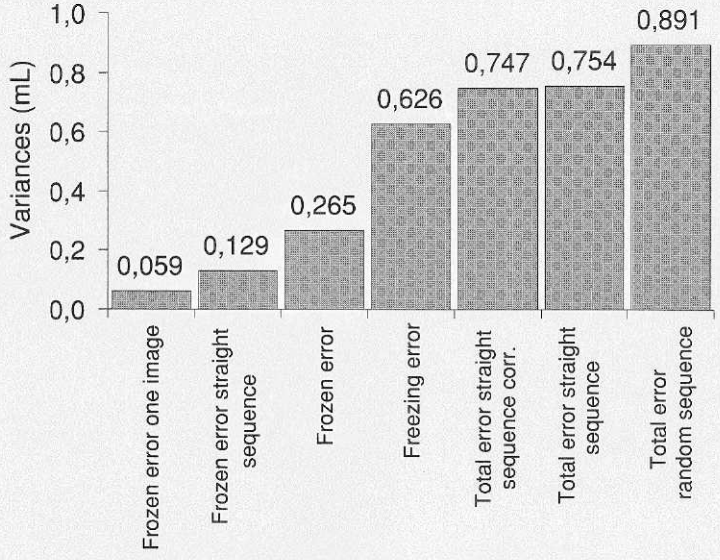


Figure 9 This figure demonstrates the influence of different errors (illustrated as variances) when assessing bladder volumes of 10 mL.

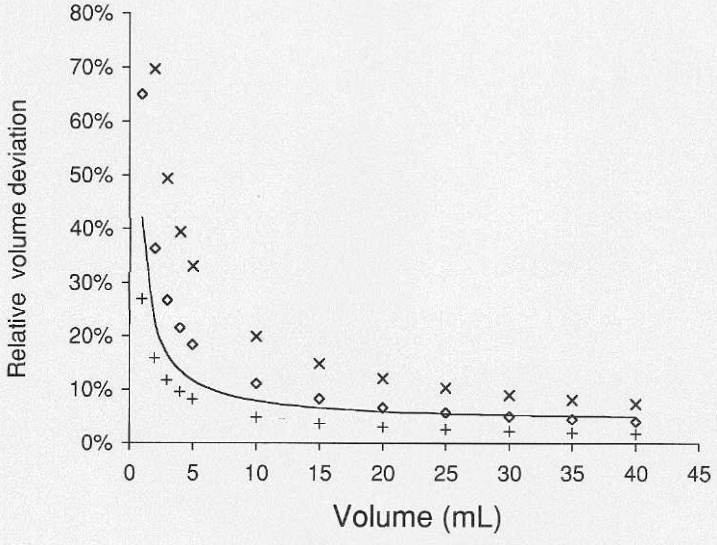


Figure 10 Using a computer model, the reduction in volume was estimated between a situation with an appropriate positioned transducer and one with a rocking angle for the ultrasound transducer. The volume differences were related to the initial bladder volume and were compared with SD for the "freezing error".

Paper III

The same longitudinal digitised bladder images were used in this study as in Paper II. However, the bladder volumes were calculated using the sum-of-cylinders method instead of the ellipsoid formula. The SD "total $error_{straight\ sequence}$" = 0.29911 + 0.02788 x Volume was therefore based on images 1, 2 and 3 from the same fetus obtained within one minute and this calculation was performed for all documented bladder images. For bladder volumes between 5 and 40 mL, the SD was: 8.8 to 3.5%. The "sum-of-cylinders" method for volume calculation tended to reduce the SD "total $error_{straight\ sequence}$" still further (p=0.0543).

SD "frozen $error_{one\ image}$" = 0.11923 + 0.01429 x Volume.

For bladder volumes between 5 and 40 mL, the SD was 3.8-1.7%.

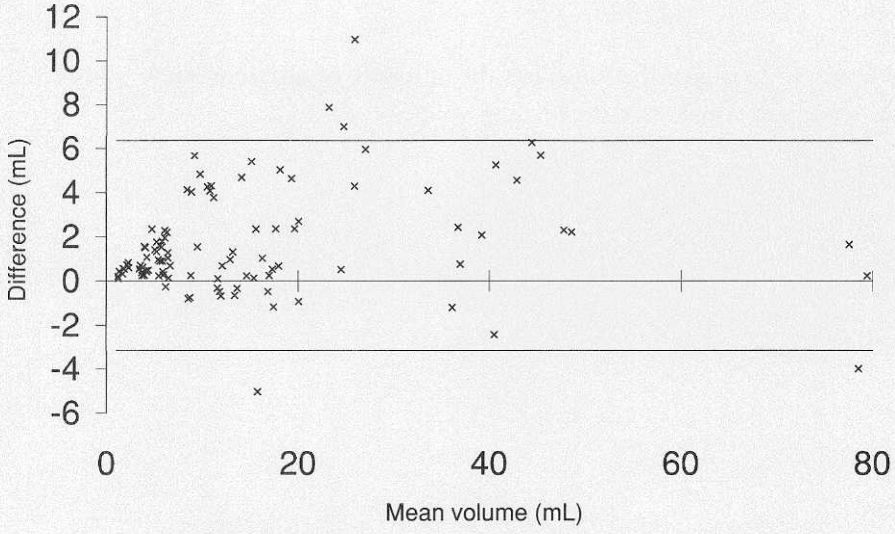


Figure 11 The calculation using the "sum-of-cylinders" method produced systematically larger volumes (p<0.0005) compared with the ellipsoid formula (Paper II). The mean difference was 1.6 mL and the SD = 2.4 mL (95% CI and TI, tolerance interval ± 0.47 and ± 4.8 mL respectively).

There was no significant relationship between the difference and the gestational age, the mean volume, the degree of filling and, finally, the ratio between the shortest and longest diameter of the longitudinal bladder image.

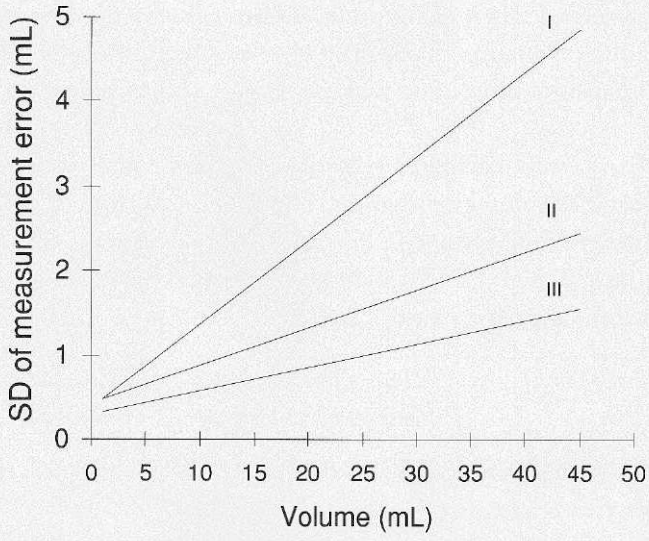


Figure 12 The volume measurement error, corresponding to the SD "total error $_{\text{straight sequence}}$" was used to compare the different volume calculation methods. There was no significant difference between the intercepts of the regression lines. The slope of 0.100 in our first study (Paper I) was significantly larger than the slope of 0.045 and 0.028, according to the method used in Paper II and Paper III respectively.

Paper IV

The measurement error when estimating the fetal urine production rate (called SD_{HFUPR} by us) was derived:

$$SD_{HFUPR} = \frac{\left[\sum (a + b \cdot x_i \cdot HFUPR)^2 \cdot (x_i - \bar{x})^2)\right]^{1/2}}{\sum (x_i - \bar{x})^2}.$$

When bladder volume is calculated by the conventional method, the constants that are used are a = 0.36516 and b = 0.09978, according to SD "total error" = 0.36516 + 0.09978 x Volume (Paper I). However, when the new method of volume estimation is used, including the "rocking" motion, the digitising of bladder images and volume calculation by the sum-of-cylinders method (Paper III), the constants are a = 0.29911 and b = 0.02788, according to

SD "total error$_{\text{straight sequence}}$" = 0.29911 + 0.02788 x Volume. To illustrate the importance of a low volume measurement error, some standardised situ-

ations are presented when using the conventional and the new method for bladder volume calculation (Table 2). However, the formula could be generally used with arbitrary numbers and measurement time points.

Table 2 SD_{HFUPR} was calculated according to the conventional and the new method of bladder volume estimation. The SD "total error $_{straight\ sequence}$" was 0.39132+0.10054*Volume and 0.29911+0.02788*Volume, respectively. The time points of 1, 2, 3, 4, 5 and 6 were assumed to be 5, 10, 15, 20, 25 and 30 minutes after an emptying phase.

Number and time points of volume estimations	*SD_{HFUPR} (mL/hour) Conventional method*	*SD_{HFUPR} (mL/hour) New method*
6 (Time points: 1, 2, 3, 4, 5, 6)	1.075 + 0.0875 x HFUPR	0.849 + 0.0228 x HFUPR
5 (Time points: 1, 2, 3, 4, 6)	1.172 + 0.1049 x HFUPR	0.923 + 0.0276 x HFUPR
4 (Time points: 1, 2, 3, 4)	2.052 + 0.1067 x HFUPR	1.597 + 0.0280 x HFUPR
3 (Time points: 1, 3)	3.275 + 0.1246 x HFUPR	2.531 + 0.0331 x HFUPR
2 (Time points: 1, 6)	1.263 + 0.1067 x HFUPR	1.002 + 0.0276 x HFUPR

Table 3 The significance of different magnitudes of SD_{HFUPR} was demonstrated by two examples. For a fetus producing about 10 mL/hour of urine, the difference, which could be caused exclusively by measurement errors, was reduced from 5 to 3 mL/hour, when using the new method.

HFUPR	*5%*		*10%*		*20%*	
(mL/hour)	*Conventional*	*New*	*Conventional*	*New*	*Conventional*	*New*
5	4.18	2.67	3.52	2.25	2.73	1.74
10	5.40	2.99	4.54	2.51	3.52	1.95
15	6.61	3.30	5.56	2.78	4.32	2.16
20	7.82	3.62	6.59	3.05	5.11	2.36
25	9.03	3.93	7.61	3.31	5.90	2.57
30	10.25	4.25	8.63	3.58	6.69	2.78
35	11.46	4.57	9.65	3.84	7.48	2.98
40	12.67	4.88	10.67	4.11	8.28	3.19
45	13.89	5.20	11.69	4.38	9.07	3.39
50	15.10	5.51	12.71	4.64	9.86	3.60

Table 4 The probability of false readings at the 2.5 percentile point was estimated, i.e. for a fetus of 24 weeks of gestational age with the true HFUPR at the 10 percentile point. When using the traditional and the new method of volume estimation, this probability was 0.08 and 0.01, respectively method.

Gestational age (weeks)	*Conventional method (probability)*	*New method (probability)*
22	0.11	0.04
23	0.09	0.02
24	0.08	0.01
25	0.06	0.01
26	0.05	0.00
27	0.04	0.00
28	0.02	0.00
29	0.01	0.00
30	0.01	0.00
31	0.01	0.00
32	0.00	0.00

Biological variation in urine production rate

In pooled material consisting of (n=414), HFUPR = -12.85 + 0.896 · min(gestational age, 27) + 2.545 · max(min(gestational age - 27, 33-27), 0) + 2.965 · max(gestational age - 33, 0).

For fetuses with a gestational age of 22, 25, 30, 35 and 40 weeks, the urine production was 6.9, 9.6, 19.0, 32.6 and 47.4 ml/hour (Table 5). For each gestational age, the HFUPR varied because of measurement errors (SD_{HFUPR}) and biological variations.

Table 5 The pooled HFUPR material was based on two previous studies, in which the bladder volumes were estimated according to the traditional method. The mean of the HFUPR, as well as the 2.5th, 10th and 25th percentile points, were calculated.

Gestational age (weeks)	*HFUPR Mean (mL/hour)*	*HFUPR 25th percentile*	*HFUPR 10th percentile*	*HFUPR 25th percentile*
22	6.86	5.19	3.67	1.98
23	7.76	5.87	4.15	2.24
24	8.66	6.55	4.63	2.50
25	9.55	7.23	5.11	2.75
26	10.45	7.91	5.58	3.01
27	11.35	8.59	6.06	3.27
28	13.89	10.51	7.42	4.01
29	16.44	12.44	8.78	4.74
30	18.98	14.36	10.14	5.47
31	21.53	16.29	11.51	6.21
32	24.07	18.22	12.87	6.94
33	26.62	20.14	14.23	7.68
34	29.58	22.38	15.81	8.53
35	32.55	24.63	17.40	9.39
36	35.51	26.87	18.98	10.24
37	38.48	29.11	20.57	11.10
38	41.44	31.36	22.15	11.95
39	44.41	33.60	23.73	12.81
40	47.37	35.85	25.32	13.66

Table 6 Although the conventional method of volume estimation was used, the biological variation accounted for 51-86% for fetuses aged 24 to 40 weeks, while the measurement errors accounted for the remaining part of the total variation.

Gestational age (week)	*HFUPR mean (mL/hour)*	*SD included (mL/hour)*	*SD excluded (mL/hour)*	*Biological variation (percentage)*
22	6.86	2.50	1.50	36
24	8.66	3.14	2.24	51
26	10.45	3.79	2.94	60
28	13.89	5.04	4.21	70
30	18.98	6.89	6.02	76
32	24.07	8.74	7.81	80
34	29.58	10.74	9.73	82
36	35.51	12.89	11.80	84
38	41.44	15.05	13.85	85
40	47.37	17.20	15.91	86

Discussion

The main findings

The hourly fetal urine production rate (HFUPR) was estimated, according to the traditional volume estimation method, for fetuses with gestational ages of 16 to 40 weeks. The HFUPR ranged from 4 to 52 mL, which is in accordance with other studies[14, 15].

It was also found that the relationship between bladder diameters in the transverse section was 0.96 and we therefore assumed that the bladder configuration during the filling phase was circular in the transverse section.

The third finding was that the dominant part of the volume measurement error was caused by the selection of bladder image. The total volume measurement error (including image selection) was therefore $SD_{\text{"total error"}}$: 17.3 to 10.9 % for bladder volumes of 5 to 40 mL. The measurement error for selected images (excluding image selection) accounted for a minor part of the total measurement error, $SD_{\text{"frozen error"}}$: 3.7 to 2.0 % for volumes of 5 to 40 mL.

A computer model of a three-dimensional (3D) ellipsoid configuration (mimicking the filled urinary bladder) and an ultrasound plane was created by two of the authors. In this model, it was possible to display images resulting from changes in bladder dimensions and transducer position, Paper II (www.ptech.se-mf). A special movement of the ultrasound transducer, the "rocking" motion, was used as a way of checking whether or not the longitudinal bladder images were sectioned appropriately. The selected bladder images were digitised before the bladder diameters were estimated on the computer screen.

The volume measurement error was significantly reduced when using the modified method to estimate bladder volume, SD "$\text{total error}_{\text{straight sequence}}$" was 12.9 to 5.5% for volumes of 5 to 40 mL in comparison with the $SD_{\text{"total error"}}$ in Paper I (17.3 to 10.9%).

The fetal urine production rate during the examination time of one minute was found to have only a small effect on the results and it can therefore be disregarded.

In Paper III, the volume calculation error (i.e. the formula that was used) was evaluated using two formulas for volume calculation: the sum-of-cylinders and the conventional ellipsoid formula. The results in Paper II were compared with the calculated volumes on identical longitudinal bladder images, when using the sum-of-cylinders formula in this study (Paper III). The

SD "total error$_{\text{straight sequence}}$" in this study was 8.8 to 3.5% (p=0.0543), as compared with the corresponding SD in Paper II (12.9 to 5.5%) for volumes of 5 to 40 mL.

In what sense does the new method, presented in Paper III make a difference compared with the conventional method, when estimating the hourly fetal urine production rate (HFUPR)? The measurement error SD_{HFUPR} when estimating the HFUPR was shown to be dependent of the measurement error when calculating the bladder volume SD "total error$_{\text{straight sequence}}$" and the time points for the volume estimations during the filling phase. The SD_{HFUPR} was reduced when comparing the HFUPR, which was estimated using the new rather than the conventional method. This therefore illustrates the significance of our previous studies (Papers I, II and III). Moreover, the SD_{HFUPR} decreased, when the number of determined volumes during the filling phase increased.

The reduction in SD_{HFUPR} consequently led to the detection of smaller real changes in HFUPR. Furthermore, the probability of false readings at the 2.5th percentile point, although the true HFUPR was at the 10th percentile point, was reduced. The SD_{HFUPR} is also generally valid when evaluating increasing volumes, no matter which method is used.

In a material comprising 414 fetuses, the mean ±2 SD in HFUPR were calculated. The 95% tolerance interval (mean ±2 SD) depended on the measurement errors and the biological variation in the fetal ability to produce urine. It was found that the measurement error accounted for a minor part and that the biological variation was therefore dominant. For example, for fetuses of 24 weeks, the measurement error accounted for 49% of the total variation, while the corresponding figure at term was 14% at term.

Appropriate bladder image

The selection of an inappropriate longitudinal bladder image will always result in the underestimation of diameters, area and calculated bladder volume. When it comes to distance measurements, however, the bladder diameters might be both over- and under-estimated.

When repeating volume calculations, there will be a range of estimated volumes, which deviate to a greater or lesser degree from the real volume. In our studies, we used the standard deviation (SD), based on this variation, to characterise the volume measurement error.

Sagittal and coronal bladder areas estimated by 2D ultrasound have been used to calculate volumes[46]. Known incremental volumes of saline solution

were infused into the urinary bladder of eleven dead fetuses in a water bath. However, the formulas for estimating identical volumes were different when using the sagittal vs. coronal bladder areas and this results in differences between the transverse diameter in the sagittal vs. coronal area, which contradicts our assumption that the fetal urinary bladder has a circular transverse shape.

However, in that study, the description of the changing bladder shapes during the filling phase deviated from the findings in studies of live fetuses[47]. The different bladder change sequence during the filling phase could indicate that the bladder shape of dead subjects is not relevant to live fetuses.

We do not know the true transverse bladder shape, but we assumed that is was circular in shape, according to the findings in Paper I. If so, the selection of an inappropriate transverse bladder image will lead to overestimations of transverse diameters and area.

To our knowledge, there are no points of orientation and no methods to test the selected transverse bladder scan. According to the traditional method, the appropriate bladder image displays the largest appearance However, the larger bladder image may be caused by a deviating transverse scan (a more or less elliptical appearance), which will lead to overestimation of the bladder volume. It was demonstrated that the use of transverse scans led to greater bladder volumes than when the simplified ellipsoid formula was used. In our opinion, the transverse bladder could introduce measurement errors which are impossible to determine by 2D ultrasound. For this reason, the transverse bladder scans were not used for our volume calculations.

Blinding of results

The estimated distances of the bladder diameters were displayed on the ultrasound screen, but this part of the screen was covered by opaque tape, to prevent the operator being influenced by the results. The bladder images (which also displayed the measurements) were printed on a video printer for subsequent statistical analysis.

When observing digitised bladder images on the computer screen, the operator placed the pointers on the bladder borders. At the same time, the corresponding co-ordinates were displayed on the screen. This part was covered by opaque tape and the operator was thus blinded to the results, whereas another investigator performed the reading of the co-ordinates.

Complementary information on website

In general, sufficient information must be presented in order to make a new procedure understood and eventually replicated. The results presented here are based on mathematical statistics, statistical derivations and simulations and a knowledge of digital image processing and programming, which may be difficult to understand in detail. However, without the support of experts in mathematics, statistics, data transmission and computer programming, these analyses of measurement errors and the introduction of the new method for estimating the fetal urinary bladder volume would not have been performed. The current studies illustrate the great value of concurrent expert knowledge.

To make these points clear to the reader, we have created a website. This medium offers new tools for illustration. The visitor can, for example, interactively use the computer simulation model mimicking the 2D ultrasound examination of a urinary bladder. Using this simulation model, the resulting reduction in bladder volume was illustrated, when the ultrasound transducer deviated by two, three and four degrees from the appropriate direction. The results were plotted in a diagram, in which the magnitude of the measurement error, SD "freezing error", was also illustrated in Paper II.

The image processing procedure (Paper III) is also illustrated on this website. This medium enables illustrations in different colours and dimensions, which will make it easier for the visitor to understand. The mathematical base for the sum-of-cylinders method is presented, but no interactive model has so far been created.

Previous investigations of volume prediction by ultrasound

Several authors have attempted to validate the ultrasound accuracy of bladder volumes by studying stillborn babies and balloons[10, 46, 44, 45]. When comparing these results with our findings, we refer to the new method described in Paper III (including volume calculation by the sum-of-cylinders method, based on digitised, longitudinal bladder images). The parameter used for the comparison was the volume measurement error SD "total $\text{error}_{\text{straight sequence}}$" and when performing repeated estimations on the identical bladder image SD "frozen $\text{error}_{\text{one image}}$".

In one study, 2D was compared with 3D ultrasound when balloons of different volumes (range 20-490 mL) were examined in a water bath[44]. The SD was not related to bladder volumes but was presented as the average SD of 8.7% and 4.4% for 2D and 3D ultrasound respectively. The results were

compared with our findings of SD "frozen $error_{one\ image}$". Although our study is based on 2D ultrasound, SD "frozen $error_{one\ image}$" = 3.5% for a volume of 40 mL, which is on a par with the finding of 4.4% for 3D in that study.

Moreover, the findings in another 3D ultrasound study of balloons[45], were in accordance with our results. The SDs were 0.48 mL and 2.26 mL for balloons with volumes of 1-10 mL and 25-45 mL, which can be related to our findings of SD = 0.35-0.58 mL for volumes of 2 to 10 mL and SD = 0.86-1.41 mL for volumes of 20 to 40 mL.

In a study of one stillborn baby in a water bath, the bladder was filled with known amounts of water in consecutive steps: 4, 6, 10, 16, 25, 30, 33 and 39 mL[10]. A comparison was then made between the known volumes and the mean and range of three ultrasound estimations of bladder volumes. The SD was calculated for these bladder volumes, based on information relating to the number of three calculations, mean and range. The SD was calculated as 1.1, 0.5, 0.3, 0.3, 0.5, 0.2, 0.8 and 0.2 mL. Our findings relating to SD "frozen $error_{one\ image}$" were 0.2, 0.2, 0.3, 0.4, 0.6 and 0.7 for volumes of 4, 5, 10, 20, 30 and 40 mL; these findings are on a par with the results of that study.

The estimation accuracy of the bladder volumes of eleven dead babies was studied[46]. The subjects (3 neonates, who died within 24 hours, and 8 stillborn infants with gestational ages ranging from 22-40 weeks) were studied in a water bath. Saline solution was infused into the bladder (2 mL increments) and the volumes were estimated by 2D ultrasound. When the ellipsoid formula was used, the estimated volumes were consistently larger compared with the true volume. Based on the results, new formulas were recommended for calculating the volume of the fetal urinary bladder: 0.46323 + 1.39394 x Sagittal Area and 1.2064 + 1.07603 x Coronal Area respectively. The results when using the sagittal area formula were a mean absolute error of approximately 0 mL (95% CI ±4.0 mL/min).

The same group used these eleven subjects to evaluate the accuracy of the estimation of urine production rate[48]. Saline solution was incrementally infused into the urinary bladder to simulate a rate of 1 ml/min. Serial fetal bladder volumes were calculated from ultrasonographic measurements using the ellipsoid formula and the two alternative area formulas. The urine production rate was estimated to be 1.68 mL/hour (instead of 1 mL/hour) when the ellipsoid formula was used. The area formulas were significantly more accurate, giving a value of 0.99 mL/min.

Also in these studies, the use of longitudinal bladder scans (sagittal area and coronal area) led to lower measurement error compared with the traditional use of the ellipsoid formula, which is based on longitudinal but also transverse

bladder scans. Although the accuracy of the measurement of bladder volumes can be improved by modifying formulas, there is still a lack of credibility due to the artificial setting. Bladders from stillborn babies are probably not representative of bladders in living fetuses.

Theoretical implications and possible practical applications

The measurement error when estimating bladder volume, SD "total error$_{\text{straight sequence}}$", provides basic information for calculating measurement error SD_{HFUPR} when estimating the HFUPR, in addition to information about the frequency and time intervals between these estimates.

The presented formula for estimating measurement errors (SD_{HFUPR}) when calculating the urine production rate is generally valid. This means that the formula can still be used using 3D ultrasound, radiology or MRI.

An accurate method for estimating the fetal urine production rate might lead to advances in our understanding of fetal physiology and of the fetal responses to impaired conditions. The varying fetal ability to produce urine accounts for the dominant part of the variation in the urine production rate, according our findings, Paper IV. It should be noted that these findings were based on HFUPR estimates according to the traditional method. Normal HFUPR values which are estimated using the new method will enable the analysis of the urine production rate with even greater accuracy.

Conclusions

1. The biological variation is responsible for the dominant part of the variation in fetal urine production rate.

2. The modification of the method for estimating the volume of the fetal urinary bladder significantly reduced the volume measurement error.

3. Volume calculation using the sum-of-cylinders method is independent of the longitudinal bladder shape when it comes to producing accurate estimations.

4. The reduced volume measurement error also resulted in a reduction in measurement error, when estimating the urine production rate. This in turn means that a small change in the estimated flow can be regarded as a true change in the fetal urine production rate. Moreover, the probability of a false HFUPR reading will be reduced.

ADDENDUM

Paper I

Convolution

1) The frequency function $f(x)$ for the measured volume was calculated by the determining the convolution of the distribution function:

$$\mathrm{Log_e}\left(\frac{4\times\pi}{3}\times \mathrm{Diameter_a}\times \mathrm{Diameter_d}\times \mathrm{Diameter_d}\right) = \mathrm{Log_e}\,(\mathrm{Diameter_a}) +$$

$2\mathrm{Log_e}\,(\mathrm{Diameter_d})$ + constant $\mathrm{Log_e}$SD, the distance measurement errors for each diameter were assumed to be independent and Normally distributed with a mean of 0.

2) Finally, the SD for volume was calculated as $\sqrt{\int x^2 f(x)dx - (\int xf(x)dx)^2}$, where $f(x)$ was the frequency function. According to the results of this study, the distribution functions of the difference between measured and true volume demonstrated a distribution very close to a Normal one. An expression of SD for the measurement error for the volume determination was obtained as a function of the volume, by applying this method of calculation and by using the result for the measurement error of a diameter.

3) The standard deviations were calculated for a set of volumes and a linear function was fitted to the points using linear regression analysis. The following relationship between the standard deviation of the measurement error and the measured volume was therefore produced: SD = 0.097 + 0.0179 × Volume.

The quotient of coefficient regression and correlation

Let X_1 and X_2 denote the two diameters without measurement error. The relationship is assumed to be: $E[\, X_1 \mid X_2 = x_2 \,] = k \times x_2$ and the correlation coefficient is assumed to be 1. Thus k is the quotient between the diameters.

With measurement error we instead consider the two random variables $X_1 = k \times (X_2 + \varepsilon_1)$ and $X_2 + \varepsilon_2$.

The measurement errors ε_1 and ε_2 are mutually independent and independent of X_2. Furthermore, $\mathrm{Var}[\, \varepsilon_1 \,] = \mathrm{Var}[\, \varepsilon_2 \,]$.

If (Z_1, Z_2) has a two-dimensional normal distribution then

$E[\, Z_1 \mid Z_2 = z_2 \,] = m_1 + (z_2 - m_2) \times \rho \times \sigma_1 / \sigma_2$, where ρ is the correlation coefficient and σ_1 and σ_2 are the standard deviations. This is a general rela-

tionship, that can be demonstrated from the definition of a two-dimensional normal distribution. This result will be used below.

From the general relationship we find that the regression coefficient is
$\rho_1 \times k \times (Var[X_2] + Var[\varepsilon_1])^{½} / (Var[X_2] + Var[\varepsilon_1])^{½} = \rho_1 \times k$
We thus have regression coefficient / $\rho_1 = k$, which is the quotient of the diameters without measurement error.

Paper III

Ellipsoid formula – sensitivity to geometric forms

Three forms are used to evaluate effect of non-elliptical forms.
All forms are rotational and fused together with the same surface with the same diameter (=d).

1. Half-sphere fused to a cone (three different heights are shown)
2. Half-sphere fused to a half-ellipsoid
3. Cylinder simulates a super-ellipsoid

d is the diameter of the short axis used in the ellipsoid formula
d/2 is also the radius of the half sphere fused to a cone or half ellipsoid.
h is the height of a cone fused to the a half sphere.
a/2 is the length of half of the half-ellipsoid fused to the half-sphere.

1. Half-sphere fused to cone

The volume of a cone with a base surface with a diameter of d and height h:

$$\text{cone\_volume} := \left(\frac{1}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot h$$

The volume of a half-sphere with radius d/2:

$$\text{half\_sphere} := \frac{1}{2}\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^3$$

The true volume of the combination of a cone and a half-sphere is:

$$\left(\frac{1}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot h + \frac{1}{2}\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^3 \text{ simplify } \rightarrow \frac{1}{12}\cdot\text{pi}\cdot d^2\cdot h + \frac{1}{12}\cdot\text{pi}\cdot d^3$$

A user of the ellipsoid formula will measure a long axis of the length (h+d/2) and the short axis d/2 and will get an estimated volume of:

$$\left(\frac{4}{3}\right)\cdot pi\cdot\left(\frac{d}{2}\right)^2\cdot\frac{\left(h+\frac{d}{2}\right)}{2}\ \text{simplify}\ \rightarrow \frac{1}{12}\cdot pi\cdot d^2\cdot(2\cdot h+d)$$

Thus the resulting ratio between the true volume and the volume estimated by the ellipsoid formula is:

$$\frac{\left(\frac{1}{12}\cdot pi\cdot d^3+\frac{1}{12}\cdot pi\cdot d^2\cdot h\right)}{\left[\frac{1}{12}\cdot pi\cdot d^2\cdot(2\cdot h+d)\right]}\ \text{simplify}\ \rightarrow \frac{h+d}{2\cdot h+d}$$

The following are three examples of cone heights and the resulting ratio between the true an estimated volumes.

If height of the cone is the same as the radius of the half-sphere h=d/2, the ratio becomes:

$$h := \frac{d}{2}$$

$$\frac{d+h}{2\cdot h+d}\ \text{simplify}\ \rightarrow \frac{3}{4}$$ which is an overestimation of 25%

If height of the cone is the same as the diameter d of the half-sphere h=d, the ratio becomes:

$$h := d$$

$$\frac{d+h}{2\cdot h+d}\ \text{simplify}\ \rightarrow \frac{2}{3}$$ which is an overestimation of 33%

If height of the cone is the same as the twice the diameter d of the half-sphere h=2d, the ratio becomes:

$$h := 2d$$

$$\frac{d+h}{2\cdot h+d}\ \text{simplify}\ \rightarrow \frac{3}{5}$$ which is an overestimation of 40%

2. Half-sphere fused to half-ellipsoid

The volume of the half-ellipsoid with height a/2 and with base radius d/2 is:

$$\text{half\_ellipsoid} := \frac{1}{2}\cdot\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot\frac{a}{2}$$

The combined volume of the half-sphere and the half-ellipsoid is:

$$\frac{1}{2}\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^3 + \frac{1}{2}\cdot\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot\frac{a}{2} \text{ simplify } \rightarrow \frac{1}{12}\cdot\text{pi}\cdot d^3 + \frac{1}{12}\cdot\text{pi}\cdot d^2\cdot a$$

A user of the ellipsoid formula will measure a long axis of the length (a/2+d/2) and the short axis d/2 and will get an estimated volume of:

$$\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot\frac{\left(\frac{a}{2}+\frac{d}{2}\right)}{2} \text{ simplify } \rightarrow \frac{1}{12}\cdot\text{pi}\cdot d^2\cdot(a+d)$$

Thus the resulting ratio between the true volume and the volume estimated by the ellipsoid formula is:

$$\frac{\left(\frac{1}{12}\cdot\text{pi}\cdot d^3 + \frac{1}{12}\cdot\text{pi}\cdot d^2\cdot a\right)}{\left[\left(\frac{4}{3}\right)\cdot\text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot\frac{\left(\frac{a}{2}+\frac{d}{2}\right)}{2}\right]} \text{ simplify } \rightarrow 1$$

This means that the estimated volume is equal to the true volume for any body composed of a half-sphere fused with a half-ellipsoid configured as described.

3. Cylinder simulates a superellipsoid

The volume of a cylinder with the length a and the diameter d is:

$$\text{cylinder} := \text{pi}\cdot\left(\frac{d}{2}\right)^2\cdot a$$

A user of the ellipsoid formula will measure a long axis of the length a and the short axis d and will get an estimated volume of:

$$\left(\frac{4}{3}\right)\cdot pi\cdot\left(\frac{d}{2}\right)^2\cdot\left(\frac{a}{2}\right) \text{ simplify } \rightarrow \frac{1}{6}\cdot pi\cdot d^2\cdot a$$

Thus the resulting ratio between the true volume and the volume estimated by the ellipsoid formula is: ,

$$\frac{\left[pi\cdot\left(\frac{d}{2}\right)^2\cdot a\right]}{\left(\frac{1}{6}\cdot pi\cdot d^2\cdot a\right)} \text{ simplify } \rightarrow \frac{3}{2}$$ which is an underestimation of 50%

Paper IV

An example of HFUPR calculation

When estimating the mean HFUPR, a stepwise calculation was performed. The formula: HFUPR = -12.85 + 0.896 · min(gestational age, 27) + 2.545 · max(min(gestational age - 27, 33-27), 0) + 2.965 · max(gestational age - 33, 0).

When estimating the mean HFUPR for a 30-week fetus:

the minimum of gestational age 27 and 30 weeks was used in the first part of the calculation :

-12.85 + 0.896 · min(gestational age, 27).

1) -12.85 + 0.896 · min(gestational age, 27) i.e. this part was:

-12.85 + 0.896 · 27.

The second part was: + 2.545 · max(min(gestational age - 27, 33-27), 0). In this part, two calculations must be performed: firstly, a subtraction between the current gestational age, in this case 30 and 27, and, secondly, a subtraction between 33 and 27. The minimum value of these two subtractions was to be selected and it was 3, because the results were 3 and 6 respectively. The maximum of 3 and 0 was the final choice.

2) + 2.545 · max(min(gestational age - 27, 33-27), 0) i.e. this part was:

+ 2.545 · 3.

The third part was: + 2.965 · max(gestational age - 33, 0). After a subtraction between the current gestational age (30) and 33, the maximum (in this case –3 or 0), 0, was chosen.

3) + 2.965 · max(gestational age - 33, 0) i.e. + 2.965 · 0. The value of this part is zero.
Consequently, the mean HFUPR for a 30-week fetus was then calculated according to the formula: -12.85 + 0.896 · 27 + 2.545 · 3 + 2.965 · 0 = 18.98 per hour.

Measurement error of the hourly fetal urine production rate
The measurement error (SD) when estimating HFUPR (by us called SD $_{HFUPR}$) was calculated on the basis of the volume measurement error for fetal urinary bladders (denoted SD "total error $_{straight\ sequence}$" by us). It was assumed that x_1, x_2, ... are the time points for bladder volume estimations during the filling phase, and Y_1, Y_2 , ... are the estimated volumes. The hourly urine production rate can be calculated as the coefficient of regression:

$$\frac{\sum Y_i \cdot (x_i - \bar{x})}{\sum (x_i - \bar{x})^2}$$

The expected volume is $Y_i = HFUPR \cdot x_i$. The standard deviation of the measurement error for volume is: SD "total error $_{straight\ sequence}$" = $a + b \times Volume$. (reference 6). The volume Y_i at the time point x_1 can be approximated as $Y_i = HFUPR \cdot x_i$ and the corresponding value is for SD "total error $_{straight\ sequence}$" = $a + b \cdot x_i \times HFUPR$. The measurement errors in the different volume estimations were assumed to be independent.

When calculating SD_{HFUPR}, the corresponding variances were used and the variance in HFUPR then equals the variance in $\frac{\sum Y_i \cdot (x_i - \bar{x})}{\sum (x_i - \bar{x})^2}$. The factors $(x_i - \bar{x})$ and $\sum (x_i - \bar{x})^2$ are constants and these factors therefore demonstrate no variability in measurement errors. Only the factor (Y_i) must be taken into consideration and the variance $[SD(Y_i)]^2$ was used. It was then replaced by$(a + b \cdot x_i \times HFUPR)^2$. The variance of the measurement error when estimating HFUPR was then $(SD_{HFUPR})^2 = \frac{\sum (a + b \cdot x_i \cdot HFUPR)^2 \cdot (x_i - \bar{x})^2}{\left[\sum (x_i - \bar{x})^2\right]^2}$

and $SD_{HFUPR} = \dfrac{\left[\sum (a + b \cdot x_i \cdot HFUPR)^2 \cdot (x_i - \bar{x})^2)\right]^{1/2}}{\sum (x_i - \bar{x})^2}$

The quantity of SD_{HFUPR} could be approximated by a linear function as $SD(HFUPR) = c + d \times (HFUPR)$. Assuming varying time points for volume estimations and the method of bladder volume estimation, different values were found for the constants c and d.

Variability in urine production caused by measurement errors and biological variability

It was assumed that the HFUPR excluding the measurement error has a Normal distribution with the mean μ and standard deviation σ and the corresponding random variable X. The measurement error when assessing HFUPR was assumed to be ε_x, with a mean value of 0 and an SD that was dependent on x. We want to estimate σ.

Pooled HFUPR material (mean and SD) for the gestational age of 22 – 40 weeks including the measurement error was used as an illustration (Figure 2) and the corresponding random variable HFUPR is denoted Y and $Y = X + \varepsilon_x$. It was assumed that the mean of HFUPR, μ, when excluding the measurement error, equals the mean value of HFUPR, when including the measurement error. For variances, the following relationships hold true:

$[SD(Y_i)]^2 = E(Y^2) - [E(Y)]^2$ and therefore $E(Y^2) = [E(Y)]^2 + [SD(Y_i)]^2$

The following notations were used:

μ symbolised $HFUPR_{mean}$ = Mean value of the investigated hourly fetal urine production rate.

$SD_{INCLUDED}$ = SD including measurement errors as well as biological variations.

σ symbolised $SD_{EXCLUDED}$ = SD excluding measurement errors, reflecting the biological variation.

c and d = constants, when estimating $SD_{HFUPR} = c + d \times (HFUPR)$

Then the expected squared value of HFUPR is:

$E(Y^2) = (HFUPR_{mean})^2 + (SD_{INCLUDED})^2$.

Thus, $E(Y^2) = E((X + \varepsilon_x)^2) = \sigma^2 + \mu^2 + E(\varepsilon_x^2)$

$\sigma^2 + \mu^2 + E(\varepsilon_x^2) = \sigma^2 + \mu^2 + E((c + d \cdot X)^2) =$

$\sigma^2 + \mu^2 + c^2 + 2 \cdot c \cdot d \cdot \mu + d^2 \cdot (\sigma^2 + \mu^2) = (\sigma^2 + \mu^2) \cdot (1 + d^2) + c^2 + 2 \cdot c \cdot d \cdot \mu$,

and we find that: $\sigma = ((E(Y^2) - (c^2 + 2 \cdot c \cdot d \cdot \mu))/(1 + d^2) - \mu^2)^{½}$

Finally, we get:

$$SD_{EXCLUDED} = \sqrt{\frac{(HFUPR_{mean})^2 + (SD_{INCLUDED})^2 - (c^2 + 2 \times c \times d \times HFUPR_{mean})}{1 + d^2} - (HFUPR_{mean})^2}$$

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