AMNIOTIC PRESSURE IN DISORDERS OF AMNIOTIC FLUID VOLUME

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ABSTRACT

Amniotic pressure (AP) has been assumed to be raised in both oligohydramnios and polyhydramnios, but has not previously been measured. The aims of this thesis were (i) to characterize AP in human pregnancies with normal amniotic fluid volume (ii) to compare with this AP in pregnancies with abnormal amniotic fluid volume and (iii) to investigate the relationship of abnormal AP with the complications of disorders of amniotic fluid volume. AP was measured during invasive procedures by a fluid manometry system attached to a needle positioned within the amniotic cavity.

In pregnancies with normal amniotic fluid volume, AP increased with advancing gestation, but was not related to amniotic fluid volume. In comparison, AP was significantly higher in pregnancies with polyhydramnios and lower in those with oligohydramnios, the degree of abnormality in AP correlating with the severity of derangement in amniotic fluid volume; furthermore, AP returned towards normal with drainage and infusion of fluid respectively.

In polyhydramnios, amniotic pressure was negatively correlated with fetal pO_2 and pH, suggesting that raised AP may impair uteroplacental perfusion. In pregnant sheep however, fetal acid/base status was unaltered when AP was acutely elevated by amnioinfusion.

In human pregnancies complicated by severe oligohydramnios, restoration of amniotic fluid volume did not alter the incidence of fetal breathing movements or Doppler indices of umbilical artery downstream resistance; together with the finding of low AP, these results challenge the concept of fetal compression in oligohydramnios that has become widely accepted in the literature. In order to determine whether lung hypoplasia in oligohydramnios is caused by low amniotic pressure disturbing the tracheal-amniotic pressure gradient, fetal sheep were subjected to chronic pharyngeal drainage at subamniotic pressures, but this had no effect on lung development. That tracheal drainage affects lung development was confirmed, but no evidence for this effect being via a reduction in fetal breathing could be obtained.

It is concluded that amniotic pressure is elevated in polyhydramnios and reduced in oligohydramnios.

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Abbreviations

AFI = amniotic fluid index
AGA= appropriate for gestational age
ANOVA = analysis of variance
AP = amniotic pressure
CI = 95% confidence interval
DNA = de-oxyribonucleic acid
EMG = electromyographic activity
FBM = fetal breathing movements
FHR =fetal heart rate
ID = inner diameter
IUGR = intrauterine growth retardation
OD = outer diameter
PG = prostaglandin
PH = pulmonary hypoplasia
PI = pulsatility index
PPROM = preterm premature rupture of the membranes
RI = resistance index
SD = standard deviation
SE = standard error
SGA = small for gestational age

Units

Means are described statistically by 95% confidence intervals for human data and standard errors for animal data, in accordance with convention.

SI units have been used throughout, with the exception of amniotic pressure, which, like blood pressure, is reported in mm Hg. Partial pressures of blood gases (pCO_2 and $pO2$) are accordingly reported in kPa, with the exception of animal studies, in which mm Hg are used as per convention.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Invasive procedures in the study of human fetal physiology

The study of human fetal physiology has until recently been limited by the relative inaccessibility of the intrauterine environment. Experimental studies in animals have provided much of what is known, although findings in predominantly non-primate species are not always applicable to human fetuses. Studies in human pregnancy have necessarily been indirect, based either on ultrasonic observation of fetal behaviour, or on inferences made from biochemical changes in amniotic fluid.

During the early 1980s, advances in ultrasound resolution, together with increasing experience with invasive procedures in continuing human pregnancies, facilitated the development of ultrasound-guided fetal blood sampling (Daffos et al 1985). The simplicity and relative safety of this procedure soon led to an expansion in its usage and indications. Similar ultrasound-guided needling procedures were applied for sampling fetal skin (Bang 1985), liver (Golbus et al 1988), urinary tract (Glick et al 1985), and body cavities (Benacerraf et al 1986), while a limited number of therapeutic procedures were introduced (Manning et al 1986, Nicolaides et al 1986, Rodeck et al 1988).

Although the amniotic cavity has been accessible for several decades, its study has been largely limited to gestations at which older sampling procedures were performed: 16 weeks for amniocentesis for karyotyping (Steele & Breg 1966) and alpha-fetoprotein determination (Brock & Sutcliffe 1972), the third trimester for amniocentesis for monitoring Rh alloimmunization (Liley 1961) and fetal lung maturity (Gluck et al 1971), and term for intrapartum pressure monitoring via an intrauterine catheter (Turnbull 1957). The advent of modern ultrasound-guided invasive procedures however, recently rendered the amniotic cavity accessible to study over a broader range of gestation. In the second and third trimesters, the amniotic cavity is frequently traversed en route to the sampling site during fetal diagnostic and therapeutic procedures, while amniocentesis for cytogenetic study is now technically possible in the first trimester (Hanson et al 1987). The recent recognition that oligohydramnios (Hackett et al 1987, Gembruch & Hansmann 1988) and polyhydramnios (Landy et al 1987, Carlson et al 1990) are themselves indications for invasive

diagnostic procedures, now permits investigation of the amniotic cavity in pregnancies with abnormal amniotic fluid volume.

The access to the fetal and intrauterine environment afforded by modern invasive procedures has opened up several areas of previously unstudied physiology and medicine. This has facilitated recent studies of fetal haematology, biochemistry, endocrinology, immunology, etc, as reviewed elsewhere (Rodeck & Nicolini 1988, Fisk & Rodeck 1992), and major contributions have been made towards understanding the pathophysiology of alloimmunization and intrauterine growth retardation (IUGR). The pressure in the amniotic fluid surrounding the fetus, although widely studied with reference to uterine contractility and maternal physiology (Csapo 1970), has not been considered from a fetal viewpoint.

1.2 Amniotic pressure

Although amniotic pressure (AP) has been extensively investigated over the last four decades, the emphasis has been almost exclusively on its change with uterine activity. Numerous studies of intrauterine pressure have been performed in labouring women at term via a needle or catheter inserted transabdominally into the amniotic cavity (Wolf 1940, Alvarez & Caldeyro 1950, Caldeyro-Barcia & Alvarez 1952, Hendricks et al 1959, Hendricks et al 1962), or transcervical catheters inserted either extra-amniotically or in patients with ruptured membranes (Williams & Stallworthy 1952, Turnbull 1957, Csapo 1970, Steer et al 1978). AP has also been studied in the first and second trimester prior to or during termination of pregnancy (Bengtsson & Csapo 1962, Wiqvist & Eriksson 1964, Turnbull & Anderson 1965, Csapo 1969). The rationale for these investigations was the characterization of pressure changes during spontaneous and pharmacologically-induced contractions. Resting AP has only been studied as a baseline against which the effects of oxytocic drugs were assessed (Hellman et al 1957, Hendricks & Gabel 1960, Csapo & Sauvage 1968). All these reports present AP readings graphically, with typical scales of 0 to 100-250 mm Hg rendering accurate interpretation of resting AP impossible. Another problem has been the lack of uniform reference point. Although some standardized readings at the maternal umbilicus (Hellman et al 1957), or against maternal intraperitoneal or intravesical pressure (Alvarez & Caldeyro 1950, Caldeyro-Barcia & Alvarez 1952), most

deemed no reference point necessary to calculate changes in AP with contractions.

The pattern of development of resting AP throughout gestation has not yet been characterized. Although a variety of geometric shapes have been used to describe the uterine cavity (Anderson et al 1967), most authors accept that, in order to apply Laplace's law, the uterus may be considered to be a sphere (Reynolds 1946, Coren & Csapo 1963, Csapo 1970), a mathematical simplification of the composite radii, which has no consequence for AP. Pressure within the sphere is thus a function of T/r, where T is the wall tension and r the radius. The initial application of this law to non-living matter held that pressure fell with increasing r, and this was the basis for an assumption that resting AP falls in late gestation (Reynolds 1946). This simple hydrostatic model however is complicated with a musculo-elastic structure like the uterus, by the effects of stretch (increasing r) on T. This in turn is modulated by wall thickness, myometrial cell length, and the effects of pregnancy hormones (Reynolds 1965). Furthermore, distension (implying an increase in pressure) is an important stimulus for uterine growth (Reynolds & Kaminenster 1936) and thus further increase in r. Therefore the development of AP in pregnancy reflects the relative rate of change of T/r. On this basis it has alternatively been suggested that AP rises in late pregnancy with tension increasing at a greater rate than radius (Anderson et al 1967). AP has not been measured systematically in human pregnancy, nor have measurements of T and r been made to allow calculation of AP.

1.3 Amniotic fluid

1.3.i Source and control

The factors determining regulation of amniotic fluid volume in normal pregnancy remain poorly understood. In early pregnancy, amniotic fluid is considered firstly a maternal dialysate, and then a fetal transudate, since its composition closely resembles that of maternal and then fetal serum (Lind 1972). After 20-25 weeks with progressive impermeability of the fetal skin to water and solutes (Parmley & Seeds 1970), amniotic fluid becomes increasingly hypotonic with greater concentrations of urea and creatinine, implicating fetal urine as the major contributor (Lind 1971, Benzie et al 1974). Certainly, failure of fetal urination results

in oligohydramnios in both clinical (Perlman & Levin 1974, Thomas & Smith 1974) and experimental studies (Minei & Suzuki 1976, Harrison et al 1983). Radioisotope studies in both human and ovine near-term fetuses suggest that 550-650 ml/day of urine enters the amniotic cavity (Tomoda et al 1987, Abramovich et al 1978). Extrapolation from ultrasonic studies reporting human fetal urine production rates of up to 50 ml/hour (Rabinowitz et al 1989, Nicolaides et al 1990) suggests a higher figure, but takes no account of the known diurnal variation in fetal urine production (Chamberlain et al 1984a).

Fetal swallowing has long been recognized as a major route of clearance of amniotic fluid. Radio-opaque dye is observed in the fetal intestines within 20 minutes of intra-amniotic injection (McLain 1963). Human fetuses with lesions preventing swallowing often develop polyhydramnios (Moya et al 1960, Pritchard 1966), although the same does not occur in experimental animals (Minei & Suzuki 1976, Wintour et al 1978). However, radio-tracer studies indicate that near-term human fetuses swallow 234-326 ml less per day than they void (Gitlin et al 1972, Abramovich et al 1979), this discrepancy suggesting additional pathways of clearance. The respiratory tract is not a site of net resorption of significant amounts of amniotic fluid (Abramovich 1970); indeed there is a net outflow of lung liquid from the fetal trachea, calculated as a 200-345 ml/24 hours in the late gestation sheep (Harding et al 1984a, Harding et al 1986a), although swallowing may prevent much of this reaching the amniotic cavity (Adams et al 1967, Harding et al 1984b).

The membranes are permeable to water and low molecular weight solutes (Seeds 1970, Abramovich et al 1976), and significant bulk flow occurs between the maternal, fetal and amniotic compartments (Hutchinson et al 1959, Seeds 1980), primarily in response to osmotic and hydrostatic gradients (Battaglia et al 1960, Schruefer et al 1972, Kerenyi & Musnai 1975, Basso et al 1977, Ross et al 1983). Consistent with this is the strong correlation between maternal plasma volume and amniotic fluid volume observed in human pregnancies with both normal and abnormal quantities of amniotic fluid (Goodlin et al 1983). Following intra-amniotic isotonic infusion, Tomoda et al (1987) calculated that bulk flow across the membranes was responsible for more than half the clearance of infused fluid.

Gilbert and Brace (1989) demonstrated in sheep that distilled water infused intra-amniotically was rapidly absorbed into the fetal circulation; this was later followed by a small decrease in maternal osmolality, which was not present in those animals in which the fetus had been killed beforehand. They therefore proposed that the *transmembranous* pathway from the amniotic cavity to the maternal circulation was not a major route for fluid flow, but that the *intramembranous* route from the amniotic cavity via vascularized membranes into the fetal circulation was. In a similar experiment involving dehydrated fetuses, Ross et al (1991) similarly considered that the rapid absorption of intra-amniotically administered saline was via the intramembranous route, since swallowing rates remained depressed. This suggests that alterations in amniotic fluid volume in response to alterations in maternal fluid balance (Bell et al 1984, Stevens & Lumbers 1987) may occur via their effect on fetal fluid balance.

Current evidence points to the fetus having considerable autonomy in fluid homeostasis. In this regard, the fetus is able to decrease its urine production by releasing vasopressin in response to fetal hypovolaemia (Schroeder et al 1984), maternal hypertonicity (Lumbers & Stevens 1983), and maternal water deprivation (Bell et al 1984, Stevens & Lumbers 1985), and increase it in response to hypervolaemia, eliminating water and electrolytes infused on a long-term basis through its kidneys rather than across the placenta (Brace 1989). Fetal swallowing and voiding rates seem tightly coupled within individual studies (Gitlin et al 1972, Abramovich 1970, Tomoda et al 1987), suggesting that, despite our lack of understanding of the factors determining fetal swallowing (Ross et al 1989), their control may be related. Furthermore, fetal swallowing and voiding rates have been shown to increase in concert in response to intra-amniotic and intravascular fluid loads (Gilbert & Brace 1988, Brace 1989).

The relative constancy of amniotic fluid volume at any one stage during pregnancy, in the presence of a high turnover rate and a large number of potential pathways of exchange, indicates remarkable coordination in its control. The literature suggests that such control may be mainly determined by the state of fetal hydration.

1.3.ii Normal volume

Amniotic fluid volume has been measured in normal human

pregnancy, by direct collection at hysterotomy (Wagner & Fuchs 1962, Abramovich 1968), and by a variety of indicator-dye dilution techniques in continuing pregnancies (Marsden & Huntingford 1965, Queenan et al 1972). As the variance in amniotic fluid volume throughout gestation could not be characterized from any single study, Brace et al (1989) recently pooled data on 705 amniotic fluid volumes from 12 publications. Mean amniotic fluid volume increased rapidly to 630 ml at 22 weeks, and then more slowly to a peak of 817 ml at 33 weeks, declining thereafter to 715 ml at 40 weeks. The 95% reference range, derived around a log transformed fourth degree polynomial, indicated a wide range of normal volumes, encompassing values within the range 1/2.57 to 2.57 times the mean volume at any given gestational age (for example 318-2100 ml at 30 weeks).

1.4 Abnormal amniotic fluid volume

1.4.i Measurement

An abnormal amount of amniotic fluid could simply be defined as an amniotic fluid volume outside the reference range (Brace 1989). However, quanititation of amniotic fluid volume is not performed in clinical practice, since it necessitates two amniocenteses with attendant risks (Queenan et al 1972, Kirshon et al 1990), and relies on the dubious supposition that complete mixing of the indicator-dye occurs within a 15-30 minute interval (Charles & Jacoby 1966, Queenan et al 1972). Accordingly, definitions of increased and decreased amniotic fluid volume are based on non-invasive criteria. Clinical assessment is considered inaccurate, while calculation of total intrauterine volume is cumbersome, poorly reproducible, and requires an outmoded static scanner (Gohari et al 1977, Grosman et al 1982). Using real time ultrasound, subjective assessment of amniotic fluid volume as normal, increased, or decreased is in widespread usage. Several semi-quantitative scoring systems have been proposed, based on the dimensions of the largest pocket of amniotic fluid (Crowley 1980, Manning et al 1981). Although their reproducibilty is little better than that of subjective classifications (Halperin et al 1985, Goldstein & Filly 1988) and they make no reference to gestational age (Bottoms et al 1986), these semi-quantitative systems have provided the objective criteria by which abnormalities of amniotic fluid volume have until recently been classified and graded.

1.4.ii Definition

Polyhydramnios has been arbitrarily defined as a deepest vertical pool measuring ≥ 8 cm (Chamberlain et al 1984b, Hill et al 1987), while oligohydramnios has been variously defined as a deepest pool devoid of cord or limbs measuring ≤3, 2, or 1 cm (Crowley 1980, Manning et al 1981, Chamberlain et al 1984c), the more stringent of these indicating moderate to severe oligohydramnios (Hoddick et al 1984). The amniotic fluid index (AFI), the sum of the deepest vertical pool in each of 4 quadrants, was initially developed for use in late pregnancy to increase the sensitivity of detection of oligohydramnios (Phelan et al 1987), but a recent modification allows it to be used throughout pregnancy with good reproducibility (Moore & Cayle 1990). The regression curve between AFI and gestational age is similar in shape to that between amniotic fluid volume and gestational age (Brace 1989), and has been used to derive a reference range for normal AFI from 16-42 weeks (Moore & Cayle 1990). The AFI appears superior to the deepest pool in diagnosis and classification of severity of both oligohydramnios and polyhydramnios (Moore 1990, Carlson et al 1990).

1.5 Polyhydramnios

1.5.i. Prevalence

The prevalence of polyhydramnios varies depending on the diagnostic criteria employed. Older studies, which used the subjective finding of ≥ 2 litres of amniotic fluid at delivery (Moya et al 1960, Queenan & Gadow 1970), report prevalences of 0.25-0.7% (Barry 1958, Murray 1964, Jacoby & Charles 1966, Queenan & Gadow 1970). Ultrasound studies, seem more sensitive, with prevalences of 1.0-3.2% reported using the 8 cm pool definition (Chamberlain et al 1984b, Hill et al 1987). Indeed, Moore et al (1990) found an AFI >95th centile in 6.5% of a high-risk population scanned for various indications. Polyhydramnios complicates 9-13% of twin pregnancies assessed on ultrasound (Hashimoto et al 1984, Schneider et al 1985). As all these figures may be influenced by referral bias, Hill et al (1987) have estimated that polyhydramnios occurs in 0.9% of their non-referred patients, and Hashimoto et al (1984) in 3.6% of non-referred twin pregnancies.

1.5.ii. Aetiology

Congenital anomalies were found in 20-27% of pregnancies with polyhydramnios in older studies (Moya et al 1960, Murray 1964, Jacoby & Charles 1966, Queenan & Gadow 1972) and 9-13% in more recent ultrasonic studies (Zamah et al 1982, Hill et al 1987). The largest category is central nervous system defects, mostly anencephaly, followed by gastrointestinal and musculoskeletal abnormalities (Jacoby & Charles 1966, Queenan & Gadow 1970, Desmedt et al 1990a). Impairment of fetal swallowing has been suggested as the predominant mechanism (Moya et al 1960). Although most anencephalic fetuses do not swallow (Abramovich 1970), only 65% develop polyhydramnios (Nichols & Schrepfer 1966), suggesting that alternative mechanisms may contribute to polyhydramnios such as transudation across meninges, or vasopressin deficiency resulting in fetal polyuria (Naeye et al 1970). The high frequency of polyhydramnios in fetuses with upper gastrointestinal obstruction (Lloyd & Clatworthy 1958), with intrathoracic space-occupying lesions such as diaphragmatic hernia (Adzick et al 1985) and pleural effusions (Rodeck et al 1988), strongly implicates impairment of swallowing in the aetiology of their polyhydramnios. Increased amniotic fluid volume can be similarly explained in fetuses with skeletal dystrophies affecting the thorax, or with neurological deficits such as myotonic dystrophy (Cardwell 1987, Phelan & Martin 1989). Nevertheless, not all fetuses so affected develop polyhydramnios, and oesophageal ligation in animals does not produce chronic polyhydramnios, as mentioned earlier.

Maternal diabetes is the second most commonly identified aetiological factor. The likely explanation for its recent decline in frequency to 5-13% (Hill et al 1987, Desmedt et al 1990a) from 22-26% in older series (Murray 1964, Jacoby & Charles 1966, Queenan & Gadow 1972) is tighter euglycaemic control, since the incidence of polyhydramnios amongst diabetics is least in those with the lowest mean glucose levels (Kitzmiller at al 1978). Although fetal polyuria secondary to an osmotic diuresis might seem an obvious mechanism, Van Otterlo et al (1977) found normal fetal urine production rates in 12 of 13 diabetic pregnancies with mild polyhydramnios. Their measurement technique is now recognised to underestimate hourly fetal urine production rates by approximately 50% (Rabinowitz et al 1989). Nevertheless, using this technique, polyuria has been

demonstrated in a fetus with diabetes insipidus and severe polyhydramnios (Kirshon 1989), and this is presumably also the cause of polyhydramnios in fetuses with Bartter syndrome (Sieck & Ohlsson 1984) or nephrogenic diabetes insipidus secondary to maternal lithium therapy (Krause et al 1990, Ang et al 1990). Similarly recipient fetuses in monochorial twin pregnancies complicated by feto-fetal transfusion syndrome have been shown to be polyuric by Kirshon (1989), who suggested increased cardiac output as the mechanism. Excessive urination as a cause of polyhydramnios is further supported by histological findings of enlarged glomeruli and dilated distal collecting tubules in recipient twins (Naeye et al 1970, Achiron et al 1987). This would also explain polyhydramnios in Rh alloimmunized fetuses and in some cases of hydrops (Phelan & Martin 1989). No evidence of increased urine output was found in two studies of patients with idiopathic polyhydramnios (Abramovich et al 1979, Kirshon 1989).

Sixty per cent of cases are idiopathic (Alexander et al 1982, Hill et al 1987), although this figure varies with the severity of polyhydramnios. Hill et al (1987) identified a cause for polyhydramnios in 91% when the deepest pool exceeded 12 cm, but in only 17% between 8-12 cm. Similarly, among 191 singleton fetuses with polyhydramnios, anomalies were identified in 75% when the polyhydramnios was subjectively classified as severe, as opposed to 29% in the remainder (Barkin et al 1987).

The term acute polyhydramnios has been used to indicate the rapid increase in amniotic fluid volume associated with uterine distension and severe maternal symptoms (Queenan & Gadow 1970, Weir et al 1979), which occurs approximately once in every 4000 pregnancies (Weir et al 1979, Steinberg et al 1990). Although there is some argument about this definition and its application to singleton pregnancies (Queenan & Gadow 1970, Desmedt et al 1990b), most cases occur before 24-26 weeks in one sac of multiple pregnancies as a florid manifestation of feto-fetal transfusion (Queenan & Gadow 1970, Weir et al 1979), a syndrome complicating 4-26% of monochorial twin pregnancies (Galea 1982, Robertson et al 1983).

1.5.iii. Maternal complications

Polyhydramnios may produce symptoms of abdominal discomfort, respiratory embarrassment, and uterine irritability (Cardwell 1987). These can be marked, especially in the presence

of severe or acute polyhydramnios. Preterm delivery occurs more frequently (Barry 1953, Hashimoto et al 1984, Hill et al 1987), although the high incidence of congenital anomalies and multiple pregnancy makes derivation of an exact relative risk for spontaneous preterm labour difficult. In this regard, Hill et al (1987) reported a preterm delivery rate of 22% corrected for congenital anomalies. In many cases, premature rupture of the membranes precedes the onset of preterm labour (Barry 1953, Boylan & Parisi 1986, Cardwell 1987). The incidence of pre-eclampsia is increased, to 17% in singleton pregnancies (Desmedt 1990a) and 21% overall (Kirkinen & Joupilla 1978). Similarly there is an increased incidence of postpartum haemorrhage, attributed to uterine atony (Cardwell 1987). Gross enlargement of the uterus in polyhydramnios may rarely result in ureteric obstruction (Seeds et al 1984, Vintzileos et al 1985a), which can be relieved by restoration of normal amniotic fluid volume (Seeds et al 1984). The above maternal complications are similar to those encountered in high-order multiple pregnancies, and many authors have attributed them to uterine distension (Caldeyro-Barcia et al 1957, Powers 1973, Boylan & Parisi 1986, Cardwell 1987, Steinberg et al 1990).

The incidence of caesarean section is also increased in polyhydramnios (Jacoby & Charles 1966, Zamah et al 1982), due largely to unstable fetal lie. A further risk is abruption, which has been associated with rapid decompression of the uterus at amniotomy (Pritchard et al 1970, Green-Thompson 1982).

1.5.iv. Fetal complications

Perinatal mortality rates of 13-29% have been reported in association with polyhydramnios (Moya et al 1960, Queenan & Gadow 1970, Hill et al 1987, Carlson et al 1990), reflecting the high incidence of congenital malformations, preterm labour, and asphyxial complications such as abruption, cord prolapse and placental insufficiency. In a series of 537 singletons with polyhydramnios, perinatal mortality was 61% in the presence of fetal or placental malformations, and 10% in the presence of maternal complications such as diabetes or pre-eclampsia, compared to only 2.4% when neither of these factors was present (Desmedt et al 1990a). Queenan & Gadow (1970) similarly noted a much higher rate in anomalous compared to structurally normal fetuses (87 vs 30%), while Hill et al's rate (1987) fell from 13 to

6% when corrected for lethal congenital anomalies. Perinatal mortality was much higher in singletons with acute as opposed to chronic polyhydramnios in Desmedt et al's review (1990b), although delivery occurred almost 7 weeks earlier in the acute group. Among 112 pregnancies with ultrasonic evidence of polyhydramnios, Carlson et al (1990) found that all 14 perinatal deaths occurred in the 49 pregnancies whose AFI exceeded 24 cm. Thus it appears that 3 main variables influence the chance of perinatal survival in polyhydramnios: presence of congenital anomalies, gestational age at delivery, and severity of polyhydramnios. It is likely that they are interrelated, although the extent to which this is so is not clear from the above studies.

Perinatal mortality exceeds 50% in feto-fetal transfusion syndrome (Pretorius et a 1988, Bebbington & Wittman 1989), when polyhydramnios is present and the diagnosis made in utero (Brown et al 1989). When detected prior to 28 weeks, mortality rates of up to 100% have been reported (Weir et al 1979, Urig 1990), although these have fallen to 56-72% in recent series employing aggressive management (Schneider et al 1985, Urig 1990). Steinberg et al (1990) in reporting only 2 post-neonatal survivors among 26 infants, suggested that acute polyhydramnios complicating feto-fetal transfusion syndrome, accounted for 17% of overall perinatal mortality in twins. Approximately one third of the losses are intrauterine deaths (Steinberg et al 1990), and in this regard fetal hypoxaemia and acidaemia have recently been demonstrated in 4 of 6 affected pregnancies investigated by fetal blood sampling (Fisk et al 1990). The remainder are due to prematurity, and consequently much attention has recently focussed on developing therapies against polyhydramnios.

1.5.v. Treatment

Treatment of polyhydramnios has two aims: firstly relief of maternal symptoms, and secondly prolongation of gestation. As many pregnancies with polyhydramnios are not at risk of either complication, treatment is usually only considered in severe or acute polyhydramnios in the mid or early third-trimester. Polyhydramnios secondary to feto-fetal transfusion syndrome is the most frequent indication.

Since the first description of therapeutic drainage of amniotic fluid (Rivett 1933), numerous case reports have been published attributing relief of maternal symptoms and

prolongation of gestation to this procedure in both singleton (Queenan & Gadow 1970) and twin pregnancies (Erskine 1944, Danziger 1948, Brown 1958, Brandt & Bates 1961, Brown & Macaskill 1961, Feingold et al 1986). Although some authors have found that rapid reaccumulation of amniotic fluid renders this procedure of little benefit (Weiner 1987, Chescheir & Seeds 1988), recent series of polyhydramnios secondary to feto-fetal transfusion syndrome suggest that it may be beneficial in prolonging gestation. In 9 pregnancies each drained a mean of 1300 ml in 1-17 procedures, Schneider et al (1985) observed that the median diagnosis to delivery interval of 43 days was greater than the 10.5 day interval reported in 18 published cases not subjected to drainage. Mahoney et al (1990) noted a significantly improved perinatal survival rate in 8 pregnancies managed by serial amniocentesis compared to those managed conservatively (69 vs 20%), despite similar mean gestational ages at diagnosis. Combining their figures with those in the literature, they confirmed a significant difference in survival between 29 pregnancies managed with, and 48 without, serial amniocenteses (69 vs 16%). The small number of such patients seen in any one centre renders it unlikely that the efficacy of this procedure, known as amnioreduction, or therapeutic or decompression amniocentesis, can be evaluated without a multicentre randomized trial. Notwithstanding this, Elliott et al (1991), in reporting a 79% perinatal survival in 17 pregnancies with feto-fetal transfusion syndrome diagnosed ≤28 weeks, recently noted that serial therapeutic amniocenteses were followed not only by ultrasonic restitution of amniotic fluid volume in the sac with polyhydramnios but also in the sac with oligohydramnios; in addition fetal hydrops resolved in 3 of 5 cases.

There is some controversy over the amount of fluid which should be removed. On the basis of their experience withdrawing 300-1200 ml in two cases, Feingold et al (1986) recommend the amount be "enough to relieve symptoms, but not enough to induce uncontrolled uterine activity". Most authors have been conservative in selecting volumes, in view of concerns regarding precipitation of abruption or preterm labour (Cabrera-Ramirez & Harris 1976). On the other hand Urig et al (1990), who drained a mean of 1826 ml (range 900-5000 ml) in 29 procedures, consider restitution of normal amniotic fluid volume the more important therapeutic goal,

and the same group's promising results in terms of reversal of hydrops in the recipient and oligohydramnios in the donor support their claim (Elliott et al 1991). Certainly patients having contractions at the time of amnioreduction are known to have a greater risk of preterm delivery following the procedure (Caldeyro-Barcia et al 1957), and in this light Urig et al (1990) reported a median procedure to delivery interval of 3.5 days in those in whom amniocentesis was performed after, compared to 80 days in those before, the onset of preterm labour.

Following recognition of decreased urinary flow rates in neonates given indomethacin for pharmacological closure of the ductus arteriosus (Cifuentes et al 1979), maternally administered indomethacin has been shown in human studies to reduce hourly fetal urine production (Kirshon et al 1988) and amniotic fluid volume as measured by para-amino hippuric acid dilution (Kirshon et a 1990). It crosses the placenta freely (Moise et al 1990), and is believed to act by either a renovascular effect (Millard et al 1979) or by reduced prostaglandin E inhibition of antidiuretic hormone (Seyberth et al 1983). In 8 singleton pregnancies with symptomatic polyhydramnios between 21-34 weeks, Cabrol et al (1987) found that indomethacin reduced fundal height, umbilical perimeter and qualitative amniotic fluid volume, all of which increased after cessation of therapy near term. Similarly Mamopoulos et al (1990) reported that the deepest pool measurement fell by almost 50% from a mean of 10.7 cm in 15 symptomatic women treated prior to 32 weeks, all of whom subsequently delivered at term. In 6 twin pregnancies with threatened preterm labour before 32 weeks, Lange et al (1989) reported a coincidental reduction with therapy in deepest pool measurement. This initial clinical experience suggests that indomethacin appears a promising drug for treatment of polyhydramnios, although there remains considerable concern regarding potential side effects of oligohydramnios (Hendricks et al 1990), premature closure of the ductus (Moise et al 1988), and cerebral vasoconstriction in the fetus (Cowan et al 1986). A further disadvantage is that amelioration in amniotic fluid volume is not rapid, one study reporting a median time to achieve normal volume of 12.5 days. Accordingly, one group advocates initial decompression amniocentesis followed by maternal indomethacin (Kirshon et al 1990).

1.5.vi. Amniotic pressure

Almost all authors attribute the complications of polyhydramnios to uterine distension. Implicit in the concept of distension of a sealed container, is a rise in internal pressure. Csapo et al (1963) incrementally increased intrauterine volume in postpartum rabbits, and although there was little initial change in resting pressure, an exponential increase accompanied further increase in volume. There has however been no scientific study of resting amniotic pressure in polyhydramnios. Rivett (1933) in his series of 10 cases treated by amniocentesis observed that the amniotic fluid "did not appear to be under pressure" in that it had to be sucked out with an aspirating syringe. Wieloch (1927) measured AP in 3 cases of polyhydramnios, finding that in 2 it exceeded that in controls by approximately 10 cm H_2O, whereas in the other it was similar to that of the 3 controls. Uranga Imaz & Gascon (1950) found pressures of 22 and 15 cm H_2O in 2 cases of polyhydramnios, but provided no details of measurements in controls. Caldeyro-Barcia et al (1957) in studying uterine contractility in 25 patients with polyhydramnios, reported on resting tonus, defined as the lowest intrauterine-intraperitoneal pressure between contractions. Notwithstanding the difficulties discerning exact details from such observational reports characteristic of the older literature, it appears that some cases of polyhydramnios had normal pressure, while in others it was markedly raised to c.25 mm Hg. However many patients in this latter study appeared to be in labour (Caldeyro-Barcia et al 1957), suggesting that these findings should not simply be extrapolated to reflect resting AP in non-labouring patients with polyhydramnios.

1.6 Oligohydramnios

1.6.i. Prevalence

The literature contains few exact estimates of the prevalence of oligohydramnios, which, as with polyhydramnios, varies with the diagnostic criteria used. Hill et al (1983) found oligohydramnios in 0.4% of 1,408 patients scanned routinely in the early third trimester, using the deepest pool ≤1 cm definition. In contrast, Philipson et al (1983) used a less stringent, qualitative definition to report an incidence of 3.9% in singleton pregnancies with intact membranes at a mean of 32 weeks; although all were referred, the main reason for this was uncertain dates, and thus the authors considered their estimate applicable to hospital

populations. However, the incidence of oligohydramnios is known to be higher in referral populations, reflecting the underlying indication for ultrasound. In this regard, Mercer et al (1984) found 5.5% of 8626 women undergoing indicated scans had a deepest pool ≤1 cm on one or more occasions. Similarly Chamberlain et al (1984c) found the deepest pool measurement to be ≤2 cm in 3.0% of 7562 high risk patients referred for biophysical profiles. The chance of detecting oligohydramnios increases with gestational age, from 0.2% in the second trimester (Barss et al 1984) to 19% at 42 weeks (Crowley et al 1984).

1.6.ii. Aetiology

Premature rupture of the membranes complicates 3-17% of pregnancies (Gunn et al 1970, Grant & Keirse 1989), and was considered the aetiology of oligohydramnios in 27% in Mercer et al's series (1984). The earlier the gestation, the lower is the incidence of prematurely ruptured membranes, with one series reporting a frequency ≤ 25 weeks of only 0.7% (Taylor & Garite 1984). Reports of reaccumulation of amniotic fluid after occlusion of the cervix with a fibrin gel (Baumgarten & Moser 1986), and of fetal urine production being unaltered in the presence of ruptured membranes (Watson et al 1991), support the mechanism of oligohydramnios in amniorrhexis being the obvious physical one of leakage through the membranous defect (Thibeault et al 1985, Nimrod et al 1984). Nevertheless, only 5-44% of patients with prematurely ruptured membranes have ultrasonic evidence of oligohydramnios (Gonik et al 1985, Vintzileos et al 1985b, Robson et al 1990). Amniorrhexis is usually obvious from the history and physical examination, except in a few very early in gestation in whom the small quantities of fluid lost vaginally go unnoticed (Fisk et al 1991). Since the latent period to onset of labour exceeds a week in only 2-5% of those with term (Kappy et al 1979, Kappy et al 1982), and 20-40% with preterm, premature rupture of the membranes (PPROM) (Taylor & Garite 1984, Johnson et al 1981, Moretti & Sibai 1988), oligohydramnios from this cause is usually short-lived.

Oligohydramnios is associated with IUGR (Manning et al 1981, Philipson et al 1983, Chamberlain et al 1984c, Patterson et al 1987). The deepest vertical pool measurement has been shown to be significantly lower throughout the third trimester in small for gestational age (SGA) compared to appropriate for gestational

age (AGA) fetuses (Bottoms et al 1986). Two studies report identical incidences, of 83%, of SGA births being preceded by oligohydramnios (Manning et al 1981, Philipson et al 1983). When oligohydramnios was detected on the last scan before delivery, 40-90% of infants born were SGA, compared to 5-8% when amniotic fluid was assessed as normal (Manning et al 1981, Philipson et al 1983, Chamberlain et al 1984c). Evidence from human studies suggests that oligohydramnios in IUGR is secondary to fetal oliguria. Wladimiroff & Campbell (1974), using intermittent sonographic observations of bladder size, found reduced hourly fetal urine production rates in all 9 fetuses studied with birthweight <5th centile, but these were also reduced in 28% of those ≥10th centile. Using the same method, Kurjak et al (1981) found low urine production rates in 70% of SGA fetuses ≤10th centile. A recent study measuring changes in bladder size at more frequent intervals, found that hourly fetal urine production rates were one standard deviation lower in severely growth retarded fetuses (mean abdominal circumference 5 standard deviations [SD] below mean for gestation) compared to controls (Nicolaides et al 1990). Fetal oliguria in IUGR probably reflects a renovascular response to hypoxia. Acute hypoxia in sheep experiments is known to produce reflex redistribution of blood flow away from viscera and kidneys towards the brain, heart and adrenals (Cohn et al 1974, Peeters et al 1979). Recent Doppler studies confirm that these circulatory adaptive changes are present in human IUGR fetuses (Wladimiroff et al 1987, Ardiuni et al 1987), and appear related to the degree of hypoxaemia (Bilardo et al 1990). Downstream resistance indices are elevated in renal artery Doppler waveforms (Vyas et al 1989, Ardiuni & Rizzo 1991), consistent with fetal lamb data showing a rise in renal vascular resistance and a fall in perfusion in response to hypoxia (Cohen et al 1974, Millard et al 1979). Significant correlations between the pulsatility index in the renal artery and semi-quantitative amniotic fluid volume (Ardiuni & Rizzo 1991) and between the reduction in urine production and the degree of fetal hypoxaemia (Nicolaides et al 1990) further support renal hypoperfusion being the mechanism. The extent to which hypoxia-induced vasopressin release (Robillard et al 1981) also contributes to fetal oliguria is not known.

Amniotic fluid volume declines rapidly after 40 weeks

(Elliott & Inman 1961, Beischer et al 1969). Crowley et al (1984) assessed amniotic fluid volume ultrasonically in 335 singleton pregnancies at ≥42 weeks, to find 19% with a deepest pool measurement ≤3 cm within 4 days of delivery. This figure is similar to the frequency with which postmaturity occurs in post-term pregnancies (Clifford et al 1954, Beischer et al 1969), and oligohydramnios has been significantly associated with other manifestations of the postmaturity syndrome (Crowley et al 1984, Phelan et al 1985, Pearce & McParland 1991). Many authors have attributed postmaturity to uteroplacental insufficiency and thus late onset IUGR (Vorherr 1975, Rayburn et al 1982). The mechanism of oligohydramnios in postmaturity has thus been suggested as being similar to that in other forms of IUGR (Vorherr 1975, Rightmire et al 1987), and in this regard fetal urine production is known to decrease after 40 weeks (Campbell et al 1973, Trimmer et al 1990). Such a renovascular mechanism for oligohydramnios in postmaturity has recently been challenged by the finding of normal renal artery pulsatility indices in post-term pregnancies with oligohydramnios, in contrast to elevated indices in SGA fetuses with oligohydramnios (Arduini & Rizzo 1991).

Pregnancies with oligohydramnios have an increased chance of a major congenital abnormality being present. Seven per cent of 339 pregnancies with oligohydramnios were associated with malformations in one study (Mercer et al 1984), and in another study (Chamberlain et al 1984c), malformations were found in 4% of 223 pregnancies with oligohydramnios compared to 0.5% of 7096 with normal amniotic fluid volume. This figure rises to 26-35% if oligohydramnios is detected in the second trimester (Mercer & Brown 1986, Moore et al 1989a). The frequency of anomalies is also greater if oligohydramnios is severe (52% vs 14% if mild/moderate) (Moore et al 1989a). Nevertheless, only 15% of congenital anomalies occur in association with oligohydramnios (Manchester et al 1988, Rosendahl & Kivinen 1989). Between 33-57% of these will be urinary tract anomalies (Mercer et al 1984, Mercer & Brown 1986, Rosendahl & Kivinen (1989), either bilateral fetal renal pathologies such as agenesis and multicystic/polycystic disease, or lower urinary tract obstruction. Gembruch & Hansmann (1988) detected such urinary abnormalities after amnioinfusion in 20 of 50 pregnancies with severe oligohydramnios. Lack of the urinary contribution to

amniotic fluid volume being the mechanism for oligohydramnios in urinary tract anomalies is supported by experimental animal studies (Minei & Suzuki 1976, Harrison et al 1983), and observation in humans of restoration of amniotic fluid volume in some fetuses with lower urinary tract obstruction after vesico-amniotic shunting (Harrison et al 1982a, Nicolini et al 1987). Some fetuses with non-urinary malformations will also be growth retarded (Nicolaides et al 1990), although in the remainder there is no obvious explanation for oligohydramnios.

The aetiology of oligohydramnios in pregnancies not complicated by IUGR, ruptured membranes or fetal urinary disorders is considered idiopathic. Moore et al (1989a) have shown that oligohydramnios in this group, when assessed qualitatively in a blind fashion by 3 independent observers, is significantly less severe than in the three other aetiological groups. With modern diagnostic techniques including transvaginal ultrasound (Benacceraf 1990) and amnioinfusion (Gembruch & Hansmann 1988), the aetiology remains unexplained in less than 10% (Fisk et al 1991).

1.6.iii. Maternal complications

Oligohydramnios per se does not lead to maternal complications, although its aetiological conditions may do. Premature rupture of the membranes is associated with an increased risk of infectious morbidity (Lanier et al 1965), which is greatest at early gestations and in severe oligohydramnios (Gonik et al 1985, Vintzileos et al 1985b). Series of mid-trimester PPROM managed conservatively yield risks of clinical amnionitis as high as 39-58%, and of endomyometritis of 11-17% (Taylor & Garite 1984, Beydoun & Yasin 1986, Moretti & Sibai 1988). Irrespective of aetiology, the mother has a significantly increased chance of being delivered by caesarean section for fetal distress (Manning et al 1981, Crowley et al 1984, Moberg et al 1984). IUGR and PPROM are both associated with increased risks of spontaneous preterm labour (Perry et al 1976, Taylor & Garite 1984, Johnson et al 1981). Other maternal morbidity may occur secondary to high rates of induction of labour and termination of pregnancy. A 25% incidence of pre-eclampsia in one study of 339 pregnancies with reduced amniotic fluid presumably reflects the underlying cause for IUGR and thus oligohydramnios (Mercer et al 1984).

1.6.iv. Perinatal mortality

Perinatal mortality is significantly increased in oligohydramnios, as a result of the underlying aetiology such as congenital malformations and IUGR, of preterm delivery, and of oligohydramnios-sequelae such as pulmonary hypoplasia (PH). In Manning et al's (1981) study of suspected IUGR, there were, among 29 pregnancies with oligohydramnios, 4 deaths, compared to none in those with normal amniotic fluid volume. Similarly in Chamberlain et al 's (1984c) report of 52 perinatal deaths in 7562 high risk pregnancies, the perinatal mortality rate was significantly higher in those with compared to those without oligohydramnios (18.8 and 0.5% respectively), even after exclusion of the 31 with congenital abnormalities (10.9% and 0.2% respectively). Perinatal mortality seems infrequent in milder degrees of oligohydramnios in the third trimester or post term, indeed no deaths being reported in 151 patients in 2 such series (Philipson et al 1983, Crowley 1984). In contrast, 43 and 88% of fetuses/infants succumbed in two series of 62 and 34 mid-trimester cases respectively of oligohydramnios (Mercer & Brown 1986, Moore et al 1989a). The poor prognosis of mid-trimester oligohydramnios seems even worse if associated with elevated maternal serum alpha-fetoprotein (Dyer et al 1987, Richards et al 1988). The severity of oligohydramnios also influences prognosis, with a mortality rate of 88% in severe compared to 11% in mild/moderate mid-trimester oligohydramnios (Moore et al 1989a). Similarly Gembruch and Hansmann (1988) reported only 3 surviving infants among 50 pregnancies with severe oligohydramnios.

Poor perinatal outcome in premature rupture of the membranes seems largely confined to preterm pregnancies with rupture prior to 29 weeks, in which mortality rates of 37-76% have been reported (Taylor & Garite 1984, Beydoun & Yasin 1986, Bengtson et al 1988, Moretti & Sibai 1988, Major & Kitzmiller 1990). Gestational age at delivery seems the most important variable, with little chance of survival before 24-25 weeks (Beydoun & Yasin 1986, Moretti & Sibai 1988, Major & Kitzmiller 1990). It is therefore not surprising that the vast majority of losses, 58-80% in these series of mid-trimester PPROM, are not stillbirths but neonatal deaths, attributable to respiratory distress syndrome and other complications of prematurity (Taylor & Garite 1984, Beydoun & Yasin 1986, Moretti & Sibai 1988, Major &

Kitzmiller 1990). Some of the deaths classified as stillbirths may also be due to extreme prematurity, although in others underlying causes are identified (Major & Kitzmiller 1990) such as intrauterine infection or abruption, the latter occurring more frequently in PPROM (Nelson et al 1986, Vintzileos et al 1987). The steady improvement in perinatal survival in reported series over the last decade has been attributed to improvements in neonatal intensive care (Major & Kitzmiller 1990). In PPROM pregnancies, perinatal death is significantly more common in association with oligohydramnios than with normal amniotic fluid volume (Vintzileos et al 1985b), although reduced amniotic fluid volume is found more frequently at earlier gestations (Gonik et al 1985). Early gestational age at rupture is also associated with poor outcome (Beydoun & Yasin 1986, Moretti & Sibai 1988). While this association may in part be mediated through gestational age at delivery, early gestation at membrane rupture significantly increases the likelihood of PH, and a recent study of prolonged preterm ruptured membranes noted more deaths in fetuses with, compared to those without, lung hypoplasia (10 of 14 vs 8 of 74 respectively) (Rotschild et al 1990).

1.6.v. Perinatal morbidity

Some of the fetal complications in oligohydramnios may be attributed to reduced amniotic fluid volume, such as PH and skeletal deformities (see next section), while others result from the underlying condition such as IUGR or PPROM. In this latter regard, series of spontaneous membrane rupture in the mid-trimester are characterized by neonatal complications of prematurity, including respiratory distress syndrome in 45-70%, intraventricular haemorrhage in 29-50%, and long term neurological sequelae in 32-71% (Beydoun & Yasin 1986, Moretti & Sibai 1988, Major & Kitzmiller 1990). Proven neonatal sepsis occurs less frequently, in 17-29%, confirming that prematurity rather than infection is the greater cause of perinatal morbidity as well as mortality in PPROM (Moretti & Sibai 1988, Major & Kitzmiller 1990). Vintzileos et al (1985b) have shown in PPROM, that pregnancies with oligohydramnios lead to a higher frequency of proven neonatal sepsis than those with normal amniotic fluid volume (1 of 54 vs 7 of 36 respectively). This difference may in partly be a gestational age effect (Gonik et al 1985), as marked reduction in bacteriostatic amniotic fluid is less likely after 32

weeks when fetal urine output increases and the frequency of chorioamnionitis declines (Russell 1979). Another explanation would be that amniotic fluid volume distinguishes those with definite PPROM and oligohydramnios from "high leaks" with preserved liquor volume, which might have a lower risk of infection due to more minor disruption in membrane integrity (Fisk 1988).

Oligohydramnios is associated with an increased risk of fetal distress and birth asphyxia. In Manning et al's study (1981), 44% of those with oligohydramnios, all associated with IUGR, required abdominal delivery for fetal distress compared to 1 of 86 with normal amniotic fluid volume. In reporting an increased frequency of meconium staining of the liquor and intrapartum fetal heart rate (FHR) decelerations in 339 pregnancies complicated by oligohydramnios, Mercer et al (1984) noted lower Apgar scores in those with severe as opposed to moderate oligohydramnios. In post-term pregnancies, Crowley et al (1984) noted significantly higher incidences of meconium staining (29% vs 2%) and abdominal delivery for fetal distress (11% vs 1%) in those with a deepest pool ≤3 cm compared to those with normal amniotic fluid volume. As IUGR and postmaturity are both known predisposing factors to fetal compromise, it is unclear from the above studies the extent to which these associations reflect the oligohydramnios, rather than the underlying condition. Although Hackett et al (1987) found abnormal aortic Doppler waveforms in 32 fetuses with severe mid-trimester oligohydramnios to be to be confined to those with IUGR, the high frequency of FHR decelerations in patients with ruptured membranes suggests that oligohydramnios per se may adversely affect fetal wellbeing. In this regard, Vintzileos et al (1985b) documented severe FHR decelerations in 58% of 36 patients with premature rupture of the membranes and oligohydramnios compared to 17% of 54 with ruptured membranes and normal amniotic fluid volume. There were similar differences in 1 and 5 minute Apgar scores, although not in cord pH. Moberg et al (1984) in reporting a significantly higher incidence of caesarean section for fetal distress in preterm patients with ruptured as opposed to intact membranes (8% vs 2% respectively), considered that 16 of these 21 PPROM patients with fetal distress had FHR patterns indicative of umbilical cord compression. Variable FHR decelerations in human pregnancy

were first attributed to cord compression by Hon in 1959, who observed them during contractions in a fetus with a prolapsed cord. Cord constriction/compression in a variety of animal species is known to produce a deceleration in FHR (Assali et al 1962, Yeh et al 1975, James et al 1976) although when the fetus is well-oxygenated or the umbilical vein alone occluded, this may be preceded by an acceleration (James et al 1976). Variable FHR decelerations have been described in a majority of human fetuses with abnormal cord position (Goldkrand & Speichinger 1975). The widely accepted concept that variable decelerations in oligohydramnios are the result of umbilical cord compression is supported by work in rhesus monkeys, in which acute drainage of amniotic fluid resulted in variable decelerations which in turn were eliminated by restoration of amniotic fluid volume (Gabbe et al 1976). Similarly in human pregnancy, the frequency of FHR decelerations is known to be increased when the membranes rupture prior to, or early in, labour compared to rupture in the second stage (Martell et al 1976, Schwarz et al 1973, Cibils 1978). Furthermore restoration of amniotic fluid volume has recently been shown to reduce the frequency of FHR deceleration patterns attributed to cord compression. In a randomized study of 96 labouring patients with repetitive variable decelerations, the FHR pattern returned to normal in 51% receiving intrapartum amnioinfusion via an intrauterine pressure catheter compared to 2% in the no-infusion control group (Miyazaki et al 1985). Nageotte et al (1985) randomly allocated 61 patients with PPROM to intrapartum transcervical amnioinfusion or no-infusion groups, to report significantly lower incidences of FHR decelerations in the first and second stage of labour, with higher umbilical venous and arterial cord pHs at delivery. Similarly Strong et al (1990a) randomized 60 women with oligohydramnios from a variety of causes to find significantly lower rates not only of variable decelerations, but also of meconium passage, end-stage bradycardia and operative delivery for fetal distress in the amnioinfusion group. Intrapartum amnioinfusion has been shown to increase quantitative ultrasonic estimates of amniotic fluid volume (Owen et al 1990), in particular in pregnancies with oligohydramnios (Strong et al 1990b). Antepartum amnioinfusion has also been reported to improve fetal condition. Imanaka et al (1989) noted the disappearance of variable decelerations with an

increase in deepest pool measurement following transcervical amnioinfusion in a patient with PPROM. Van den Wjingaard et al (1987) attributed the normalization of a highly resistant umbilical artery waveform after amnioinfusion in a patient with severe oligohydramnios to relief of cord compression, documenting recurrence of the Doppler abnormality with return of oligohydramnios five days later. An alternative explanation to cord compression for these cardiovascular changes might be fetal head compression, which, in fetal lambs, is also followed by bradycardia (O'Brien 1984). In this light, Van den Wjingaard et al (1988) have shown increased peripheral resistance in Doppler studies of the cerebral arteries in human fetuses with oligohydramnios. Although considered to be the result of fetal head compression in that study, these changes can also be induced by excessive transducer pressure (Vyas et al 1990), an unwitting possibility in any study requiring high ultrasonic resolution in the presence of severe oligohydramnios.

1.6.vi. Oligohydramnios-sequelae

Prolonged oligohydramnios is associated with a variety of fetal manifestations including PH, characteristically flattened facies, and postural deformities such as contractures and talipes equinovarus. These phenomena were first considered with bilateral renal agenesis to be part of a specific syndrome (Potter 1946a, Potter 1946b), but reports of their presence in association with reduced amniotic fluid volume from other causes, and their absence in fetuses with bilateral renal agenesis and normal amniotic fluid volume, led to their recognition as oligohydramnios-sequelae (Perlman & Levin 1974, Thomas & Smith 1974, Fantel & Shephard 1975, Perlman et al 1976). Drainage of amniotic fluid in a variety of animal species has been shown to reproduce both the pulmonary (Moessinger et al 1983, Adzick et al 1984, Hislop et al 1984, Moessinger et al 1985, Collins et al 1986, Moessinger et al 1986, King et al 1986, Dickson & Harding 1989) and soft tissue/skeletal abnormalities (Kendrick & Field 1967, DeMyer & Baird 1969, Persaud 1973, Symchych & Winchester 1978). Accordingly many authors have attributed oligohydramnios-sequelae to compression of the fetus by the uterine walls in the absence of amniotic fluid (Gruenwald 1957, Thomas & Smith 1974, Perlman & Levin 1974, Fantel & Shephard 1975, Wigglesworth et al 1977, Nakayama et al 1983, Thibeault et al

1985, Rotschild et al 1990).

PH is a disorder of impaired lung growth, characterized by diminished size, generational branching and vasculature. It is found in 10-15% of perinatal autopsies, the commonest aetiology being oligohydramnios (Wigglesworth & Desai 1982, Knox & Barson 1986). Hypoplastic lungs associated with oligohydramnios show predominantly evidence of delayed growth, as evidenced by reduced lung de-oxyribonucleic acid (DNA) content and elaboration of airspaces (Wigglesworth & Desai 1981), but also have features of arrested respiratory epithelial maturation such as diminished alveolar elastic tissue and phospholipid concentration (Wigglesworth et al 1981). The undersized lungs have normal lobation consistent with PH being due to impaired lung growth rather than a defect in organogenesis. Human and animal studies suggest that the most vulnerable period of lung development to lack of amniotic fluid occurs during the canalicular phase (Nimrod et al 1984, Moessinger et al 1985, Moessinger et al 1986, Rotschild et al 1990), when the lung changes from being a gland-like secretory organ to a network of vascularized epithelial airway spaces capable of gas exchange. In man, the canalicular period extends from 17-26 weeks (Burri 1984), during which time lung DNA, an index of cell number (Enesco & LeBlond 1962), trebles (Wigglesworth & Desai 1981). Consistent with this are the findings in lungs of anuric near-term human fetuses of a reduced number of bronchial generations, suggesting that the pulmonary insult may commence as early as 16 weeks (Hislop et al 1979), and of low lung DNA contents equivalent to those normally found at 20-22 weeks (Wigglesworth & Desai 1981).

In severe pulmonary hypoplasia death occurs at or soon after birth, and the diagnosis based on necropsy demonstration of reduced lung:body weight ratio (Askenazi & Perlman 1979, Wigglesworth & Desai 1981), radial alveolar count (Emery & Mithal 1960, Cooney & Thurlbeck 1982), or lung DNA content (Wigglesworth & Desai 1981). In milder cases infants survive after recovering from respiratory insufficiency characterized by high ventilatory pressures (Thibeault et al 1985, Bhutani et al 1986) and radiographic evidence of reduced lung size (Leonidas et al 1982); in these the diagnosis is necessarily based on less exact clinical criteria.

Clinical studies indicate that the likelihood of PH after

oligohydramnios depends on three variables: gestation at onset, duration and severity of oligohydramnios (Harrison et al 1982a, Nimrod et al 1984, Moore et al 1989a, Rotschild et al 1990). Thus almost all infants with renal agenesis die from respiratory insufficiency (Potter 1946a), having had virtually no amniotic fluid present from 14-16 weeks (Hislop et al 1979, Ratten et al 1973, Moore et al 1989a). The most severe degrees of PH at necropsy are found in fetuses with renal agenesis (Reale & Esterly 1973), suggesting that oligohydramnios due to other renal pathologies such as incomplete lower urinary tract obstruction, may be less severe or occur later in gestation and thus for a shorter duration. In contrast, amniorrhexis is unlikely to result in PH when membrane rupture occurs after the second trimester (Nimrod et al 1984, Rotschild et al 1990). Among 100 pregnancies with PPROM for ≥1 week, Nimrod et al (1984) found that 8 of 9 with PH were ≤25 weeks at membrane rupture, with only 1 of these 9 leaking fluid for ≤5 weeks. Rotschild et al (1990) confirmed gestational age at rupture statistically to be the most important variable determining PH. Even with membrane rupture prior to 26 weeks, the majority of fetuses will not develop pulmonary hypoplasia, which occurred in only 8 of 30 in Nimrod et al's series (1984). Rotschild et al's (1990) regression equation of the probability of PH based on gestational age at rupture, indicates that this risk exceeds 50% only before 19 weeks, declining steeply thereafter. The severity of oligohydramnios also seems important (Thibeault et al 1985, Glick et al 1985), with 9 of 15 of fetuses with severe, compared to 1 of 18 with mild/moderate oligohydramnios, developing PH in one mid-trimester series (Moore et al 1989a). However, at least in patients with ruptured membranes, severity as well as duration of oligohydramnios are related to gestation (Johnson et al 1981, Gonik et al 1985), and in this regard Rotschild et al (1990) used logistic regression analysis on 88 mid-trimester cases of prolonged PPROM to show that severity and duration were not significantly associated with PH after correction for gestational age. These findings should be interpreted with caution, as they were based on only 14 cases of PH, and severity was assessed on retrospective review of films from a single ultrasound examination. In utero decompression of experimentally induced urethral obstruction in fetal lambs produced larger lungs and less respiratory insufficiency than in

non-decompressed controls (Harrison et al 1982b), providing evidence that duration is an important variable in PH. Nevertheless, a small but significant increase in neonatal respiratory morbidity has been reported in some (MRC Working Party 1978, Tabor et al 1986) but not all studies (National Institute of Child Health & Human Development 1976, Hunter 1987) after amniocentesis, consistent with a study in monkeys showing decreased alveolar number after a single amniocentesis (Hislop et al 1984). However, the fact that these changes were also seen when the membranes were punctured with no fluid removed, that single needle puncture in rats causes chronic leakage and oligohydramnios (Moessinger et al 1983), and that up to 2% of amniocenteses in humans are complicated by chronic leakage of fluid (Tabor et al 1986), raises the possibility that any respiratory sequelae which follow a single amniocentesis may be due to chronic oligohydramnios.

Inhibition of fetal breathing movements (FBM) is known to cause PH, both from animal studies (Wigglesworth & Desai 1979, Alcorn et al 1980, Liggins et al 1981, Fewell et al 1981) and from reports of human fetuses with neurological deficit (Cunningham & Stocks 1978, Goldstein & Reid 1980, Dornan et al 1984). Compression by intrathoracic space occupying lesions such as viscera in diaphragmatic hernia (Campanale & Rowland 1955, Pringle et al 1984), pleural effusions (Castillo et al 1987), tumours (Reece et al 1987), or balloon inflation in experimental studies (Harrison et al 1980), is also known to impair fetal lung growth. As discussed earlier, the fetus is widely held to be compressed in prolonged oligohydramnios, and thus extrathoracic compression has been assumed as the mechanism for oligohydramnios-related PH (Thomas & Smith 1974, Fantel & Shephard 1975, Nakayama et al 1983, Thibeault et al 1985, King et al 1986). Normally, the upper airways produce resistance to the egress of lung liquid, creating a standing tracheal pressure of 1.5-3.0 mm Hg above amniotic pressure (Vilos & Liggins 1982, Fewell & Johnson 1983, Moessinger et al 1985). As lung development is impaired when this resistance is bypassed in tracheostomy experiments (Alcorn et al 1977, Fewell et al 1983), it has been suggested that lung liquid acts as an internal stent around which the lung grows (Alcorn et al 1977, Adzick et al 1984). If fetal lungs are compressed in oligohydramnios, this might be expected to facilitate lung liquid

escape. Loss of lung liquid from the airways into the amniotic cavity is implicated in oligohydramnios-related PH by the following: (i) lung volume is reduced in chronic oligohydramnios (Dickson & Harding 1989), (ii) tracheal ligation in experimental studies prevents the adverse pulmonary effects of oligohydramnios (Adzick et al 1984), and (iii) human fetuses with renal agenesis do not develop PH if laryngeal atresia is also present, as in Fraser syndrome (Wigglesworth et al 1987, Scurry et al 1989). Extrathoracic compression in oligohydramnios might also produce PH by inhibiting fetal breathing movements (Gruenwald 1957, Wigglesworth et al 1977). In this regard, Blott et al (1987, 1990, Blott & Greenough 1988) recently noted in oligohydramnios secondary to preterm premature rupture of the membranes that FBM were absent in fetuses subsequently shown to have PH, and present in those which survived the neonatal period. These findings were directly contradicted by another group who found the incidence of FBM in fetuses with oligohydramnios and PH to be the same as in controls, and greater than those with oligohydramnios and no PH (Fox & Moessinger 1985, Moessinger et al 1987). This disparity has been suggested as simply due to different definitions of FBM (Greenough et al 1988). Whether FBM are inhibited in oligohydramnios remains controversial. In animals, the incidence of FBM is reported to be unaltered by either acute (Moessinger et al 1985) or chronic (Harding et al 1990) oligohydramnios. In humans, numerous cases have been reported of FBM continuing in the presence of oligohydramnios-related PH (Kilbride et al 1988, Van Eyck et al 1990). Even if FBM are inhibited in oligohydramnios, the fact that experimental inhibition of FBM in addition to oligohydramnios produces more severe PH than oligohydramnios alone, suggests that the mechanism for oligohydramnios-related PH is not simply a lack of FBM (Adzick et al 1984).

Lack of understanding of the pathogenesis of oligohydramnios-related PH, hampers the development of preventative/therapeutic strategies against PH other than simple restitution of amniotic fluid volume. In this regard, there are currently no controlled data to support the use in human oligohydramnios of the novel practices of serial amnioinfusion (Imanaka et al 1989, Fisk et al 1991) or vesico-amniotic shunting (Manning et al 1985), although in animal studies, restoration of

amniotic fluid volume in fetuses with bladder outlet obstruction has been shown to have significant beneficial effects on lung development (Harrison et al 1982b, Nakayama et al 1983).

The soft tissue/skeletal deformities found after oligohydramnios include chin recession, a flattened nose, a prominent inner canthal fold, aberrant folding of the ears, spade-like hands, flexion contractures of the knees, elbows and feet, and talipes equinovarus (Potter et al 1946b, Thomas et al 1974). Their relationship to oligohydramnios seems different from that of PH. In pregnancies complicated by PPROM, Thibeault et al (1985) found deformities largely confined to those with severe PH, while in contrast two other studies noted that 33-50% of deformities occurred in fetuses without evidence of PH. Applying multiple regression analysis to 18 cases of deformities among 88 infants born after mid-trimester PPROM, Rotschild et al (1990) showed that the presence of deformities, in contrast to PH, was significantly related to the severity and duration of oligohydramnios, but not to gestation at membrane rupture. Severe degrees of deformity however, defined as Potter facies with limb abnormalities, were only found in association with PH.

1.6.vii. Amniotic pressure

The complications of oligohydramnios have been widely attributed to compression of the fetus and umbilical cord. Implicit in this concept is a rise in amniotic pressure. There has however been no study in either animals or man, of amniotic pressure in pregnancies complicated by oligohydramnios.

1.7 Aims

As outlined in this introduction, it has been previously been speculated that AP is raised in both polyhydramnios and oligohydramnios. This is in contrast to the theoretical situation within an inelastic container, where one would expect a rise in pressure with increase in volume, and fall in pressure with decrease in volume. As discussed earlier, this simple hydrostatic model is not necessarily applicable to AP within a musculo-elastic structure like the uterus.

The aims of this work were:

i. to measure amniotic pressure (AP) throughout gestation in normal human pregnancy
ii. to determine the clinical variables influencing AP
iii. to compare AP in pregnancies complicated by abnormal amniotic fluid volume with that in pregnancies with normal amniotic fluid volume
iv. to determine in pregnancies with abnormal amniotic fluid volumes the effects on AP of restoration of amniotic fluid volume
v. to investigate the relationship between raised AP and fetal blood gas status in human pregnancy, and to determine experimentally in sheep the effect of raising AP on fetal blood gas status
vi. to investigate the presumed compressive effects of oligohydramnios by restoring amniotic fluid volume
vii. to examine in fetal sheep the contribution of low AP in oligohydramnios to impairment of lung development.

Although this was predominantly a pathophysiological study of human pregnancy, animal models were used where it was not possible for ethical and practical reasons to test in humans the hypotheses generated above. The work is presented in logical order, which is not necessarily the chronological order in which it was undertaken.

CHAPTER 2: MANOMETRIC TECHNIQUE IN HUMAN PREGNANCY

2.1 Method

2.1.i. Apparatus

Amniotic pressure was measured during invasive procedures by means of a fluid-filled line attached at one end to a needle positioned within the amniotic cavity, and at the other to a silicon strain gauge transducer. Needles were 20G and either 10 or 15 cm in length (Vygon, Ecouen France). After removal of the stylet, the hub was connected via a Luer lock to 25 cm of polyvinyl tubing, 2.5 mm in inner diameter, and in turn via a 3-way tap to 150 cm of polyvinyl tubing, 1 mm in internal diameter. After connection to the dome of one of two pressure transducers (EM 750, Elcomatic Ltd, Glasgow), the system was filled with normal saline via a syringe attached to the 3-way tap. New sterile equipment was used for each procedure, with the exception of the transducer dome which was sterilized by soaking in aqueous hibitane and then cleansed with sterile saline. After amplification and conversion of pressure readings to analogue direct current signals, the output was displayed both on a meter, and on a chart recorder run at 0.5 cm/sec. Each of these devices had adjustable zero and gain controls. The electrically isolated electronic circuits were powered by a nickel-cadmium battery recharged prior to each recording session; fully charged its operating time exceeds 5 hours.

2.1.ii. Reference point

Amniotic pressure (AP) was defined as the pressure within the amniotic cavity over and above that exerted gravitationally by the height of the amniotic fluid column. Ideally therefore the reference point for zeroing AP should be the top of the amniotic cavity. Using this reference point, the pressure measured by a fluid-filled system will in addition to the pressure due to uterine tone, reflect the sum of the gravitational pressure in the the column of fluid above the needle (shown as Y in Figure 2.1) plus the gravitational pressure in the tubing between the needle and transducer (shown as Z). Thus variation in position of the needle within the amniotic cavity will not alter the pressure recorded at the transducer. Referencing at the top of the amniotic cavity was considered impractical, and potentially inaccurate; this point

would need to be determined ultrasonically, and then somehow translated laterally to reference the transducer. However, as the maternal soft tissue overlying the top of the amniotic cavity comprises both muscle (more dense than water) and fat (less dense), their combination was considered to approximate the density of water. Accordingly the skin surface was chosen as the reference point (Figure 2.1), and considered to be the highest point of a virtual amniotic cavity whose vertical dimension was larger by the overlying soft tissue thickness.

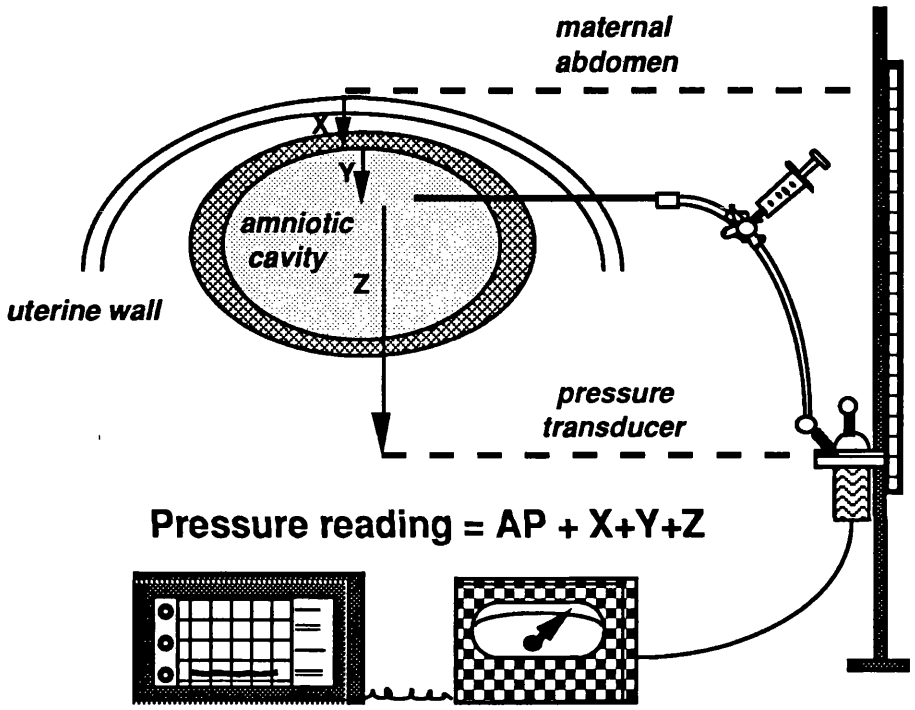

Figure 2.1: Fluid-filled manometry system for AP measurement in human pregnancies undergoing invasive procedures. For ease of interpretation of the components of measured AP, this example shows the transducer positioned beneath the amniotic cavity: the pressure recorded at the level of the transducer equals, in addition to that due due to uterine tone (AP), the sum of the height of the fluid column in the catheter below the needle (Z) and the height of the fluid column within the amniotic cavity above the needle (Y). Given that the density of overlying fat and muscle approximates that of water, fluid pressure at the transducer equals AP+X+Y+Z. When the transducer is elevated on the sliding attachment pole to be level with, and thus referenced to the maternal skin, X+Y+Z=0 and the recorded pressure will equal AP. Referencing the apparatus at the maternal skin surface thus eliminates the gravitational component.

2.1.iii. Recordings

The electrical system and chart recorder were calibrated prior to each procedure by raising a fluid-filled line connected to the manometer vertically in 6.8 cm (corresponding to 5 mm Hg) increments, which had been marked on a ruler fixed to the

attachment pole. Prior to recording, the entire system was again flushed to ensure the absence of bubbles. Using sterile technique, the needle was guided into the amniotic cavity under ultrasound control. After removal of the stylet, the intra-amniotic position of the needle was confirmed by aspirating 0.3 ml of amniotic fluid. The connecting end of the tubing was then positioned at the uppermost level of the skin overlying the uterus, and the vertical position of the pressure transducer altered on its attachment pole to zero the recording. Prior to connection, this end of the tubing was gently squeezed to remove any air trapped within it. Similarly, if amniotic fluid did not emerge spontaneously from the needle hub, gentle pressure on the maternal abdomen was applied so as to prevent introduction of air bubbles during connection. Pressure readings were accepted if stable for 15 seconds; in the presence of a contraction or maternal distress resulting in excessive respiratory activity, recording was abandoned after 30-45 seconds if a stable reading could not be obtained. All readings were made to the nearest 0.5 mm Hg.

2.2 Validation

2.2.ii. Reference point

In order to validate the above assumptions regarding the reference point, pressure recordings were made via multiple needles inserted at different vertical positions into sealed containers. As shown in Figure 2.2, two plastic sealed containers containing 1 litre of normal saline (Viaflex, Baxter, Norfolk) and measuring approximately 26 cm in height were suspended vertically. Ten 21 G needles were than introduced horizontally into each container at a vertical spacing of 1.4 cm (corresponding approximately to 1 mm Hg increments). The hub of each needle was then occluded with a plug, and the manometry system referenced to the fluid level within each container. A further needle was inserted into the top of the container above the fluid level, and the pressure within the container allowed to equilibrate passively with atmospheric pressure. The plugs at the hub of each of the needles (except the 2 at the top) were then removed in turn, and pressure recordings made (n=20). A sphygmomanometry cuff was next placed around the top of each container and inflated to a pressure of 10 mm Hg. Manometry readings were then repeated at each of 20 sites. Readings were similarly obtained after

further cuff inflation to 20, 30, and 40 mm Hg. Results are shown in Table 2.1. Essentially, readings were independent of the position of the needle tip, and all observed pressures were within ± 0.5 mm Hg of expected, which was the limit of resolution of readings on the apparatus.

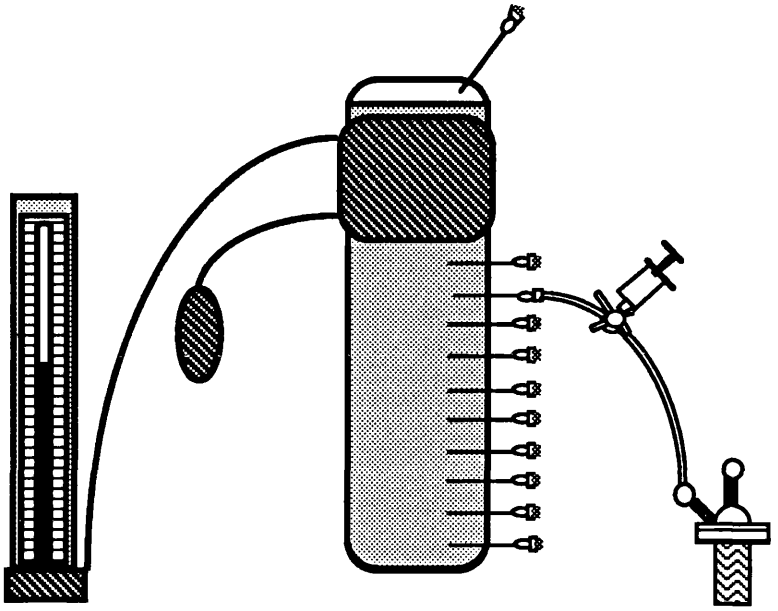

Figure 2.2: System for validation of lack of effect of needle position on measured pressure (not to scale). After allowing the inside of the container to equilibrate with atmospheric pressure, the hub of the top needle was sealed. Pressure readings were made via each needle in turn by removing the plug and connecting the manometry tubing, before resealing each hub. This was repeated with the sphygmomanometry cuff inflated to 10, 20, 30 and 40 mm Hg.

Table 2.1: Range of pressure recordings measured via different needle positions at various baseline pressures as illustrated in Figure 2.1. Inexact readings were those not exactly equal to (i.e. within 0.5 mm Hg of) the cuff pressure.

Cuff pressure (mm Hg)	No. of exact readings (out of 20)	Range of recorded pressures (mm Hg)
0	17	-0.5 to +0.5
10	14	9.5 to 10.5
20	16	20.0 to 20.5
30	13	29.5 to 30.5
40	14	39.5 to 40.5

2.2.ii. Sources of error

Fluid-filled transducers, in contrast to pressure tip transducers, may produce damping if the tubing is of excessive length. This however is only a problem with respect to pulse pressures, and is not considered to affect stable pressures (Rudolph & Heyman 1985). Another disadvantage of fluid-filled systems is the potential for inaccuracy in the presence of bubbles. Accordingly great care was taken to avoid this potential source of error by repeated flushing and visual inspection of both transducer dome and pressure lines.

The calibration of pressure readings in mm Hg was referenced against a fluid column of known height. The conversion factor used (1 mm Hg=1.354 cm H_2O) was based on the reference value for density of mercury measured in atmospheric pressure at 20°C (Kaye & Laby 1973), the approximate ambient temperature under which recordings were made. In order to quantify the degree of error introduced by calibrating with 0.9% sodium chloride instead of distilled water, the specific gravity of normal saline at 20°C was measured with a hygrometer to be 1006. This would account for an error of <1%, which for the range of AP readings reported in the thesis, was well below the resolution of the measurement technique.

A further potential source of error was electrical drift. This was beneath the level of resolution of the apparatus for readings made within 15 minutes of each other, but increased over 2 hours to +0.5 and -2.0 mm Hg for the two transducers used. Accordingly great care was taken to rezero the system before each reading and recalibrate before each new patient. The manufacturers specifications indicate thermal drifts of <1% for both zero and sensitivity.

The reproducibility of the manometry system was ascertained by each of 3 observers making 4 readings each of pressures generated by raising a column of fluid to 2 separate fixed vertical heights (corresponding to 5 and 10 mm Hg) above the same reference point. The apparatus was re-zeroed before each reading. For each set of 4 readings, the column of fluid in the tubing was positioned 8 times, with the observer making the reading unaware as to which were correct (n=4) and incorrect (n=4) positionings. Recordings at each height were identical indicating that for readings made within a short time interval, inter and intraobserver variability was within the resolution of the

apparatus.

2.3 Discussion

The ideal system for measuring AP would involve subtraction of amniotic fluid pressure from surrounding maternal intraperitoneal pressure. Although suitable for animal studies, the additional insertion of a paracentesis needle (Caldeyro et al 1950) in women is now considered unethical. Caldeyro et al (1950, Caldeyro-Barcia & Alvarez 1952, Caldeyro-Barcia et al 1957) circumvented this problem by measuring intravesical pressure via a urethral catheter, considering this to reflect maternal intra-abdominal pressure when intravesical volume was <20 ml. Subtraction of intra-abdominal from intrauterine pressure was deemed important in these older studies of uterine activity during labour, to distinguish contractions from maternal straining and respiratory effort. In the non-labouring patient at rest, it seems unlikely that maternal intra-abdominal pressure comprises a significant component of measurements of stable AP, especially in view of the low APs found in normal human pregnancy (see Chapter 3.3). In a recent report by another group on human AP measurement during invasive procedures (Weiner et al 1989), which appeared after work on this thesis commenced, a similar assumption was made.

Pressure recordings made through a single needle system however, require a reproducible reference point. The accuracy of readings zeroed at the level of anatomical landmarks in the fetus is likely to be impaired by variations in maternal and fetal position, and by changes in uterine and fetal size with gestation. Accordingly a simple referencing system (Figure 2.1) has been adopted in this work, using the top of the maternal abdomen over the uterus as the zero. Fluid-filled rather than pressure-tip catheters were used to remove any error due to the varying position of the needle tip within the amniotic cavity. Although it might be argued that AP as a reflection of uterine tone should be distributed uniformly within the uterine cavity, the gravitational effect of the fluid column on a pressure tip transducer is likely to be substantial in the presence of the small amniotic pressures found in early gestation and in oligohydramnios (see Chapters 3.3 and 6.3 respectively). Fluid-filled systems have been widely used in studies of uterine and fetal physiology in both animals and man.

With the precautions described to minimize potential

sources of error such as electrical drift and bubbles, the reproducibility of measurements obtained in the laboratory with this simple manometry system was within its 0.5 mm Hg limit of resolution. The slightly greater variation in the readings found within the container shown in Figure 2.2, is thus more likely to be a reflection of the accuracy of the sphygmomanometer. It was not possible to test reproducibility in vivo, in view of the ethical difficulties that multiple needle insertions, and/or multiple recordings would have engendered. Furthermore, any change in measured AP in vivo over repeated readings, may have reflected alteration in uterine tone, rather than poor reproducibility.

CHAPTER 3: AMNIOTIC PRESSURE IN PREGNANCIES WITH NORMAL AMNIOTIC FLUID VOLUME

3.1 Aims

This chapter reports the results of measurement of basal AP in human pregnancies with normal amniotic fluid volume, using the technique described in Chapter 2.1. The majority of recordings were made during clinically-indicated invasive procedures in the second and third trimesters. Due to the lack of clinical indication for amniocentesis procedures in the first trimester, recordings at this gestation were instead made on patients undergoing termination of pregnancy.

The aims of this study of human pregnancies with normal amniotic fluid volume were:

i. to characterize AP throughout gestation
ii. to examine the extent to which clinical variables influence AP
iii. to construct a reference range for AP throughout gestation
iv. to determine the effect on AP of removal of small quantities of amniotic fluid, as performed for diagnostic purposes.

3.2 Methods

The study population comprised patients scheduled to undergo a transamniotic invasive procedure, either for diagnostic reasons, or during termination of pregnancy in patients who consented to participation in a institutionally-sanctioned trial of early-pregnancy sampling techniques. Each had (i) certain menstrual dates confirmed by ultrasound either at the routine 18-20 week examination, or at the time of the procedure if prior to this, and (ii) amniotic fluid volume subjectively assessed as normal on ultrasound immediately prior to the procedure. Patients were excluded for any of the following findings on the pre-procedure scan: (i) fetal death (ii) a deepest vertical pool measurement devoid of cord or limbs either <3.0 or >8.0 cm in those ≥17 weeks (iii) a fetal abdominal circumference measurement< 5th centile in those ≥18 weeks (iv) absence of end-diastolic frequencies on pulsed-wave Doppler waveforms (Acuson, Mountain View, California) of the umbilical artery in those ≥18 weeks.

One hundred and ninety four women were enrolled in the study, 186 with singleton, and 8 with twin pregnancies. In

singletons, AP was measured at the time of surgical termination of pregnancy for social indications (≤15 weeks) in 40, at cytogenetic amniocentesis (15-18 weeks) in 42, at fetal skin biopsy (15 weeks) in one, during transamniotic fetal blood sampling (18-38 weeks) in 92 , and at amniocentesis for measurement of optical density (24-33 weeks) in 11 with alloimmunization. The indications for fetal blood sampling were as follows: rapid karyotyping of ultrasonically detected fetal malformations in 42, assessment of fetal haematocrit or platelet count in 41 with alloimmunization, prenatal diagnosis of ß-thalassaemia in 3, and cytogenetic study in 6 presenting too late for amniocentesis, or in whom amniocentesis failed or showed mosaicism. In twins, AP was measured at termination in one, prior to selective fetocide in 3 discordant for fetal abnormality, and at fetal blood sampling in 4 (rapid karyotyping for discordant anomaly in 1, amniotic fluid mosaicism in 1 and maternal age in 2). All gave informed consent to participation in this study approved by the institutional ethics committee.

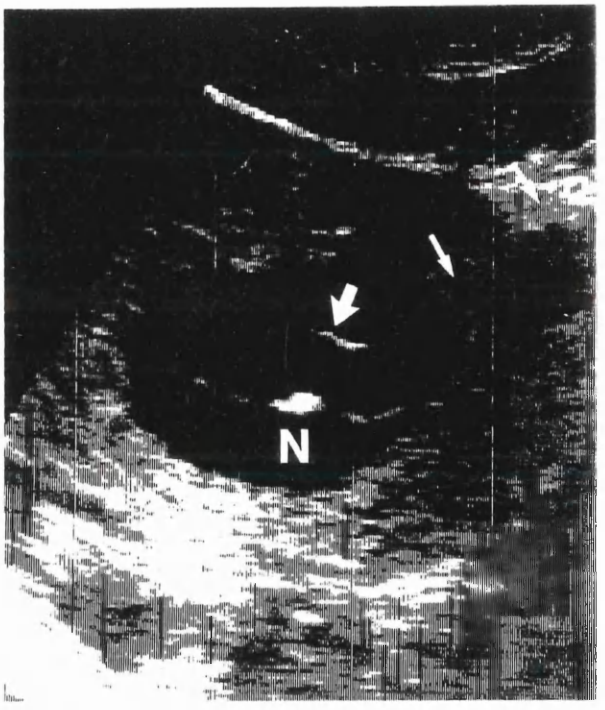

Figure 3.1: Ultrasound picture of 9 week singleton pregnancy showing the tip (N) of the needle (thin arrows) located centrally within the amniotic cavity having pierced the amniotic membrane (thick arrows), rather than within the extra-embryonic coelom.

Parity ranged from 0 to 6 (median 1) using international definitions (i.e. parity=no. of deliveries with birthweight ≥ 500 g or at ≥22 weeks), gravidity from 1 to 8 (median 3), and maternal age from 16 to 43 (mean 30 years). The deepest vertical pool of amniotic fluid was recorded in all patients, and in 128 pregnancies

≥ 10 weeks, the amniotic fluid index was also determined using the modification described by Moore & Cayle (1990) to divide the pre-third trimester uterus into 4 equal quadrants.

All procedures were performed under ultrasound guidance, and pressure measurements obtained on entry of the needle into the amniotic cavity prior to the clinical procedure. In those undergoing termination, cervical priming agents were not used, and transabdominal amniocentesis was performed after induction of general anaesthesia with 1.0-1.5 mg/kg propofolol, 0.5-1.0% isoflurane, and a 2:1 nitrous oxide/oxygen mixture. Sedation was not used in the remainder. As the amniotic membrane does not usually fuse with the chorionic membrane until the early second trimester, care was taken in first trimester pregnancies to visualize the amniotic membrane separately so as to ensure that the needle tip was placed within the amniotic cavity rather than the extra-embryonic coelom (Figure 3.1).

A saline manometry technique was used as described in Chapter 2.1 in all except those undergoing cytogenetic amniocentesis. In these patients a water manometry system was used for two reasons: firstly, the simpler apparatus was considered less intimidating for these often highly anxious women, and secondly these procedures were done at a different location. When amniotic fluid emerged freely from the needle hub, a 25 cm sterile plastic line, 2.5 mm in inner diameter was attached via a Luer lock, and the patient's own amniotic fluid aspirated by means of a 20 ml syringe to a height of 15 cm. The 3-way tap at the distal end was then opened to the atmosphere, and the tubing held vertically against a ruler positioned on the most vertical point of the abdomen overlying the gravid uterus. The reciprocal of the conversion factor in Chapter 2.2 (1/1.354=0.739) was used to convert readings in cm H_2O to mm Hg. As per Chapter 2.2.ii, the specific gravity of amniotic fluid was measured at 37°C in pooled specimens from 10 patients at 16 weeks, which had been stored frozen at -70°C for 1-4 weeks since collection. As with normal saline, the reading of 1003 was very close to that of water, suggesting an error engendered in pressure measurement based on the height of a column of amniotic fluid rather than water of considerably less than 1%, well below the resolution of the measurement technique. Readings made with this system were similarly referenced at the top of the maternal abdomen, as

previously validated and readings made to the nearest 0.5 cm of H_2O.

Measurements were repeated after removal of 20-23 ml of amniotic fluid in 22 patients undergoing cytogenetic amniocentesis. Serial readings were made on 2-4 occasions in 27 patients being monitored for Rh alloimmunization at 1-12 weekly intervals.

After delivery, details of outcome were obtained from the case notes or from the referring hospital. These were not available in 22 lost to follow up or in 14 continuing pregnancies. Procedure related complications were defined as those occurring within a week of the procedure.

A reference range for singleton pregnancies was calculated from a single reading from 171 patients, after exclusion of 7 with unstable recordings and 6 with fetal aneuploidy. Two further pregnancies at 36 and 38 weeks were also excluded in view of the potential for such outliers on the x axis to skew the regression curve. The last reading was used in those with serial recordings in order to optimize scatter over the 3 trimesters. The reference range was derived using standard polynomial regression techniques with least squares analysis. The Shapiro Francia W' test was used to check normality of distribution and determine the optimal transformation required (Royston 1991). A shifted $\log_e$ transformation was required to ensure a normal distribution at each week of gestational age (Royston 1991). After exclusion of heteroscedasticity, the reference range was constructed as 95% data intervals (estimated mean $\pm$ 1.96 x standard deviation [SD]) and confidence intervals (CI) for the upper and lower limits determined.

Relationships were assessed from the data used in the reference range between amniotic pressure expressed both in mm Hg and z scores (no. of SDs from the mean), and other variables using Pearson and Spearman correlation coefficients for normally and non-normally distributed data as appropriate. Statistical comparisons were made by paired or unpaired t -testing as indicated.

3.3 Results

Although AP increased significantly with gestational age ($y=2.8 + 0.11x$ where y=AP in mm Hg and x=gestational age in weeks, $R^2=0.17$, $p<0.001$) in the singleton pregnancies, the

residuals from this equation were not normally distributed (W' test=0.884, $p<0.001$). Log_e/constant transformation corrected this (W' test=0.994, $p>0.9$), and the resulting cubic equation produced a significantly better fit than lower order polynomials ($\log_e(y+1)=0.12 + 0.23x - 0.010x^2 + 0.00015x^3$, $R^2=0.19$, $p<0.001$). There was no significant variation in the standard deviation of the residuals (heteroscedasticity) over gestational age tertiles.

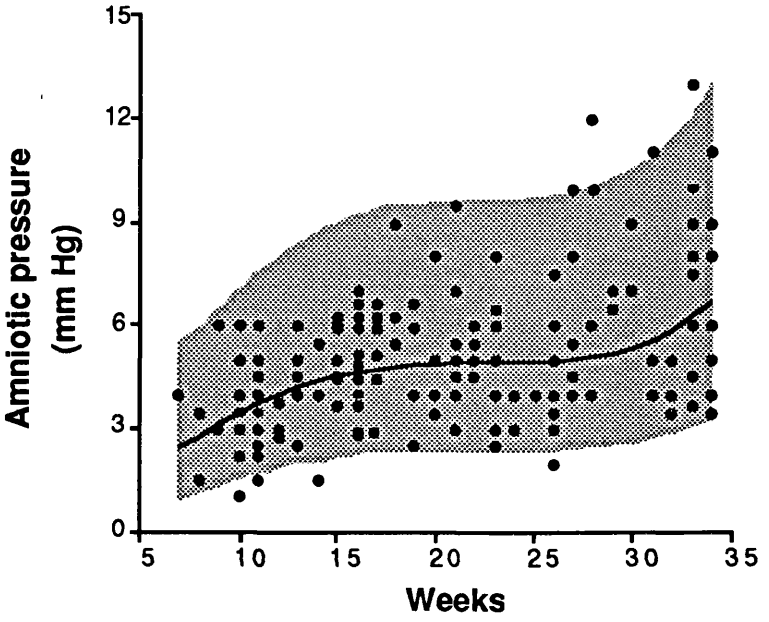

Figure 3.2: Amniotic pressure in 171 singleton pregnancies with normal amniotic fluid volume between 7-34 weeks. The line represents the estimated mean ($\log_e(y+1)=0.12 + 0.23x - 0.010x^2 + 0.00015x^3$, $R^2=0.19$, $p<0.001$ where y=amniotic pressure in mm Hg and x=gestation in weeks) and the shaded area the 95% reference range.

The reference range together with the raw values are shown in Figure 3.2. Eight values (4.7%) lay outside the 95% reference range. At the mean gestational age of 20.5 weeks, the 95% confidence intervals (CIs) of the lower and upper limits of the reference range were 2.1 to 2.5 and 8.9 to 10.5 respectively. Data points for the upper and lower limits of the reference range are shown in Table 3.1.

Among patients with serial readings (Figure 3.3), there was no significant difference between the last AP and the mean of the earlier readings corrected for gestational age (mean difference -0.2 z scores, CI=-0.8 to +0.3, p=0.4). The use of the last rather than first serial AP reading in constructing the reference range was further vindicated by the lack of correlation between serial APs expressed as z scores and the order of reading (Spearman r=-

0.10, p=0.4).

In singleton gestations, there was no significant correlation between AP expressed as z scores and maternal age (Pearson r=0.02), gravidity (Spearman r=0.11) or parity (Spearman r=0.20). There was no significant difference in AP between nulliparous (mean z score +0.2, CI -0.04 to +0.4) and parous women (mean z score 0.0, CI -0.2 to +0.2). Similarly, there was no significant difference between primigravidae (mean z score 0.1 CI -0.2 to +0.4) and multigravidae (mean z score 0.0, CI -0.1 to +0.2). Figure 3.4 shows mean AP as a function of parity. AP was similar in pregnancies with male (mean z score 0.3, CI -0.1 to +0.5) compared to those with female fetuses (mean z score -0.1, CI -0.3 to+ 0.2).

Table 3.I: Upper and lower limits of the reference range for amniotic pressure in mm Hg and cm H_2O between 8-34 weeks.

Weeks	Lower limit		Upper limit	
	mm Hg	cm H_2O	mm Hg	cm H_2O
8	1.1	1.5	6.1	8.2
10	1.5	2.0	7.2	9.7
12	1.8	2.4	8.1	11.0
14	2.0	2.7	8.8	11.9
16	2.2	2.9	9.3	12.6
18	2.2	3.0	9.5	12.9
20	2.3	3.1	9.6	13.1
22	2.3	3.1	9.7	13.1
24	2.3	3.1	9.7	13.1
26	2.3	3.1	9.8	13.3
28	2.4	3.2	10.1	13.6
30	2.5	3.4	10.6	14.3
32	2.8	3.8	11.5	15.6
34	3.2	4.4	13.1	17.7

Although raw AP was correlated with both amniotic fluid index (r=0.24, p=0.01) and the deepest pool (r=0.22, p=0.02),

these relations were not significant when AP was expressed in z scores (p>0.5 for both). Multiple regression of raw AP on gestational age and amniotic fluid index (AFI) or deepest pool confirmed that only gestational age had any significant effect on AP (p=0.01).

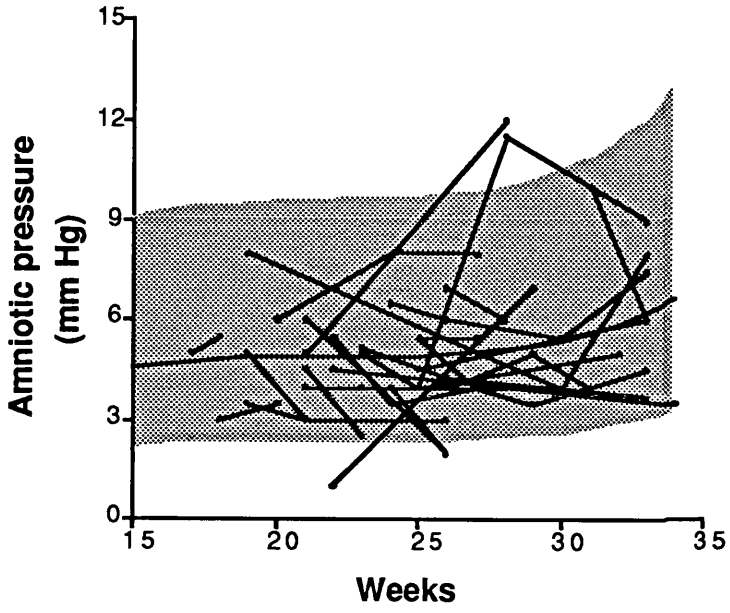

Figure 3.3: Amniotic pressure readings in 27 patients with singleton pregnancies in whom serial readings were made. The shaded area represents the 95% reference range.

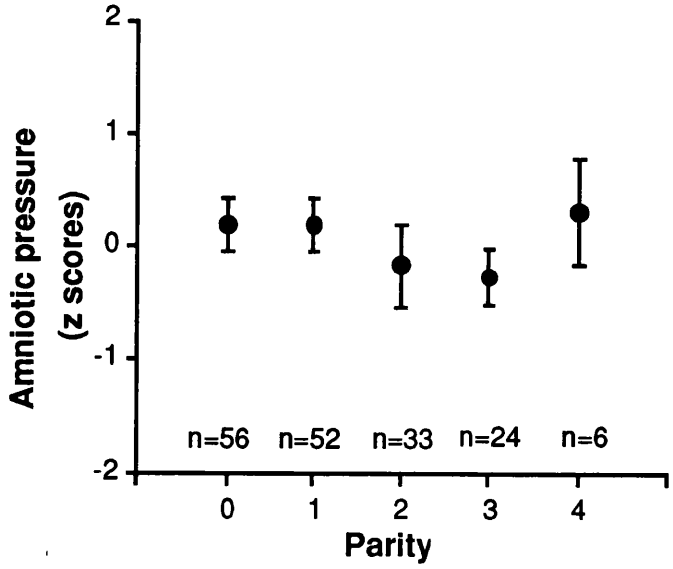

Figure 3.4: Mean and 95% confidence intervals for amniotic pressure z scores (SDs from the mean) in relation to maternal parity.

Amniotic pressure was not significantly elevated in twin pregnancies (mean z score 0.4 CI -0.5 to 1.2, p=0.4), as shown in

Figure 3.5. Although three of the twin pregnancies included fetuses with aneuploidy, this was not felt to affect AP, as AP was not altered by fetal aneuploidy in singleton pregnancies (mean z score 0.7, CI -0.6 to +2.0, p=0.3).

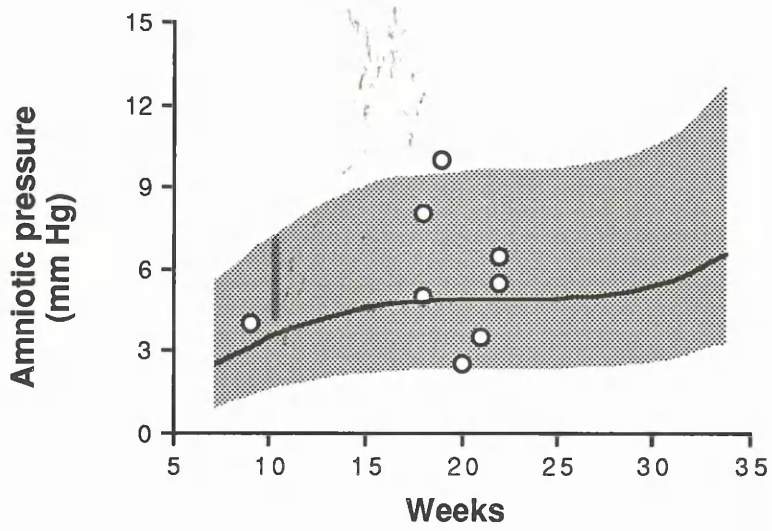

Figure 3.5: Amniotic pressure in 8 twin pregnancies shown against the reference range for singleton pregnancies.

At a median of 16 weeks, acute drainage of fluid (approximately 10-12% of amniotic fluid volume) (Brace & Wolf 1989) did not alter AP (mean Δ AP 0.1 mm Hg, CI -0.3 to +0.6, p=0.6).

After exclusion of procedure related losses, there were no spontaneous abortions but 12 pregnancies were complicated by spontaneous preterm labour leading to delivery. However, 9 of these were patients with Rh alloimmunization, 8 of whom underwent multiple procedures, while the remaining 3 had fetal abnormalities. Their APs (mean 0.2, CI -0.4 to +0.7) were similar to those of patients delivered at term (0.1, CI -0.2 to +0.3, p=0.7).

3.4 Discussion

This study documents the normal changes in basal AP throughout gestation. Although some of the pregnancies studied were not strictly "normal" by virtue of the indication for their invasive procedure, all had ultrasonically normal amniotic fluid volume. Care was taken to ensure stable readings taken during a period of uterine quiescence. On several occasions, the introduction of a needle through the uterine wall was noted to provoke a contraction, while unstable artefactually high readings

were sometimes observed in anxious women who tensed their abdominal muscles. Isoflurane was chosen as the inhalational anaesthetic agent in those undergoing termination rather than the more conventional halothane, as it has significantly less effect on gravid uterine muscle both in-vivo (Tjeuw et al 1986) and in-vitro (Ghaly et al 1988). Although it remains theoretically possible that the anaesthetic agents used may have affected uterine tone, halogenated anaesthetics have only been shown to diminish uterine contractility, not resting tone.

Although serial longitudinal observations are preferred for determining time-specific changes in a variable, the paucity of clinical indications for serial transamniotic invasive procedures necessitated the use of cross-sectional data. In deriving a cross-sectional reference range, it may methodologically be preferable to use the first rather than last value in those with serial observations (Royston 1991); however in this study the lack of difference between the last and earlier readings, and the lack of correlation between AP and the order of reading suggest that this did not materially affect the results. Use of the later gestation readings was necessitated by the relative lack of invasive procedures in third trimester, now that amniocentesis for fetal lung maturity is rarely performed (James et al 1983).

These results for AP are consistent with a preliminary report published since this study was commenced, of AP measured over the last half of pregnancy, showing a linear increase with advancing gestation (Weiner et al 1989). Values obtained in the late third trimester are in accord with older data obtained of basal AP in the mid-trimester (6-10 mm Hg) (Bengsston & Csapo 1962) and in early labour at term (8-12 mm Hg) (Caldeyro-Barcia & Alvarez 1952). However, they are only half as high as those of Weiner et al (1989), who recorded AP after fetal blood sampling, and used a variable reference point, the fetal heart. In contrast, AP was measured in this work prior to other procedures to avoid stimulating contractions, and a fixed reference point used, comparable between patients. There are two components to AP: firstly that due to uterine tension, which according to Pascal's Law is equal and uniform within the uterus (Coren & Csapo 1963), and secondly a gravitational component that varies vertically. AP referenced to a variable point will thus vary with the position of that point within the cavity, as discussed in Chapter 2.1.ii.

After this study was completed, a paper appeared purporting to document amniotic pressure in 200 pregnancies between 10-38 weeks gestation (Sideris & Nicolaides 1990*). However, the methodology involved a pressure-tip transducer attached to the needle hub with measurements referenced to its tip, and consequently measured pressure at the variable position of the needle tip, rather than gravity-independent pressure within the amniotic cavity attributable to uterine tone. Thus it is not surprising that these workers found a decline in pressure with gestation, given that it is likely that an increasingly larger proportion of the needle would be introduced with advancing gestation beneath the reference point used in this thesis.

The characterization of the pattern of AP development with gestation, and the mathematical equation so derived allows comparison of AP measurements in various conditions with those obtained in singleton pregnancies with normal amniotic fluid volume. Such comparisons can be done at differing gestations by expressing AP values from pregnancies of interest as standard deviations around the reference mean. Alternatively, the 95% reference range may be used to determine whether AP readings in pregnancies of interest lie outside what may be considered a normal or reference range. Both these techniques will be used later in evaluating pregnancies with abnormal amniotic fluid volumes and in monitoring pressure changes during amniotic fluid fluid infusion and drainage procedures. The mathematical modelling employed in construction of the reference range correctly resulted in 5% of values being outside the 95% limits. The numbers used were considered sufficient, as the error for the lower limit of the reference range lay within, and that for the upper limit was only 3 times higher than, the minimum resolution of the technique. This range is independent of maternal age, parity and gravidity, and allowing for limited numbers studied, may also be applicable to twin pregnancies.

The lack of influence of amniotic fluid index, which reflects amniotic fluid volume (Moore & Cayle 1990), on AP suggests that intrauterine volume is not a primary determinant of AP. Indeed, the lack of effect of drainage on AP in singletons together with the absence of raised pressure in twin gestations supports this contention. Instead it seems that gestational age is the main factor

* Published June 1991

influencing AP. Furthermore the relationship of AP to increasing gestation is different from that of amniotic volume, which increases to 22 weeks and does not change significantly thereafter (Brace & Wolf 1989). Our results demonstrate that AP rises in late pregnancy, whereas it had previously been assumed to fall, due to a stable uterine radius and thus volume (Reynolds 1965). As discussed in Chapter 1.2, pressure within the uterine cavity is a function of T/r, where T is the uterine wall tension and r the radius. Tension is affected by increasing stretch with uterine growth, and modulated by myometrial thickness and cell length, and the effects of pregnancy hormones. If the development of AP with advancing gestation is assumed to reflect the relative change of T/r, the weakly sigmoid-shaped curve found in this study suggests that P, and thus T relative to r, increases more rapidly in the first and third trimesters than in mid-pregnancy. It was not possible to deduce T, since uterine radius was not measured. However, a planimetric ultrasound study has suggested that uterine radii increase in a near-linear fashion throughout pregnancy (Gohari et al 1977). It is speculated that AP is mediated by gestation-specific anatomical and hormonal influences on gravid uterine musculature.

As uterine distension may be implicated in some causes of preterm labour such as multiple pregnancy and polyhydramnios, AP in pregnancies complicated by spontaneous preterm labour was compared with that of pregnancies progressing to term. The lack of difference suggests that chronic elevation in AP does not precede spontaneous preterm labour, although these findings need to be interpreted with caution in view of the high frequency of multiple procedures and fetal abnormality encountered in this study.

3.5 Summary

Amniotic pressure was measured on 232 occasions in 194 pregnancies with normal amniotic fluid volume undergoing invasive procedures. In singleton pregnancies between 7-34 weeks, AP increased with advancing gestation (R^2=0.19, $p<0.001$, $\log_e(y+1)=0.12 + 0.23x - 0.010x^2 + 0.00015x^3$, where y=AP in mm Hg and x=gestation). However, the sigmoid shaped curve indicated a tendency for AP, unlike volume, to plateau in the mid-trimester. Volume-related phenomena such as twin gestation, the deepest vertical pool or amniotic fluid index did not influence AP.

Furthermore AP was not affected by parity, gravidity, maternal age, fetal sex, or subsequent spontaneous preterm delivery. These findings suggest that AP is not primarily determined by intrauterine volume. It may be speculated that AP, which reflects change in uterine tension as function of radius, is instead determined by gestation-specific anatomical and hormonal influences on gravid uterine musculature. A reference range for AP was constructed.

CHAPTER 4: AMNIOTIC PRESSURE IN POLYHYDRAMNIOS

4.1 Aims

This chapter reports the results of measurement of basal AP in human pregnancies with increased amniotic fluid volume, using the technique described in Chapter 2.1. Recordings were made in the second and third trimesters; their number, frequency and clinical circumstance were necessarily limited by the need for a clinical indication for amniocentesis or other transamniotic procedure.

The aims of this study were:

i. to compare AP in pregnancies with polyhydramnios with AP in those with normal amniotic fluid volume

ii. to examine the extent to which clinical variables influence AP in pregnancies with polyhydramnios

iii. to determine in pregnancies with polyhydramnios the effect on AP of removal of quantities of amniotic fluid, as performed for therapeutic purposes.

4.2 Methods

The study population comprised patients with increased amniotic fluid volume scheduled to undergo a transamniotic invasive procedure, either for diagnostic or therapeutic reasons. Each had (i) certain menstrual dates confirmed by ultrasound at 18-20 weeks, and (ii) amniotic fluid volume subjectively assessed as increased on ultrasound immediately prior to the procedure. Patients were excluded for either of the following findings on the pre-procedure scan: (i) fetal death (ii) a deepest vertical pool measurement devoid of either cord or limbs $\leq$8.0 cm.

Thirty six women between 17-34 weeks were enrolled in the study. Of 27 singletons, 16 were associated with fetal structural anomalies, and 3 with Rh alloimmunization; hydrops was present in 8 and 2 of these respectively. Polyhydramnios in the remaining 7 was unassociated with hydrops, fetal abnormality or alloimmunization. AP was measured at the time of transamniotic fetal blood sampling, the indications being rapid karyotyping alone (n=16), rapid karyotyping plus investigation of hydrops (n=8), and suspected fetal anaemia (n=3). AP was also measured in 7 di-amniotic twin (6 monochorial) and 2 tri-amniotic triplet pregnancies (both monochorial); fetal structural abnormalities

were present in 2, and hydrops in 3. The indications for fetal blood sampling in the 9 multiple pregnancies were rapid karyotyping in all cases in addition to investigation of feto-fetal transfusion syndrome in 7. All gave informed consent in accordance with institutional ethics committee requirements.

The deepest vertical pool of amniotic fluid was recorded in all patients, and in the last 15 cases, the amniotic fluid index (AFI) was also determined. Fetal blood sampling was performed via a transamniotic approach, either because of a posterior or lateral placental cord insertion or because the fetal intrahepatic vein was sampled. All procedures were performed under ultrasound guidance, and pressure measurements made on entry of the needle into the amniotic cavity, as described in Chapter 2. Sedation was not used. In multiple pregnancies, pressure was measured in the sac with polyhydramnios.

In 17 cases, after the initial pressure reading, a quantity of fluid was removed. A 25 cm long portion of polyvinyl tubing, 2.5 mm in inner diameter, was connected to the needle hub at one end, and a 50 ml syringe, which was attached to a three-way tap at the other end, was used serially to remove 100-1100 ml of amniotic fluid. This was done for clinical reasons in order to reduce the risk of uterine activity and/or amniorrhexis; accordingly the volume drained was selected by the operator with regard to minimizing the potential for complications associated with removal of excessive quantities of fluid as discussed in Chapter 1.5.v. After drainage of fluid, the manometry apparatus was flushed to ensure the absence of bubbles, re-referenced and then reconnected to the needle hub and a further reading made.

Serial readings were made on 2-3 occasions in 7 patients at 1-4 weekly intervals, the indication for subsequent procedures being further drainage of fluid (n=6), determination of fetal lung maturity (n=1), and fetal blood sampling for direct administration of antiarrythmic drugs (n=1).

Data on basal AP and the effect of drainage were analysed with respect to the first procedure only. To remove the effect of gestational age, suggested in Chapter 3 to be the primary variable determining AP, comparisons of APs between groups were made using z scores (no. of SDs from the mean). Changes in variables with drainage are expressed as Δ variable (=post-drainage value minus pre-drainage value). Relationships were assessed between

AP and other variables using Pearson and Spearman correlation coefficients for normally and non-normally distributed data as appropriate. Statistical comparisons were made parametrically by paired or unpaired t -testing as indicated, or non parametrically by the Mann-Whitney test.

4.3 Results

As shown in Figure 4.1, amniotic pressure was elevated in polyhydramnios (mean z score 2.7, CI 2.1 to 3.4, $p<0.001$). AP exceeded the upper limit of the reference range in 20 (56%), while in the remainder it lay on (within 0.2 mm Hg), or above, the mean for gestational age in pregnancies with normal amniotic fluid volume.

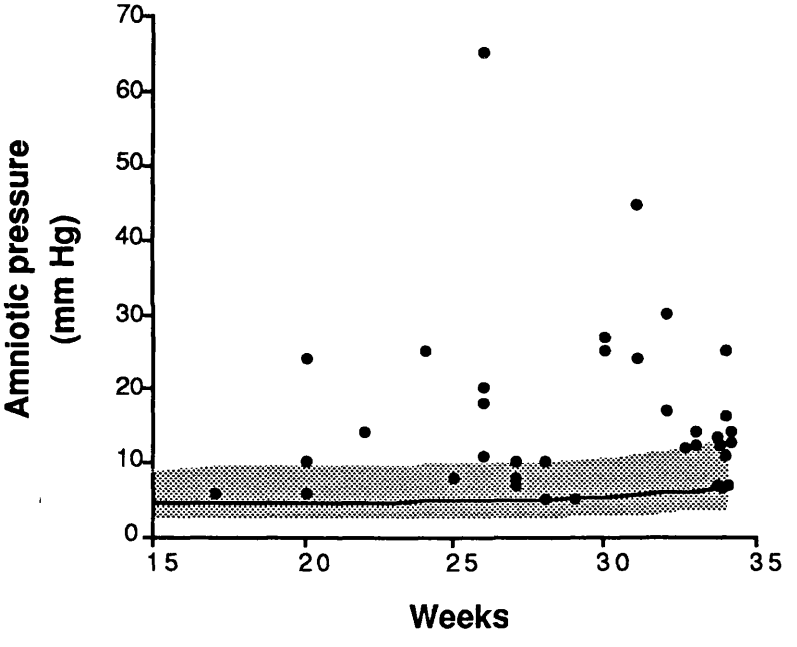

Figure 4.1: Amniotic pressure in 36 pregnancies complicated by polyhydramnios shown against the reference range (=shaded area, continuous line=mean) for pregnancies with normal amniotic fluid volume.

Unlike pregnancies with normal amniotic fluid volume, AP in those with polyhydramnios correlated positively with both the deepest vertical pool measurement ($y=-4.2 + 0.5x$, where y=AP z score and x=deepest pool in cm, $r=0.76$, $p< 0.001$, Figure 4.2) and the AFI ($y=-3.9 + 0.13x$, where y=AP z score and x=amniotic fluid index in cm, $r=0.88$, $p<0.001$, Figure 4.3). These semi-quantitative indices of amniotic fluid volume were both significantly greater in polyhydramnios pregnancies with raised (defined as above the reference range) AP (mean deepest pool 14.6 cm CI 12.7 to 16.4,

and mean AFI 53.2 cm, CI 43.9 to 62.4) compared to polyhydramnios pregnancies with normal (defined as within the reference range) AP (mean deepest pool 11.9 cm, CI=10.9 to 13.0, p=0.02, and mean AFI 35.1 cm, CI=28.5 to 41.6, p=0.007).

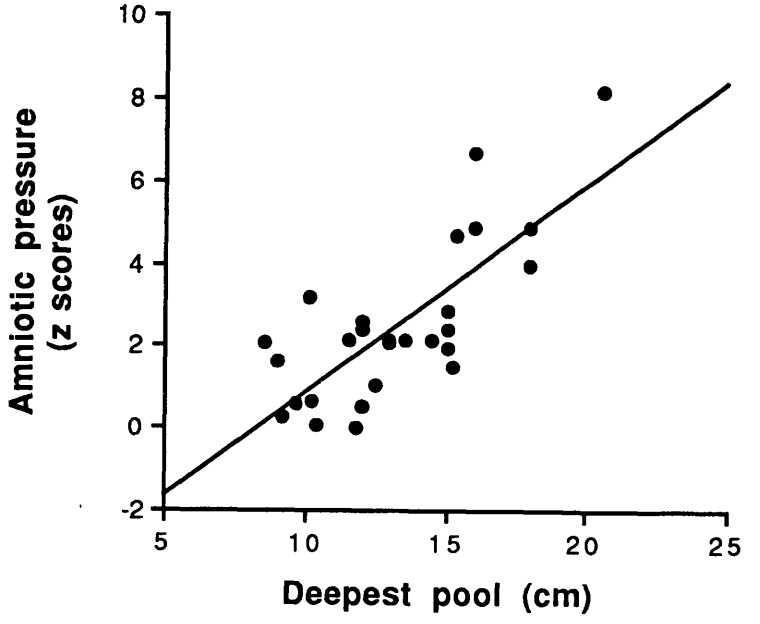

Figure 4.2: The relationship in pregnancies with polyhydramnios between amniotic pressure in z scores and semi-quantitative amniotic fluid volume expressed as the deepest vertical pool measurement. y=-4.2 + 0.5x, where y=AP z score and x=deepest pool in cm, r=0.76, p< 0.001.

In pregnancies with polyhydramnios, there was no significant correlation between gestational age and the degree of elevation in AP (i.e. z scores); furthermore gestational age in those with raised AP was similar to those with normal AP (mean 28.3 weeks, CI 26.3 to 30.3, and 29.8 weeks, CI 27.2 to 32.6, respectively). Although recordings were made significantly earlier in gestation in the multiple compared to singleton pregnancies (mean 24.3 weeks, CI 22.3 to 26.3, and 30.6 weeks CI 28.9 to 32.3, p=0.001), it was thus not surprising that there was no significant difference in AP between multiple and singleton pregnancies (mean z scores 3.8, CI 2.3 to 5.3 and 2.4, CI 1.7 to 3.0 respectively). Similarly, there was no significant correlation between AP z scores and fetal number (=number of fetuses per pregnancy). The deepest pool measurement was similar in singleton (mean 12.7 cm, CI 11.7 to 13.8) and multiple pregnancies (14.7 cm, CI=11.5 to 18.0), as was the AFI (median 3.9 cm, range 2.2 to 5.6 and 5.9 cm, range 3.8 to 7.7 respectively).

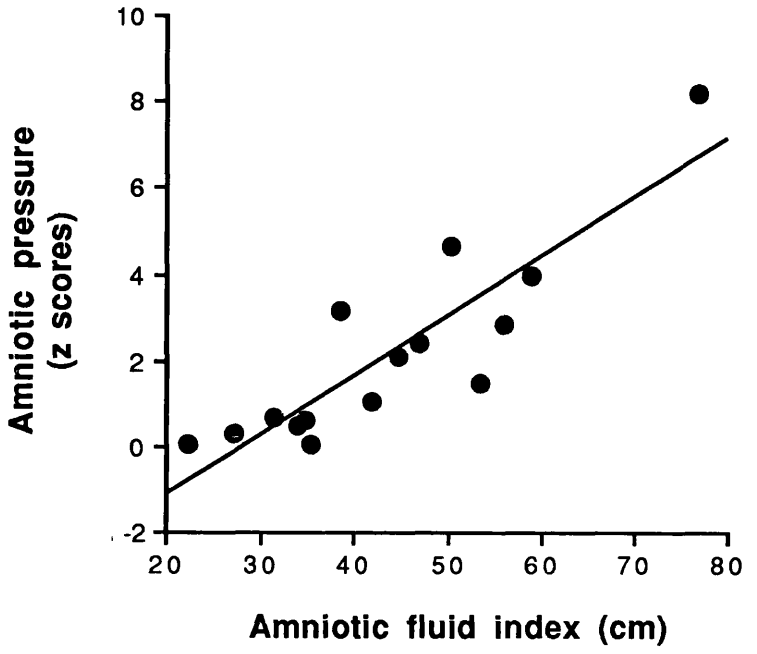

Figure 4.3: The relationship in pregnancies with polyhydramnios between amniotic pressure in z scores and semi-quantitative amniotic fluid volume expressed as the amniotic fluid index. y=-3.9 + 0.13x, where y=AP z score and x=AFI in cm, r=0.88, p< 0.001.

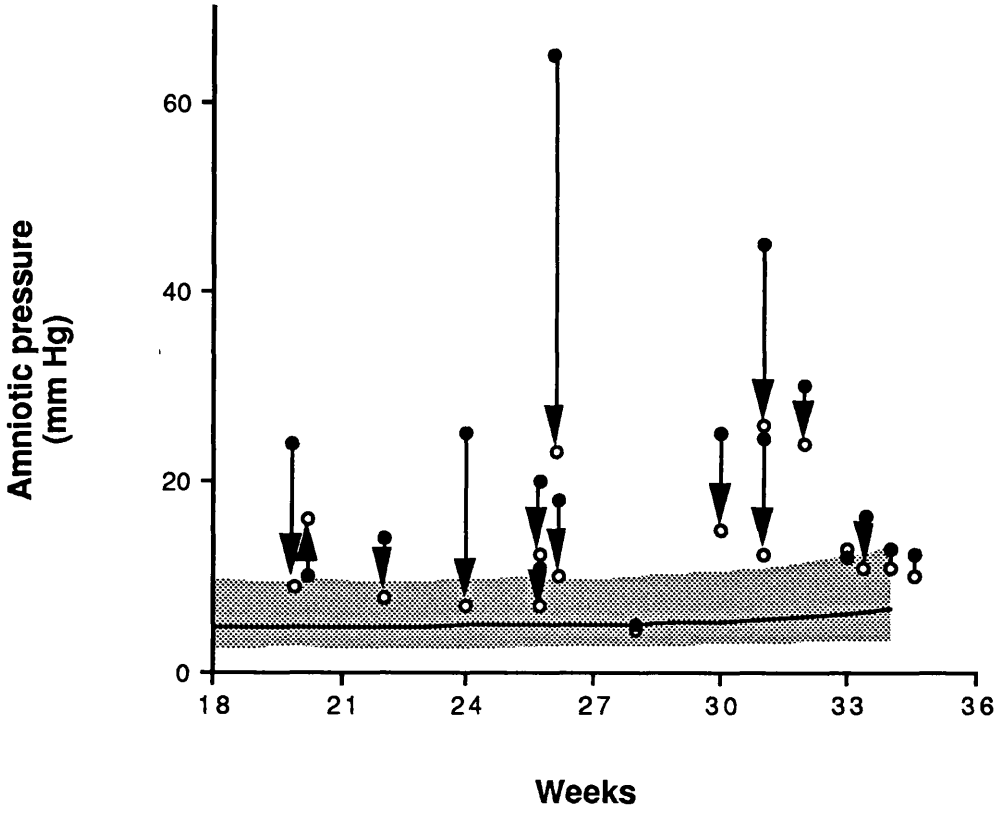

Figure 4.4: The acute effect of drainage of 100-1100 ml of amniotic fluid on amniotic pressure. (filled circles=pre-drainage, open circles=post-drainage, shaded area=reference range).

AP fell significantly with drainage of fluid as shown in Figure 4.4 (mean Δ AP in mm Hg=-8.9, CI -14.0 to -3.4, p=0.004, and mean Δ AP in z scores=-1.4, CI -2.1 to -0.8, p<0.001). Amniotic pressure did not change with drainage in the normal pressure group (mean Δ AP -0.3 z scores, CI -0.6 to +0.8, p=0.23), in contrast to the fall in AP in the high pressure group (mean Δ AP -1.8 z scores, CI -2.5 to -1.0, p<0.001), the difference in Δ AP between these two groups being significant (p=0.048). The volume of fluid drained however was greater in the high than in the normal pressure group (mean 679 ml, CI 542 to 816, and mean 275, CI 59 to 490, respectively, p=0.01). In addition, there was a clear difference in the degree of polyhydramnios between these groups as mentioned previously, which may have influenced selection of volume. Although volume drained was significantly higher in multiple (819 ml, CI 673 to 1492) than singleton pregnancies (376 ml, CI 256 to 496, p<0.001), there was no significant difference in Δ AP (-1.9 z scores, CI -3.1 to -0.8, and -1.0, CI -1.5 to -0.4, respectively, p=0.13).

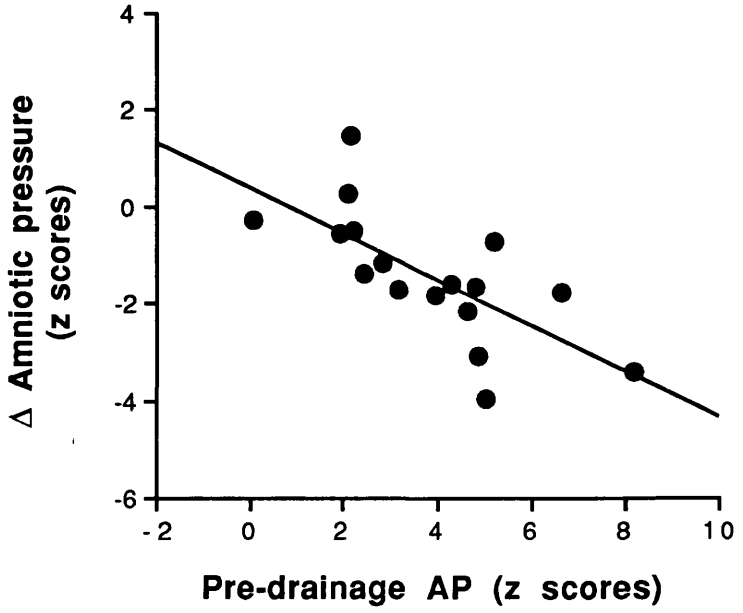

Figure 4.5: The relationship between the change in amniotic pressure with fluid drainage and the initial amniotic pressure, when both are expressed in z scores. (y=0.38 - 0.47x, r=0.70 p=0.002, where y=Δ AP z score and x=pre-drainage AP z score).

There was no significant correlation between Δ AP in z scores and gestational age or fetal number. Δ AP expressed in z scores showed significant negative correlations with volume

(y=0.032 - 0.0025x, r=0.55, p=0.02, where y=Δ AP z score and x=volume in ml), and with the deepest pool measurement (y=1.7 - 0.22x, 0.61, p=0.03, where y=Δ AP z score and x=volume in ml). The fall in amniotic pressure z score was also significantly related to the pre-drainage AP z score (y=0.38 - 0.47x, r=0.70 p=0.002, where y=Δ AP z score and x=pre-drainage AP z score, Figure 4.5), but not to the post-drainage AP z score. Multiple regression indicated that this association remained significant (p=0.002), after accounting for volume and the deepest pool measurement, which themselves were not independently significantly correlated with Δ AP.

The pattern of change in AP with drainage, i.e. substantial falls in those with raised pressure prior to drainage and no change in those with normal pressure prior to drainage, was further borne out in the analysis of those with serial readings (Figure 4.6).

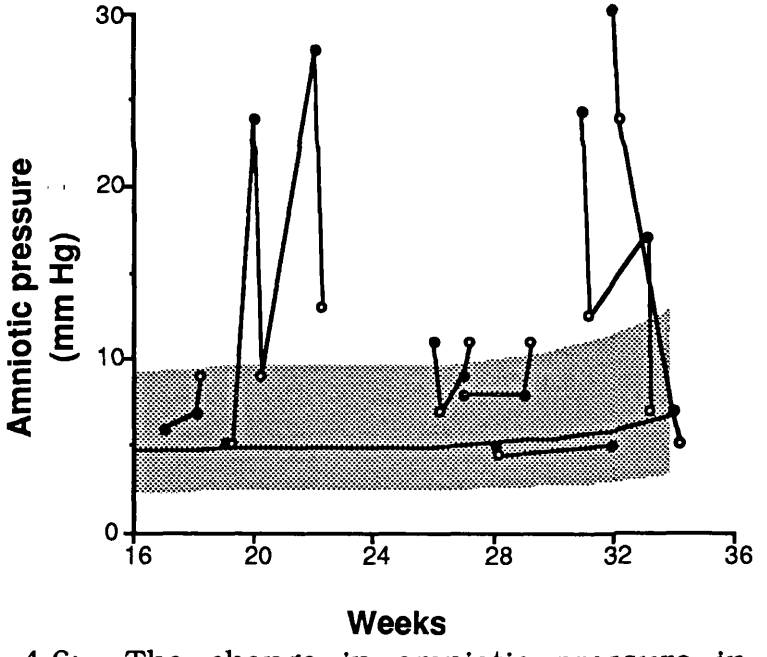

Figure 4.6: The change in amniotic pressure in patients with polyhydramnios who underwent serial recordings (filled circles=pre-drainage, open circles=post-drainage).

4.4 Discussion

This study demonstrates that amniotic pressure is raised in polyhydramnios. Since commencing this study, Weiner et al (1989) have reported similar findings in a smaller number of patients: mean AP in 8 cases of clinically-diagnosed polyhydramnios was approximately 6 mm Hg higher than that in 42 with normal amniotic fluid volume, although differences in

gestation were not taken into account, nor differences in the position of their reference point (fetal heart), as discussed in Chapter 3.4.

Although in this study, AP readings in polyhydramnios lay on or above above the reference mean, they exceeded the upper limit of the reference range in only just over half; this may explain previous conflicting reports of AP in polyhydramnios (as discussed in Chapter 1.5.vi). In contrast to Caldeyro-Barcia et al (1957), who contended that there were two separate types of polyhydramnios (normal and high pressure), this work suggests that raised pressure may instead simply reflect the degree of increased amniotic fluid volume. AP seemed a function of severity, as assessed semi-quantitatively by both the deepest pool measurement and the AFI. In those with mildly increased amniotic fluid volume, AP often lay within the reference range, while gross elevations in AP (z score >4) were found only where the deepest pool exceeded 15 cm and the AFI 50 cm. This is consistent with the known properties of skeletal and uterine muscle, in which resting tension increases initially only slightly with increasing length as the elastic and inelastic muscle fibres slide over each other, until the resting length is exceeded producing a large rise in tension due to stretching of the inelastic fibres. This was first demonstrated in vitro by Hill (1953) using a frog sartorius preparation. Csapo (1962) later reported similar findings in strips of rabbit myometrium stretched in 2 mm increments: tension remained below 4 g as the strip was lengthened from 12 to 30 mm, but increased exponentially to exceed 50 g when the length was increased only a little more to 40 mm. Similarly, Csapo et al (1963) confirmed this in vivo by incrementally infusing fluid into parturient rabbit uteruses: there was little if any initial increase in resting intrauterine pressure, but then an exponential rise with further increase in uterine volume.

Thus the relationship between AP and amniotic volume, although not measured in this study, is on theoretical grounds unlikely to be linear. However, the relationship found between AP and semi-quantitative assessments of amniotic fluid volume in this study was linear. This should not be interpreted as suggesting that AP increases linearly with increasing amniotic fluid volume, since the relationship between such ultrasonic indices and amniotic fluid volume has not been determined and is, in any case, unlikely

to be linear on geometrical grounds. Figures 4.2 and 4.3 suggest that in lesser degrees of polyhydramnios, such as when the deepest pool measurement is ≤13 cm or the AFI ≤40 cm, there is little if any relationship between AP and these semi-quantitative indices of amniotic fluid volume, similar to the situation in pregnancies with normal amniotic fluid volume (Chapter 3.3).

Elevated AP in gross polyhydramnios is consistent with the clinical features of this condition, i.e. maternal discomfort, uterine irritability, preterm labour and preterm premature rupture of the membranes (Barry 1953, Caldeyro-Barcia et al 1957, Powers 1973, Boylan & Parisi 1986, Cardwell 1987, Hill et al 1987, Steinberg et al 1990), as discussed in Chapter 1.5.iii. It is thus speculated that the complications of polyhydramnios may be mediated through raised AP. It was not possible in this clinical study to examine scientifically the relationship between the degree of elevation in AP and the above complications, in view of the the variety of therapies used (drainage, indomethacin, transfusion, antiarrythmic drugs), the differing management policies employed at the various institutions in which these referred patients were subsequently managed, and the high incidence of confounding variables (multiple pregnancy, fetal abnormality, hydrops etc).

In polyhydramnios pregnancies with high AP (above the reference range), pressure was restored towards normal by drainage of amniotic fluid. In contrast, there was no significant change in AP with drainage in those with normal AP (within the reference range), similar to the lack of effect of drainage of small quantities of fluid in pregnancies with normal amniotic fluid volume (Chapter 3.3). There were however clear differences in the degree of polyhydramnios and the volume drained between these two groups. Data on the effect of removing quantities of amniotic fluid were necessarily limited by the clinical circumstances of each procedure, thereby introducing potential bias in selection of volume. Multiple regression analysis was thus used to control for confounding variables, indicating that pre-drainage AP was the only variable independently correlated with the fall in AP with drainage, after accounting for semiquantitative amniotic fluid volume, volume drained, and multiple pregnancy.

In this study, the drainage volumes associated with significant reductions in AP were relatively small (mean 679 ml), considering that amniotic fluid volumes of up to 7-10 litres have

been reported in association with gross polyhydramnios (Caldeyro-Barcia et al 1957, Weir et al 1978). This is in keeping with the muscle tension experiments discussed above, whereby a small reduction in length in a muscle strip stretched just beyond its resting length would be expected to be accompanied by a relatively larger reduction in tension. If the complications of polyhydramnios are mediated through raised AP, then restoration of normal AP rather than volume might be a more appropriate therapeutic goal. This would have advantages in minimizing the potential for complications such as abruption and preterm labour, attributed to rapid removal of large volumes of amniotic fluid (Cabriera-Ramirez & Harris 1976, Feingold et al 1986).

4.5 Summary

In 36 pregnancies with polyhydramnios undergoing invasive procedures, amniotic pressure was elevated compared to pregnancies with normal amniotic fluid volume (mean z score 2.7, CI 2.1 to 3.4, $p<0.001$), and was significantly positively correlated with semiquantitative amniotic fluid volume (deepest pool and AFI). Drainage of fluid (mean 584 ml, CI 443 to 725) produced a fall in AP (mean Δ z score -1.4, CI -2.1 to -0.8, $p<0.001$). Δ AP was significantly related to the pre-drainage AP z score ($y=0.38 - 0.47x$, $r=0.70$, $p=0.002$), but not independently to the volume drained. Amniotic pressure did not change with drainage in the 16 with a pre-drainage pressure within the reference range (mean Δ AP -0.3 z scores, CI -0.6 to +0.8), in contrast to the fall in AP in the 20 (56%) with a pre-drainage AP outside the reference range (mean Δ AP -1.8 z scores, CI -2.5 to -1.0, $p<0.001$). These findings suggest that AP is elevated in polyhydramnios, and may be restored towards normal by drainage of amniotic fluid.

CHAPTER 5: ASSOCIATION OF RAISED PRESSURE IN POLYHYDRAMNIOS WITH IMPAIRED FETAL BLOOD GAS STATUS

5.1 Background

The excess perinatal morbidity and mortality associated with polyhydramnios is in part attributable to an increased incidence of preterm delivery secondary to preterm labour and ruptured membranes (Barry et al 1953, Hashimoto 1984, Boylan & Parisis 1986, Cardwell 1987, Hill et al 1987), and in part to the presence of congenital malformations (Queenan & Gadow 1970, Hill et al 1987), as discussed in Chapter 1.5.iv. These associations however, do not entirely account for the adverse perinatal outcome attributed to polyhydramnios: eight (6%) of 141 perinatal deaths in Queenan & Gadow's large series occurred antepartum in normally formed singletons, as did 2 (9%) of 22 deaths in a more recent series of severe polyhydramnios (Barkin et al 1987). Similarly, Carlson et al (1990) reported that 2 (14%) of 14 perinatal deaths in 49 pregnancies with an AFI >24 cm were normally formed antepartum stillbirths, although it was not stated whether these were twins or singletons. Certainly feto-fetal transfusion syndrome when diagnosed antenatally in the presence of polyhydramnios, is associated, as discussed in Chapter 1.5.iv, with perinatal mortality rates in excess of 50%, a substantial proportion of which occurs antepartum in normally formed fetuses. Eight (50%) of the 16 perinatal losses in Weir et al's (1979) series of 8 monozygous twin pregnancies with acute polyhydramnios were intrauterine deaths, as were 14 (61%) of 23 deaths in a later series from the same institution (Steinberg et al 1990). Some of these occurred in association with hydrops, although the contribution of hydrops to fetal compromise in feto-fetal transfusion syndrome and its pathophysiology remains poorly understood.

Tabor & Maier (1987) speculated that raised amniotic pressure impairs uteroplacental perfusion in polyhydramnios; this was based on their observation of a case of iatrogenic polyhydramnios during intrapartum amnioinfusion. A previously normal cardiotocograph showed a baseline bradycardia and decelerations as amniotic pressure rose with the development of polyhydramnios from 10 to 50 mm Hg, these changes disappearing with drainage of fluid and restoration of normal

pressure. Impaired uteroplacental perfusion in polyhydramnios might also explain the high frequency of fetal hypoxaemia and acidaemia recently documented in utero in pregnancies complicated by feto-fetal transfusion syndrome (Fisk et al 1990). Indeed, it has recently been suggested that raised pressure in the recipient's sac secondary to polyhydramnios impairs placental perfusion in the donor twin, and that this results in further hypovolaemia and oliguria in the donor (Urig et al 1990, Elliot et al 1991). This suggestion arose from an unexpected observation in recent series of feto-fetal transfusion syndrome treated by serial amniocenteses. Removal of sufficient fluid to normalize volume in the recipient's sac was accompanied by normalization of amniotic fluid volume in the previously anhydramniotic donor's sac (Mahoney et al 1990, Urig et al 1990, Elliot et al 1991), and in some cases, by resolution of hydrops. Using this technique of "aggressive therapeutic amniocentesis", survival rates of 69-79% have recently been reported (Mahoney et al 1990, Urig et al 1990, Elliot et al 1991), leading one group to suggest that decompression "effectively reverses the physiology of twin-twin transfusion syndrome" (Elliott et al 1991).

5.2 Aims

The aims of this part of my work were:

i. to investigate the relationship between raised AP and fetal blood gas status in human pregnancies with polyhydramnios undergoing clinically-indicated fetal blood sampling

ii. to investigate the relationship between raised AP and Doppler measurements of downstream resistance in the uteroplacental circulation in human pregnancies complicated by polyhydramnios

iii. to determine experimentally in sheep the effect on fetal blood gas status of increasing amniotic pressure by fluid infusion.

5.3 Clinical data

5.3.i. Methods

Sufficient blood to allow blood gas analysis was obtained at fetal blood sampling in 22 of the pregnancies with polyhydramnios described in Chapter 4.2. Of 18 singletons, 9 were associated with

fetal structural anomalies, and 1 with Rh alloimmunization; hydrops was present in 5. All 4 multiple pregnancies had ultrasonic signs consistent with feto-fetal transfusion syndrome; in each case the fetus in the sac with polyhydramnios (the recipient) underwent blood sampling, only one of these being hydropic. Gestational age ranged from 20-34 weeks (median 30).

After recording the amniotic pressure as described previously, fetal blood sampling was performed under ultrasound guidance from the umbilical vein at either the placental cord insertion (n=11), the fetal intrahepatic vein (n=9), or a free loop of cord (n=2). After entry of the needle into the fetal vessel, 1-3 ml of fetal blood were collected for cytogenetic and haematological investigations. An additional 100-150 µl was placed into a heparinized syringe, and acid-base balance determined within 10 minutes (ABL 330, Radiometer, Copenhagen, Denmark). Fetal blood sampling was repeated for clinical reasons in one fetus immediately following drainage of amniotic fluid, as described in Chapter 4.2.

The purity and fetal source of the blood sample were confirmed (i) at the time by demonstration of separate mean cell volume peaks on a Coulter Channelyzer (Coulter Electronics, Luton, Beds) and (ii) later by examination of the blood film stained by the Kleihauer-Betke method. When the site of sampling was the placental cord insertion or a free loop, 1-2 ml of saline was injected to confirm the venous rather than arterial origin of the sample by ultrasonic visualization of the direction of turbulent flow.

Blood gas results were compared to published reference ranges (Soothill et al 1986). From the graphs in that publication, regression lines and standard deviations were derived manually to allow gestation-independent expression of pCO_2 and pO_2 as z scores ($y=4.04 + 0.024x$ where $y=pCO_2$ in kPa and x=gestation in weeks, SD=0.46, and $y=10.8 - 0.16x$ where $y=pO_2$ in kPa and x=gestation in weeks, SD=1.0). Although fetal venous pH does not vary with gestation (Soothill et al 1986), z scores were similarly calculated (SD=0.033, mean pH=7.385) so that all three variables could be compared to reference data by means of the one sample t test. Relationships were assessed between amniotic pressure and other variables using Pearson correlation coefficients for normally distributed data. Linear relationships were used only where supported by least-squares analysis. AFI was not used in the

analysis in view of the small number of patients in whom this measurement was obtained (n=9).

5.3.ii. Results

Blood gas results in the fetuses with polyhydramnios are shown in Table 5.1. Comparison of gestation-independent z scores with published data on normal fetuses indicates that as a group, fetuses with polyhydramnios were significantly acidaemic, hypercapnic, and hypoxaemic. Of the 22 fetuses, 8 (36%) had a pH value, 10 (45%) had a pCO_2 value and 16 (73%) had a pO_2 value below the reference range.

Table 5.1: Mean and 95% confidence intervals for fetal blood gas variables in 22 fetuses with polyhydramnios, expressed both in the original units and in z scores. Significance was determined by one sample t testing of z scores.

Mean (CIs)	Units	z scores	p value
pH	7.32 (7.28 to 7.38)	-1.7 (0.2 to 3.1)	0.04
pCO_2 (kPa)	5.7 (4.9 to 6.5)	2.1 (0.3 to 3.8)	0.03
pO_2 (kPa)	3.2 (2.5 to 3.8)	-3.0 (-2.3 to -3.7)	<0.001

As shown in Table 5.2, there were significant linear correlations between amniotic pressure and fetal pH and pO_2, but not pCO_2. Fetal pH decreased significantly with increasing AP (y=7.43 - 0.0063x, r=0.54, p=0.01, where y=pH and x=AP in mm Hg), and this relationship was of slightly greater significance when AP was expressed in z scores (y=7.43 - 0.036x, r=0.56, p=0.006, Figure 5.1). Similarly the degree of hypoxaemia was negatively correlated with amniotic pressure (y=-1.9 - 0.069x, r=0.41, p=0.05, where y=pO_2 z scores and x=AP in mm Hg), this relationship again being of greater significance when AP was expressed in z scores (y=-1.6 - 0.48x, r=0.54, p=0.01, Figure 5.2).

There was no significant correlation between any of the fetal blood gas variables and the deepest pool measurement. As AP was significantly correlated with this semi-quantitative index of amniotic fluid volume, multiple linear regression analysis was used to demonstrate that fetal pH and pO_2 z scores were both

significantly correlated with AP z scores (p=0.04 and 0.02 respectively) independent of the deepest pool measurement.

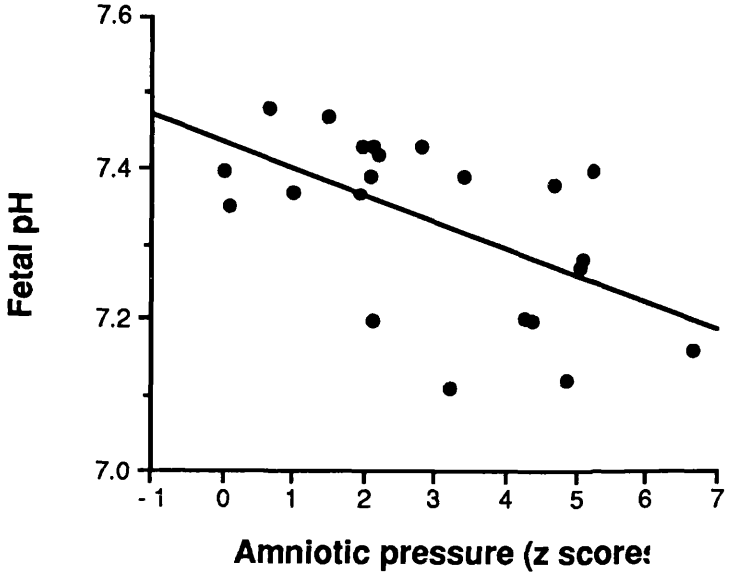

Figure 5.1: The relationship between fetal pH and the degree of elevation in amniotic pressure. (y=7.43 - 0.036x, r=0.56, p=0.006, where y=pH and x=AP z scores).

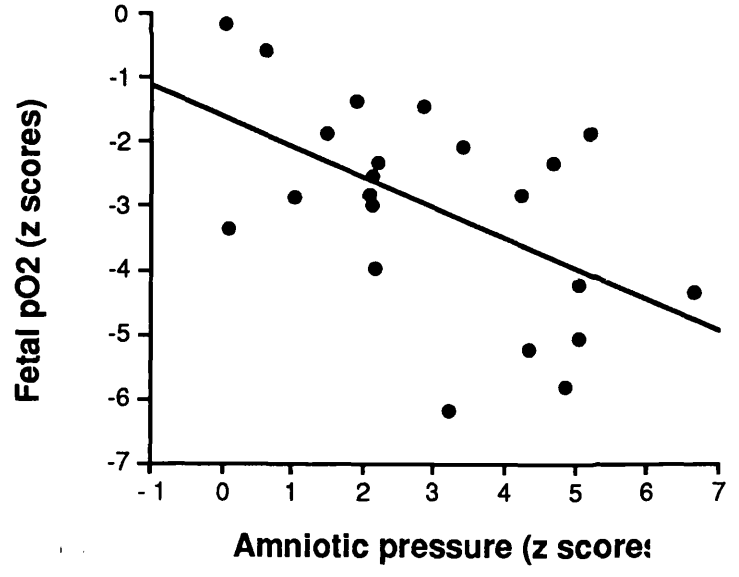

Figure 5.2: The relationship between the degree of fetal hypoxaemia and the degree of elevation in amniotic pressure. (y=-1.6 - 0.48x, r=0.54, p=0.01, where y=pO_2 z scores and x=AP z scores).

The variances of the regression equations in Figures 5.1 and 5.2 were only 0.31 and 0.29 respectively, suggesting that factors other than AP explain most of the variation in fetal pH and pO_2. Accordingly blood gas status was compared between fetuses with and without several potential confounding factors: the presence of

hydrops, the presence of structural anomalies, and multiple pregnancy. There was no significant difference in pH or pO_2 between fetuses with (mean pH 7.30, CI 7.22 to 7.39, and mean pO_2 -3.2 z scores, CI -4.5 to -1.9) and without structural anomalies (mean pH 7.35, CI 7.29 to 7.40, and mean pO_2 -2.9 z scores, CI -3.7 to -2.1). Similarly there was no difference in pH or pO_2 z score between those with (7.27, CI 7.19 to 7.35, and -3.5, CI -4.5 to -2.5 respectively) and without (7.35, CI 7.29 to 7.41, and -2.8, CI -3.7 to -1.9) hydrops. Although fetal pH values were similar in singleton (mean 7.35, CI 7.30 to 7.40) and multiple (7.25, CI 7.12 to 7.38) pregnancies, pO_2 z scores were lower in multiple (-5.1, CI -4.2 to -6.0) compared to singleton pregnancies (-2.5, CI -1.9 to -3.2, p=0.002). Notwithstanding any effect of the above, the correlations between AP z score and pH or pO_2 z score remained significant after exclusion of those with anomalies (r=0.75, p=0.003, and r=0.65, p=0.02 respectively), those with hydrops (r=0.49, p=0.05, and r=0.58, p=0.02 respectively), and those with multiple pregnancies (r=0.57, p=0.01, and r=0.56, p=0.02 respectively).

Table 5.2: Pearson correlation coefficients and p values for linear regression equations between blood gas variables and amniotic pressure in 22 fetuses with polyhydramnios. Gestation dependant variables are also expressed as z scores.

Blood gas variable	Amniotic pressure in mm Hg	Amniotic pressure in z scores
pH	r=0.54, p=0.01	r=0.56, p=0.01
pCO_2 (kPa)	r=0.31, p=0.15	r=0.39, p=0.10
pCO_2 z scores	r=0.30, p=0.16	r=0.38, p=0.10
pO_2 (kPa)	r=0.55, p=0.008	r=0.62, p=0.002
pO_2 (z scores)	r=0.41, p=0.06	r=0.54, p=0.01

As these potential confounders (hydrops, anomalies and multiple pregnancy) were dichotomous variables, it was not possible to assess their effect on the relationship between AP and fetal blood gas status using multiple linear regression analysis. Accordingly fetal, pH and pO_2 were expressed as dichotomous

variables (values either within or outside the published reference range), and entered as the outcome variable in a multiple logistic regression model which comprised AP z score, presence or absence of hydrops, presence or absence of congenital anomalies, and presence or absence of multiple pregnancy. None of these were significantly related to the presence of fetal acidaemia or hypoxaemia; however in each model AP was the variable which most closely approached statistical significance (p=0.07 for acidaemia and p=0.29 for hypoxaemia).

Anecdotally, the reduction in AP (20 to 12 mm Hg) which followed removal of 1100 ml amniotic fluid from the sac of a hydropic triplet fetus was accompanied by correction of fetal acidaemia (pH 7.19 to 7.36) and hypoxaemia (pO_2 1.4 to 3.3 kPa). The interval between the two samplings was 24 minutes.

5.3.iii. Discussion

This study documents abnormal blood gas status in fetuses with polyhydramnios, and suggests that derangement in fetal venous pH and pO_2 is at least in part, a function of the degree of elevation in amniotic pressure.

This work provides no information on the mechanism of abnormal fetal blood gas status in polyhydramnios, other than it is related to raised AP, and that anecdotally it appears acutely reversible. In the case sampled before and after removal of fluid, the rapid amelioration in fetal pH and pO_2 which accompanied return of AP towards normal, is consistent with the hypothesis proposed by Tabor & Maier (1987) that raised AP impairs uteroplacental perfusion. As discussed in Chapter 5.1, the improvement in amniotic fluid volume discordance reported in series of feto-fetal transfusion syndrome treated by "aggressive" amniocentesis (Urig et al 1990, Elliott et al 1991), is also consistent with such hypothesis, although AP was not measured in those studies.

Animal studies indicate that uteroplacental perfusion needs to be reduced by $\geq$ 50% before any major effect of fetal blood gas status is demonstrated (Greiss 1967, Skillman et al 1985). These latter authors used a vascular occluder placed around the maternal internal iliac artery in sheep to achieve graded reductions in uteroplacental blood flow. Although a 24% reduction was accompanied by a minor change in pO_2 (c.-2 mm Hg), and a 49% reduction also by a minor change in pH (c.-0.03), fetal hypoxaemia

and acidaemia were observed only after a reduction in uteroplacental blood flow of 63%. If the association of abnormal blood gas status with raised AP in human polyhydramnios is due to impaired uteroplacental perfusion (Tabor & Maier 1987), the greater frequency of hypoxaemia than acidaemia found in this human study is consistent with such animal evidence that pO_2 is the most sensitive of the acid/base parameters in response to reduced uteroplacental perfusion (Skillman et al 1985). pCO_2 did not change over 1 hour in the sheep study; in contrast 45% of these human fetuses with polyhydramnios were hypercapnic. It is possible that a longer-lasting reduction in perfusion is needed to produce an alteration in pCO_2. Nevertheless, the degree of hypercapnia in the human fetuses was not related to AP, suggesting that it may be due to causes other than reduced uteroplacental perfusion. Another mechanism might be umbilical venous compression; again experiments in fetal lambs suggest that acute reductions in umbilical venous flow of <50% produce only a slight reduction in pO_2 (Itskovitz et al 1983) Reductions of 50-75% show a fall in pO_2 comparable to that found with reduced uteroplacental perfusion (Skilman et al 1985), but no similar change in pH rendering this an unlikely mechanism.

The significant association observed between raised AP and fetal hypoxaemia and acidaemia is likely to have been influenced by the presence of confounding variables such as congenital anomalies, hydrops and multiple pregnancy. Indeed only 29-31% of the variance in fetal pH and pO_2 was explained by AP. This clinical observational study was necessarily limited by the presence of the underlying conditions responsible for the polyhydramnios, and it is known that such conditions are identified more frequently in those with severe as opposed to mild polyhydramnios (Carlson et al 1990), which in turn are more likely to be associated with higher APs (Chapter 4.3). Although a powerful tool in accounting for the effect of potentially confounding variables, multiple logistic regression analyses usually require sample sizes larger than the 22 in this study; nevertheless AP in the multiple logistic model used was the factor most closely approaching statistical significance in determining fetal blood gas status. Within the constraints of this study however, it was possible to show that the association between raised AP and fetal hypoxaemia and acidaemia still applied in singleton fetuses, in non-hydropic

fetuses, and in structurally normal fetuses. Determination of the exact relationship of elevated amniotic pressure to fetal blood gas status independent of these confounding variables requires controlled experiments in an animal model.

5.4 Uteroplacental Doppler studies

5.4.i. Methods

Doppler studies of the uteroplacental circulation were performed in the last 11 pregnancies with polyhydramnios studied in Chapter 4. Amniotic pressure readings in these patients (mean z score=2.5, CI 1.1 to 4.0) were representative of the findings in the larger group reported in the previous chapter, lying within the reference range in 5, and above its upper limit in 6. Fetal pH and pO_2 were measured in 8 patients as described in Chapter 5.2.1.

Immediately prior to the invasive procedure, the patient was studied in a recumbent position with a wedge positioned under the right hip to achieve approximately 15° of lateral tilt. Doppler waveforms were obtained with a continuous wave machine (Vasoflo 4, Oxford Sonicaid, Oxford) which provides continuously updated computations of Doppler indices, their coefficients of variation, and maternal heart rate averaged over 5 consecutive waveforms. Waveforms were obtained from left and right sides of the uterus and recognized as being from the uteroplacental circulation by their characteristic appearance and synchrony with the maternal pulse (Pearce 1987). The angle-independent Doppler index of downstream resistance used in this study was the resistance index (RI=[x-y]/x where x=peak systolic velocity and y=end-diastolic velocity), which by convention is the index used to characterize waveforms from the uteroplacental circulation. Each reading was obtained by "freezing" the image when the coefficient of variation of the RI values in 5 consecutive waveforms was <5%. Three readings were obtained on each side. In view of considerable variation between RI values on the placental and non-placental side of the uterus (Bewley et al 1989), and the lack of uniformity of placental position, the RI value used in this study was calculated as the average of the means of values from each side (Schulman et al 1986, Steel et al 1990).

Drainage of amniotic fluid was performed in 5 patients as described in Chapter 4.2 and the uteroplacental Doppler study

then repeated.

Relationships were assessed between AP and other variables using Pearson and Spearman correlation coefficients for normally and non-normally distributed data respectively. Linear relationships were used only where supported by least-squares analysis. AP and pO_2 were expressed as z scores to control for the effect of gestational age. It was not possible to express RI values as z scores in view of the absence of an adequate published reference range over the gestational ages of this study (22-34 weeks), based on data obtained with continuous wave Doppler from either side of the uterus. Although the RI is known to decline significantly with gestational age (Pearce et al 1988), the effect is only slight, accounting for a difference of <0.05 over the gestational ages in this study. Similarly variation in heart rate is known to have a slight effect on Doppler indices (Mires et al 1987), although correction for heart rate is not considered necessary by most authors (Kofinas et al 1989, Brar et al 1989), especially in studies of maternal vessels (Pearce 1987). Accordingly relationships between AP or blood gas status and the uteroplacental RI were assessed by multiple linear regression analysis to exclude any effect of gestational age or maternal heart rate. Several authors have suggested that a RI value ≥0.58 indicates a highly resistant waveform (Campbell 1986, Steel et al 1990), and accordingly RI values were classified as normal or raised using this cut-off. Changes in variables with drainage are expressed as Δ variable (=post-drainage value minus pre-drainage value). Statistical comparisons were by paired or unpaired t-testing as appropriate.

5.4.ii. Results

The mean RI in the uteroplacental circulation of patients with polyhydramnios was 0.48, (CI 0.41 to 0.54) and was similar on the left (0.45, CI 0.39 to 0.51) and right sides of the uterus (0.52, CI 0.44 to 0.60, p=0.2). There was no significant correlation between RI values and maternal heart rate or gestational age. There was no significant linear relationship between the RI and AP z scores (Fig 5.3, r=0.54, p=0.09), and this remained non-significant after controlling for gestational age (p=0.13) or heart rate (p=0.25) by multiple linear regression. Three patients had raised RI values, each of whose AP reading lay above the reference range (Fig 5.3). The difference in proportion of normal and raised RI values in pregnancies with normal compared to raised APs was

not significant (Fisher exact p=0.12). Similarly there was no significant correlation between the RI and either fetal pH (Spearman p=0.2) or pO_2 z scores (Spearman p=0.1).

Drainage of amniotic fluid reduced AP (mean Δ z score -4.1, CI - 5.7 to -2.6, p=0.006), but had no effect on RI (mean Δ -0.06, CI -1.3 to +0.1, p=0.15) or maternal heart rate (mean Δ 6/min, CI -2 to +14, p=0.2). Drainage of fluid in those with raised RIs produced a fall in AP of 7.0, 3.1 and 4.2 z scores which was accompanied by a fall in RI of 0.07, 0.07 and 0.17 respectively, these changes not being significant at the 5% level in view of small numbers.

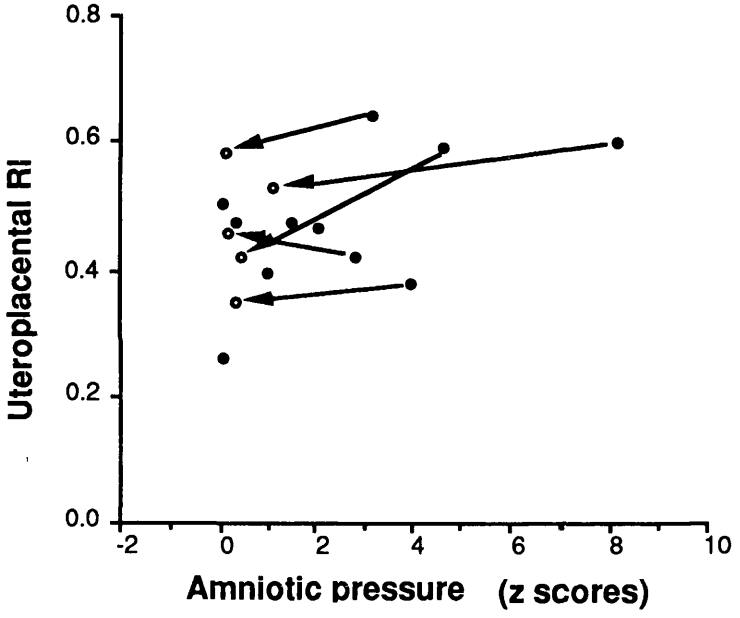

Figure 5.3: Resistance indices in the uteroplacental circulation of 11 patients with polyhydramnios shown as a function of amniotic pressure in z scores (filled circles). Values are also shown after drainage of amniotic fluid in 5 patients (open circles).

5.4.iii. Discussion

In order to determine the effect of elevated AP on uteroplacental perfusion, the uteroplacental circulation was examined non-invasively in this study to determine downstream resistance in polyhydramnios. The hypothesis was that if raised AP impairs uteroplacental perfusion, then this should be reflected in increased Doppler indices of downstream resistance (Spencer et al 1991). Transient elevations in AP, such as those that accompany contractions during labour, are known to be associated temporally

with significant increases in downstream resistance in uteroplacental waveforms, in the absence of change in umbilical waveforms (Fleischer et al 1987, Brar et al 1988). Although AP in polyhydramnios is presumably chronically elevated, fetal blood gas status changed semi-acutely with reduction in AP in Chapter 5.3.ii, suggesting that any abnormality in uteroplacental Doppler waveforms in polyhydramnios should return to normal relatively soon after reduction in AP with fluid drainage.

The values found were broadly similar to those reported in normal pregnancies (Pearce et al 1988), did not correlate significantly with AP or fetal blood gas status, and did not change with reduction in AP. However, the numbers in this study were small, and the results must therefore be considered subject to potential type 2 error, and thus, inconclusive. Three of the 11 studied did have raised RI values, which fell with reduction of AP. Indeed the p value (=0.09) for the Pearson correlation between the uteroplacental RI and AP, suggests that these findings may have been significant if larger numbers were studied. There were two reasons for the small sample size. Firstly, uteroplacental Doppler studies of patients with polyhydramnios were not commenced until after the preliminary analysis of the AP data had suggested an association with impaired fetal blood gas status (Chapter 5.2.ii). Secondly, recruitment was hampered by a decline in the indications for invasive procedures in polyhydramnios, as a result of (i) the recognition that fetal aneuploidy was unlikely in the absence of structural anomalies (ii) the recognition that fetal blood sampling had little role in the diagnosis of feto-fetal transfusion syndrome, and (iii) the increasing use of indomethacin to reduce amniotic fluid volume non-invasively.

Although Doppler waveforms of the uteroplacental circulation have been extensively studied over the last 5 years, these have been almost exclusively with reference to the chronic changes in uteroplacental vasculature that accompany intrauterine growth retardation and pre-eclampsia (Campbell et al 1986, Hanretty et al 1988, Steel et al 1990). Pathologies other than diminished trophoblastic invasion of the spiral arterioles have yet to be studied. Although angiotensin II infusion is known to elevate uteroplacental downstream resistance (Erkkola et al Pirhonen 1990), there is little information available on the effect of acute alterations in downstream resistance on uteroplacental Doppler

waveforms. In addition, there remains considerable controversy with regard to uteroplacental Doppler studies as to the optimal location and nature of the vessel insonated, and the variability within each patient between high and low resistance waveforms (Hanretty et al 1988, Campbell et al 1988, Bewley et al 1989). Little is known of the exact relationship between uteroplacental perfusion and Doppler indices in the uteroplacental circulation, and it remains possible that the technique used in this study was too insensitive to detect any change in uteroplacental perfusion. Nevertheless, Brar et al (1988) demonstrated in labour a linear relationship during contractions between AP, measured via a transcervical intrauterine pressure catheter, and the uteroplacental systolic/diastolic ratio. Peak AP in that study however, ranged from 35-60 mm Hg, whereas in this study, notwithstanding differences in referencing, only one patient had a basal AP ≥35 mm Hg.

5.5 Animal experiments

5.5.i.Methods

Chronically-instrumented fetal sheep were prepared using standard procedures approved by the host institution and in accordance with UK legislation (Hanson et al 1988). Anaesthesia was induced in 9 time-dated cross-bred ewes (Suffolk x mule) at 116-126 days gestation with thiopentone (1g intravenously) and maintained with halothane (1.5-2.0%) and a 2:1 mixture of nitrous oxide and oxygen. One horn of the gravid uterus was exposed at laparotomy and the fetal head delivered through a uterine incision. Polyvinyl catheters were implanted into a fetal carotid artery (inner diameter (ID) 1.0 mm, outer diameter (OD) 2.0 mm), a fetal jugular vein (ID 1.0 mm, OD 2.0 mm), and into the amniotic cavity (ID 2.0 mm, OD 3.0 mm). An additional polyvinyl catheter of ID 2 mm was implanted for fluid infusion into the amniotic cavity in 7 ewes, and in 2 ewes, which served as controls, into the maternal peritoneal cavity. The fetus was replaced, the uterus closed in 2 layers, and all catheters exteriorized through the ewe's right flank. In 3 ewes a catheter was also placed into a maternal carotid artery. At least 5 days were allowed for postoperative recovery, during which time antibiotics were administered daily to ewe (dihydrostreptomycin 1g intramuscularly) and fetus (crystalline penicillin 300 mg intravenously and 300 mg intra-amniotically),

and the catheters kept patent by daily flushing with 2 ml 0.9% saline with 50 units/ml heparin.

Experiments were performed between 121-133 days gestation with the ewe in the standing position. Before starting, 200 µl of fetal arterial blood were withdrawn for blood gas analysis (Instrumentation Laboratories IL 1302, Cheshire UK, temperature corrected to 39.5°C), and normal fetal pH (mean ± standard error [SE] pH 7.33 ± 0.01), pCO_2 (38 ± 3 mm Hg), and pO_2 (22 ± 1.3 mm Hg) values demonstrated. Normal saline (0.9% NaCl) warmed to 38-39°C was instilled intra-amniotically or intraperitoneally via an infusion pump at 100 ml/min. Pressures in the fetal vascular or amniotic compartments were measured via saline filled polyvinyl catheters connected to standard biomedical transducers (L221, Bell & Howell, Hants), and recorded on a polygraph run at 0.25 mm/sec. Amniotic pressure was referenced at the lowermost point of the maternal abdomen, and vascular pressures were displayed after electronic subtraction of amniotic pressure. Fetal heart rate was determined electronically from arterial pressure fluctuations and charted as above. Arterial samples for blood gas determination were withdrawn at 10 minute intervals, and analysed within 5 minutes of collection.

As amniotic fluid volume in the sheep at this gestation approximates 1 litre (Tomoda et al 1985), intra-amniotic infusion of 5 litres was estimated to increase amniotic fluid volume by more than 500%. However, in the absence of change in fetal pH and blood gases or maternal disturbance with intra-amniotic infusion of this volume, a further 5 litres was infused in one ewe and a further 10 litres in 3 ewes. The maternal flank was examined after each litre for evidence of leakage around the catheters and the perineum inspected for signs of rupture of the membranes. The volume infused intraperitoneally in control fetuses was 15 litres. One hour after the infusion, animals were killed with pentobarbitone (4g intravenously) and the gross distribution of infused fluid confirmed at necropsy. There was no evidence of intraperitoneal leakage in the 7 ewes which underwent intra-amniotic infusion.

Mean amniotic pressure was calculated after every litre from point estimates made every 10 seconds over 2 minutes. In the presence of a contraction, the point estimate was obtained after the next return to baseline amniotic pressure. Fetal heart rate

(FHR) and mean arterial pressure (MAP) were similarly recorded in 5 fetuses. Two-way analysis of variance was used over 5 and 15 litres to analyse changes in the various parameters (Δ=postinfusion value minus preinfusion value), after their distributions were confirmed as normal by histograms. The non-parametric Wilcoxon test was used for testing the significance of overall changes (post-pre infusion) in animals undergoing infusions of varying volumes. Linear regression analysis was used only after demonstration of lack of residual relationship with the least squares method.

5.5.ii. Results

Amniotic pressure increased with all 7 intra-amniotic and 2 maternal intraperitoneal infusions. As evident from Figure 5.4, the slopes of the individual regression equations were similar, suggesting that differences in absolute AP were attributable to differences in the pre-infusion AP, and therefore likely to be the result of variation in the position of the reference point in relation to the amniotic cavity. Accordingly changes in AP with infusion were re-expressed as Δ AP (Figure 5.5), and the slopes of the individual regression equations calculated assuming an intercept of zero (Table 5.3).

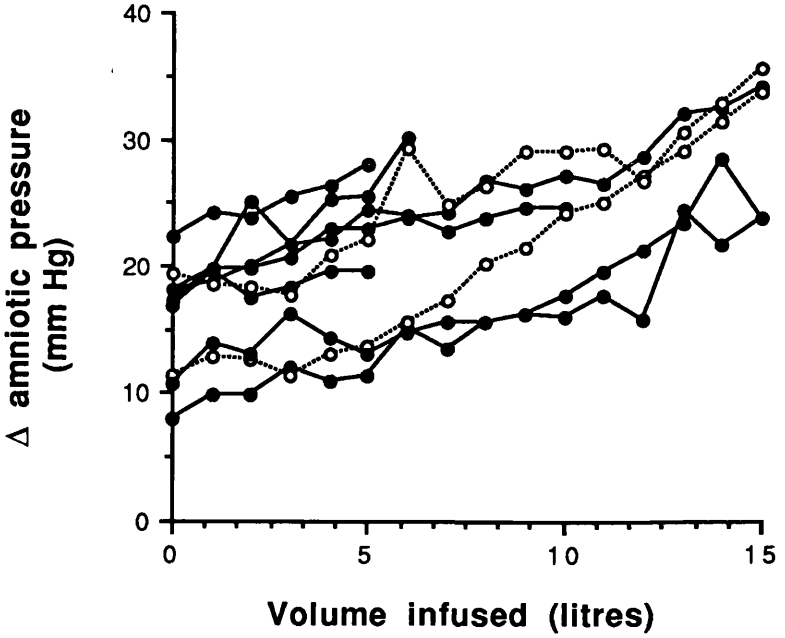

Figure 5.4: The change in amniotic pressure referenced at the lowermost point of the maternal abdomen with infusion of normal saline into the amniotic cavity (n=7, closed circles) or the maternal peritoneal cavity (n=2, open circles) in sheep.

The mean slope in the intra-amniotic infusion group (1.00 ±

0.13) was similar to that in the intraperitoneal infusion group (1.09 ± 0.19). Although in the intra-amniotic infusion group the mean slope was steeper in singleton than twin pregnancies (1.15 ± 0.19 and 0.80 ± 0.13 respectively), this difference was not significant (p=0.21). In intra-amniotic infusions, the slopes were similar in those infused to 5 litres compared to those infused to 15 litres (1.11 ± 0.32 and 0.96 ± 0.11 respectively). There was a significant linear relationship in the intra-amniotic infusion group between mean Δ amniotic pressure calculated after each litre, and the volume infused (y=0.96x, r=0.96, p<0.001, where y=Δ AP in mm Hg and x=volume in litres). Rupture of the membranes was noted at 15 litres in 2 of the 3 undergoing intra-amniotic infusion of this volume, leading to falls in pressure over the next hour of 9.8 and 6.1 mm Hg (58 and 39% respectively of the overall Δ amniotic pressure with infusion). The corresponding fall after infusion in the pregnancy with intact membranes infused to 15 litres was 3.7 mm Hg (16% of overall Δ AP).

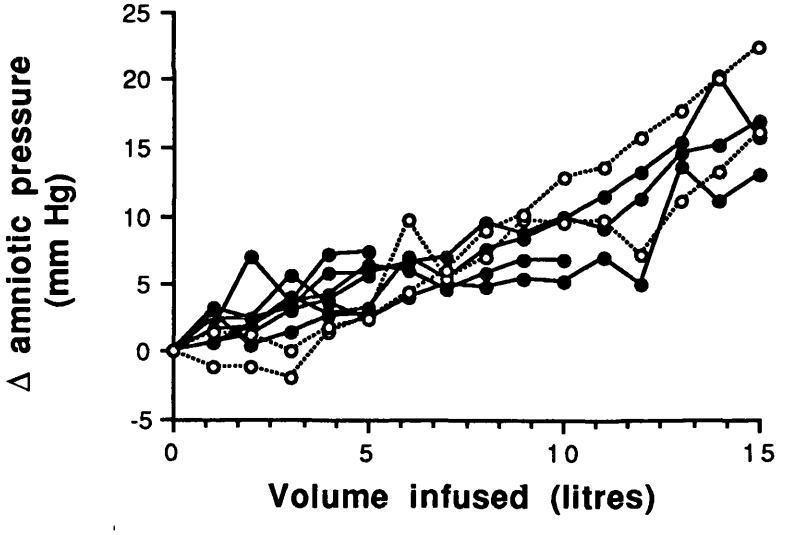

Figure 5.5: The rise in amniotic pressure (Δ) with infusion of normal saline into the amniotic cavity (n=7, closed circles) or the maternal peritoneal cavity (n=2, open circles) in sheep.

Intra-amniotic infusion of the first 5 litres over 50 minutes increased mean amniotic pressure by 4.8 ± 0.7 (SE) mm Hg (F ratio=12.6, p<0.001), but did not produce any significant change in fetal pH (Δ=0.01 ± 0.02, F=0.7), pCO_2 (Δ=2.2 ± 3.9 mm Hg, F=0.4) or pO_2 (Δ=-0.6 ± 0.6 mm Hg, F=1.7). As infusion of a further 5 litres in one ewe similarly failed to produce any significant change in fetal blood gases, a total of 15 litres was next

infused intra-amniotically in 3 ewes, producing a mean rise in amniotic pressure of 15.2 ± 1.2 mm Hg (F ratio=20.7, p<0.001) but again no significant change in fetal pH (Δ=-0.04 ± 0.03, F=0.7), fetal pCO_2 (Δ=-0.2 ± 7.3 mm Hg, F=0.4), or pO_2 (Δ=-3.3 ± 2.0 mm Hg, F=1.7). There was similarly no change in the intraperitoneal infusion group (Δ pH=0.002 ± 0.002 F=2.0, Δ pCO_2=0.8 ± 5.5 F=0.5, Δ pO_2=0.5 ± 0.5 F=1.2) following infusion of 15 litres. The median overall change in pH in 7 intra-amniotic infusions was 0.00 (Wilcoxon p=0.7), the change in pCO_2 was 7.9 mm Hg (p=0.3), and in pO_2 -1 mm Hg (p=0.2). Mean values for fetal pH and blood gases after each intra-amniotic litre are shown in Figure 5.6 as a function of the volume infused, and in Figure 5.7 as a function of the rise in amniotic pressure.

Table 5.3: Details of the linear relationships between volume infused and the rise in amniotic pressure with amnioinfusion. SE=standard error.

Ewe	Type	Site	Vol infused (litres)	Correlation coefficient	Significance	Slope (± SE)
A	twin	amniotic	5	0.54	p=0.018	0.60 ± 0.16
B	singleton	amniotic	5	0.83	p=0.003	1.69 ± 0.27
C	singleton	amniotic	5	0.96	p<0.001	1.04 ± 0.07
D	singleton	amniotic	10	0.88	p<0.001	0.79 ± 0.06
E	twin	amniotic	15	0.98	p<0.001	1.05 ± 0.03
F	singleton	amniotic	15	0.96	p<0.001	1.10 ± 0.05
G	twin	amniotic	15	0.84	p<0.001	0.74 ± 0.06
H	twin	peritoneal	15	0.91	p<0.001	0.90 ± 0.07
I	twin	peritoneal	15	0.96	p<0.001	1.28 ± 0.06

There was no significant change in FHR or MAP (F ratio 2.0 and 1.8 respectively) with intra-amniotic infusion of 10 litres. Furthermore, infusion of another 5 litres in 2 of these fetuses produced no subsequent change in FHR or MAP (F 1.5 and 1.1 respectively). Intraperitoneal infusion similarly had no effect on FHR or MAP (F 0.4 and 1.0 respectively). Intra-amniotic infusion of 15 litres produced no significant change in maternal arterial pH (mean Δ -0.05, CI -0.13 to +0.02, F=1.8) pCO_2 (Δ -3.4 mm Hg, CI -9.6 to +2.9, F=1.1) or pO_2 (Δ -1.7 mm Hg, CI -21.6 to +18.3, F=

0.6).

In the intra-amniotic infusion group, there was no significant relationship between changes in blood gas variables and the overall rise in AP achieved or the volume infused, as shown in Table 5.4.

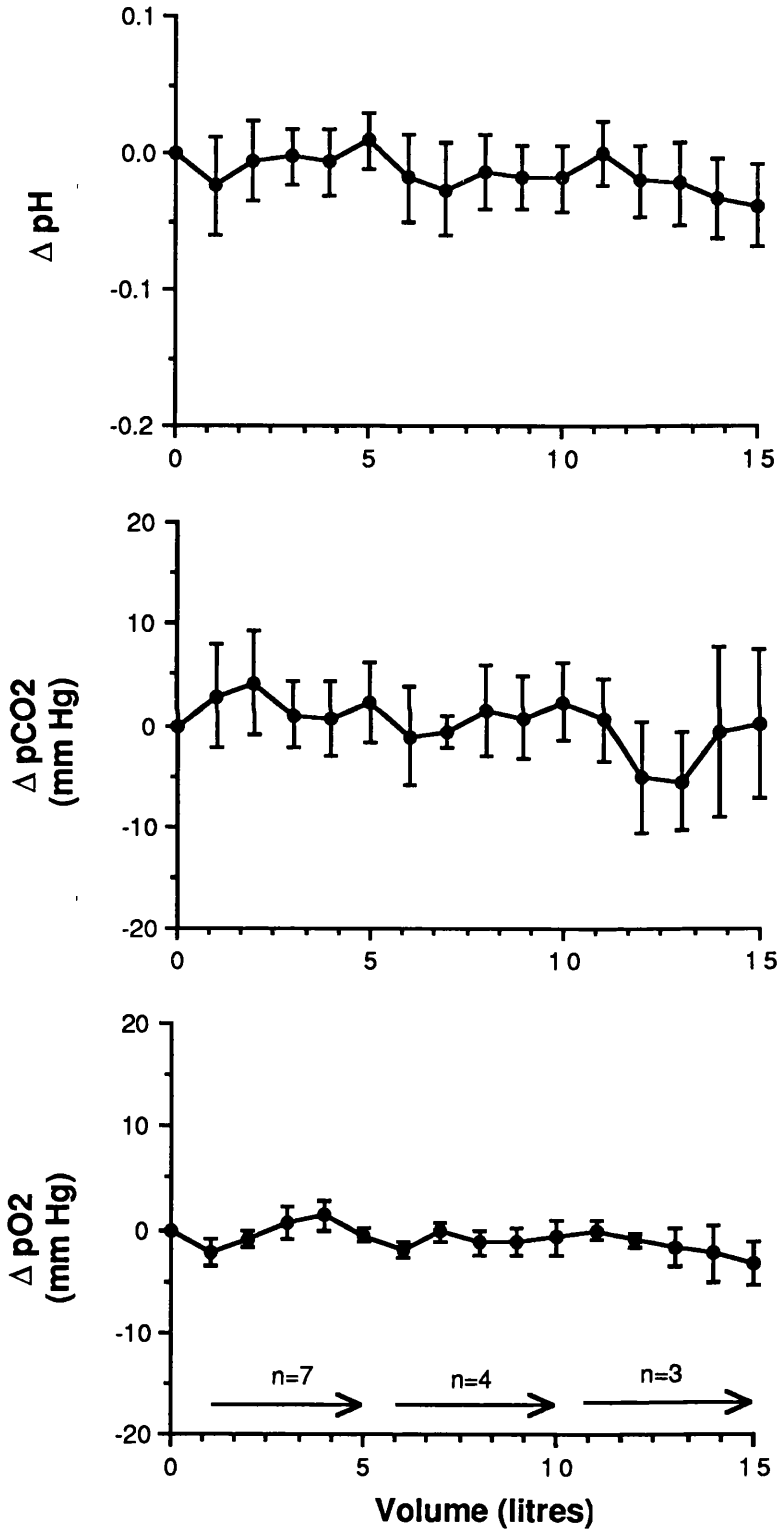

Figure 5.6: Mean (± SE) change in fetal arterial pH, pCO_2 and pO_2 with intra-amniotic infusion. Note that the number of animals (n) declines with increasing volume.

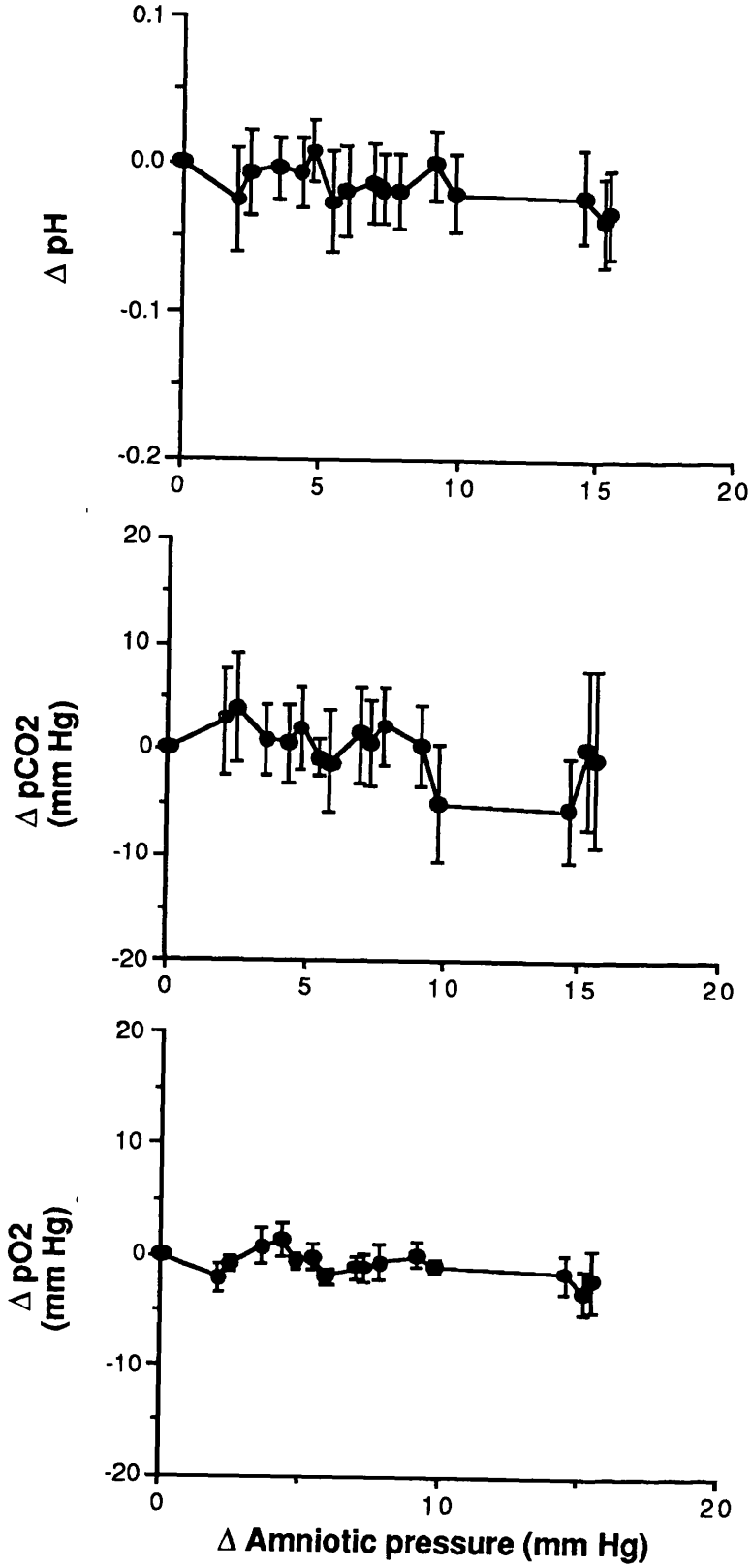

Figure 5.7: Mean (± SE) change in arterial pH, pCO_2 and pO_2 as a function of the mean rise in amniotic pressure, calculated after each litre of intra-amniotic infusion.

5.5.iii. Discussion

The lack of effect on fetal blood gas status of raising amniotic pressure over the acute period of this study does not support the

hypothesis that raised amniotic pressure compromises fetal blood gas status. This discrepancy could be due to (i) use of inappropriate methodology in this animal study (ii) species differences between man and sheep or (iii) the hypothesis being incorrect.

Table 5.4: Pearson correlation coefficients and p values for linear associations between the overall change in blood gas variables with intra-amniotic infusion and Δ amniotic pressure and volume infused.

Blood gas variable	Δ amniotic pressure (mm Hg)	Volume infused (litres)
Δ pH	r=0.66, p=0.11	r=0.59, p=0.16
Δ pCO_2 (mm Hg)	r=0.08, p=0.86	r=0.54, p=0.21
Δ pO_2 (mm Hg)	r=0.40, p=0.37	r=0.25, p=0.58

This study only elevated amniotic pressure acutely, and it remains possible that chronic elevations in pressure may effect fetal blood gas status. Nevertheless, the rapid improvement in fetal condition noted anecdotally with drainage of amniotic fluid in human polyhydramnios suggests a more immediate effect (Chapter 5.2.iii and Tabor & Maier 1987). As normal amniotic volume in the late gestation sheep is approximately 1 litre (Tomoda et al 1985, Tomoda et al 1987), 5 litres was initially infused so that the estimated fivefold increase in amniotic volume would be analogous to human pregnancies complicated by gross polyhydramnios (Pritchard 1966). Having achieved no effect on fetal blood gas status and only a modest rise in amniotic pressure, the volume infused was increased to 10 and then to 15 litres. Intra-amniotic infusion of 15 times normal volume not only increased amniotic pressure substantially, but also appeared to achieve excesses of amniotic fluid considerably greater than found in human polyhydramnios. Nevertheless, the Δ amniotic pressure achieved by infusion of 15 litres (15 mm Hg) was only at the lower end of the APs found in human pregnancies complicated by gross polyhydramnios (c.8-60 mm Hg in those with raised pressure as per Figure 4.1). It was not possible to elevate pressure further by this method, since rupture of the membranes occurred in 2 of 3 ewes infused intra-amniotically with this volume.

In the absence of signs of intraperitoneal leakage, it seems highly likely that some of the fluid infused intra-amniotically was absorbed into the maternal circulation, either directly or via the fetal circulation (Tomoda et al 1987, Gilbert & Brace 1988, Gilbert & Brace 1989). Consistent with this was the anecdotal observation of maternal polyuria during the larger volume infusions. Amniotic volume was not measured, in view of the difficulties of ensuring adequate mixing of tracers or dyes in an enlarged and acutely expanding amniotic cavity. Nevertheless, the aim of this study was to increase pressure rather than volume, and the net effect of any absorption that may have occurred did not prevent the continued rise in pressure with increasing volume, or the uterine distension observed at necropsy.

Uteroplacental perfusion was not measured in this experiment, the aim of which was merely to determine whether raising AP affected fetal blood gas status. If an adverse effect had been demonstrated, it would then have been important to determine the mechanism. Measurement of uteroplacental perfusion in sheep is technically very difficult, necessitating the application of flow probes around uterine arteries. Indices of umbilical venous compression (Chapter 5.2.iii) such as FHR and MAP are easier to study, and showed no change with rising AP in these experiments.

These findings are not necessarily applicable to human pregnancies complicated by polyhydramnios, in view of considerable interspecies differences. In contrast to the human uterus, the ovine organ is thin-walled and bicornuate. In addition, sheep have a T-shaped allantoic cavity, one arm of which extends into the non-pregnant horn in singleton gestations, and its presence might conceivably dissipate the effects of increasing amniotic pressure. The linear relationship between AP and amniotic fluid volume (measured as infused volume) in sheep seems different from that suggested by the observational data (Chapter 4.3) in humans. The rise of only 15 mm Hg in amniotic pressure after amnioinfusion of 15 litres together with the finding that intraperitoneal compliance is similar to amniotic compliance suggests that the ovine uterus may be relatively more distensible than the human uterus. Indeed, during an epidemic of ovine hydramnios, amniotic volumes of 8-18 litres were recorded in pregnancies of prolonged gestation (Coetzer & Barnard 1977); in

contrast human polyhydramnios, although rarely associated with this degree of excess amniotic fluid volume, is frequently complicated by preterm labour and PPROM. Finally, the sheep placenta is cotyledonary and epitheliochorial. Notwithstanding these marked differences in placentation, the sheep has been widely used in the study of fetoplacental circulatory physiology, and was chosen here in view of its relative availability, and the local expertise available with fetal catheterization techniques.

These results are also consistent with the hypothesis that raised AP compromises fetal blood gas status being incorrect. Accordingly, the association demonstrated in human pregnancies may instead still be due to confounding variables, reflecting underlying pathologies such as multiple pregnancy, fetofetal transfusion syndrome, hydrops, and congenital anomalies. A further possibility is that raised AP only affects fetal blood gas status in the presence of abnormal fetal cardiovascular physiology found in association with such conditions, but not in the presence of normal cardiovascular physiology.

This study also shows that amnioinfusion increases amniotic pressure. In sheep, Gilbert & Brace (1989) noted an increase in amniotic pressure of 1 to 2 mm Hg after infusing 1.5 litres of water, suggesting an ovine uterine compliance of 1 mm Hg per litre of water. The slope of the linear relationship we observed between amniotic pressure and volume infused not only confirms this suggestion but extends it to infusions of 15 litres of normal saline.

5.6 Summary

Of 22 fetuses from pregnancies with polyhydramnios investigated by fetal blood sampling, 8 (36%) had a pH value and 16 (73%) a pO_2 value below the reference range. Both fetal pH and pO_2 were significantly negatively correlated with the degree of elevation in amniotic pressure ($y=7.43 - 0.036x$, $r=0.56$, $p=0.006$, where y=pH and x=AP z score, and $y=-1.6 - 0.48x$, $r=0.54$, $p=0.01$, where $y=pO_2$ z score respectively). Although some of these fetuses were hydropic, had congenital anomalies, or were from multiple pregnancies, univariate and multiple logistic regression analyses indicated that the above associations could not be accounted for by these potentially confounding variables. In order to investigate the suggestion that uteroplacental perfusion is impaired in

polyhydramnios with elevated AP, Doppler waveforms were obtained from the uteroplacental circulation in 11 pregnancies complicated by polyhydramnios; there was no evidence of increased downstream resistance. In order to determine the effect of raising AP on fetal blood gas status in the absence of confounding variables, 5-15 litres were infused intra-amniotically in sheep, producing a rise in AP of 1.0 $\pm$ 0.013 (mean $\pm$ SE) mm Hg/litre infused, but no significant change in fetal pH, pCO_2, pO_2, fetal heart rate or mean arterial pressure. This work suggests that abnormal fetal blood gas status in human pregnancies with polyhydramnios is associated with elevated amniotic pressure, although the failure to reproduce this effect in sheep by raising AP suggests that this may not be due to compressive effects of raised AP on the uteroplacental circulation.

CHAPTER 6: AMNIOTIC PRESSURE IN OLIGOHYDRAMNIOS

6.1 Aims

This chapter reports the results of measurement of basal AP in human pregnancies with decreased amniotic fluid volume, using the technique described in Chapter 2.1. Recordings were made in the second and third trimesters; their number, frequency and clinical circumstance were necessarily limited by the need for a clinical indication for amniocentesis or other transamniotic procedure.

The aims of this study were:

i. to compare AP in pregnancies with oligohydramnios with AP in those with normal amniotic fluid volume
ii. to examine the extent to which clinical variables influence AP in pregnancies with oligohydramnios
iii. to determine in pregnancies with oligohydramnios the effect on AP of intra-amniotic instillation of fluid, as performed for therapeutic purposes.

6.2 Methods

The study population comprised patients with decreased amniotic fluid volume scheduled to undergo a transamniotic invasive procedure, either for diagnostic or therapeutic reasons. Each had (i) certain menstrual dates confirmed by ultrasound at 18-20 weeks, or at the time of the procedure if prior to this, and (ii) amniotic fluid volume subjectively assessed as decreased on ultrasound immediately prior to the procedure. Patients were excluded for either of the following findings on the pre-procedure scan: (i) fetal death (ii) a deepest vertical pool measurement devoid of either cord or limbs >3.0 cm.

Sixty women with singleton pregnancies between 16-36 weeks were enrolled in the study. Thirty three were associated with fetal structural anomalies, 27 of which involved the urinary tract. Intrauterine growth retardation, defined as an abdominal circumference measurement <5th centile, was present in 18, while in 10 there was a history suggestive of ruptured membranes. The indication for the transamniotic invasive procedure was investigation of oligohydramnios by amnioinfusion in 9, rapid karyotyping by fetal blood sampling or transabdominal chorion villus sampling in 11, whereas in 40 cases, both indications

applied. All gave informed consent in accordance with institutional ethics committee requirements.

The deepest vertical pool of amniotic fluid was recorded in all patients, and in the last 34 cases, the AFI was also determined. Oligohydramnios was severe (defined as the deepest pool ≤1.0 cm) in 40, moderate (1.1-2.0 cm) in 10, and mild (2.1-3.0 cm) in 10. All procedures were performed under ultrasound guidance, and pressure measurements made on entry of the needle into the amniotic cavity, as described in Chapter 2. Care was taken to avoid loops of cord by first insonating potential pockets of amniotic fluid with pulsed wave Doppler. In the absence of an identifiable pool, the needle was guided toward a potential pool of amniotic fluid in the vicinity of fetal limbs. The intra-amniotic position of the needle was confirmed before pressure measurement, either by aspirating 0.3 ml of amniotic fluid or, in cases of anhydramnios, by observing sonographically 1-2 ml of saline flushed down the needle disperse within the amniotic cavity. Sedation was not used.

In 49 cases, after the initial pressure reading, amnioinfusion was performed for clinical indications to facilitate ultrasonic visualization of fetal anatomy and determination of membranous integrity. Oligohydramnios was classified as severe in 39, moderate in 5 and mild in 5. A 25 cm long portion of polyvinyl tubing (2.5 mm ID) was connected to the needle hub at one end, and a 50 ml syringe, which was attached to a three-way tap at the other end, was used to inject warmed normal saline (0.9% sodium chloride at 37°C) intra-amniotically at a rate of 25-50 ml/minute. The volume infused (range 40-640 ml) was selected by the operator to be the minimum needed to improve the ultrasound view. Following amnioinfusion, the manometry apparatus was flushed to ensure the absence of bubbles, re-referenced and then reconnected to the needle hub and a further reading made. Vaginal fluid leakage was observed immediately following the procedure in 8 patients, indicating the presence of ruptured membranes.

In 43 of the 49 cases which underwent fetal blood sampling following measurement of AP, sufficient blood was obtained to allow blood gas analysis as described in Chapter 5.3. In 37 cases, fetal blood sampling was performed after the amnioinfusion.

Serial readings were made on 2-6 occasions in 13 patients at 1-7 weekly intervals, the indication for subsequent procedures being repeat urine aspiration for fetal renal function (n=5), and

prophylactic amnioinfusion either to promote lung development (n=) or facilitate vesico-amniotic shunt placement (n=2). The deepest pool prior to each infusion measured ≤3.0 cm.

Data on basal AP and the effect of infusion were analysed with respect to the first procedure only. To remove the effect of gestational age, comparisons in AP between groups were made using z scores (no. of SDs from the mean). Changes in variables with infusion are expressed as Δ variable (=post-infusion value minus pre-infusion value). Relationships were assessed between amniotic pressure and other variables using Pearson and Spearman correlation coefficients for normally and non-normally distributed data as appropriate. Statistical comparisons were made parametrically by paired or unpaired t -testing as indicated, or non-parametrically by the Mann-Whitney test.

6.3 Results

Amniotic pressure was significantly reduced in 60 pregnancies with oligohydramnios compared to those with normal amniotic fluid volume (mean z score -1.4 CI -1.7 to -1.0, $p<0.001$). As shown in Figure 5.1, AP readings were beneath the lower limit of the reference range in 16 (27%), and beneath the mean for gestational age in 51 (85%). There was no significant correlation between AP z scores and gestational age.

The degree of reduction in AP was directly related to the degree of oligohydramnios, expressed both as the deepest pool measurement (Spearman $p=0.001$) and the AFI (Spearman $p=0.03$). AP was reduced in pregnancies with severe oligohydramnios (mean -1.8 z scores CI -2.3 to -1.4, $p<0.001$), but not in those with mild (-0.5 z scores CI -0.9 to +0.1, $p=0.1$) or moderate oligohydramnios (-0.3 z scores CI -1.1 to +0.6, $p=0.5$). In those with severe oligohydramnios however, there was no correlation between AP z scores and either the deepest pool measurement (Spearman $p=0.7$) or AFI (Spearman $p=0.6$). In the 40 pregnancies with severe oligohydramnios, AP was on (within 0.2 mm Hg) or below the mean for gestational age in all cases, and beneath the lower limit of the reference range in 16 (40%).

Intra-amniotic infusion of saline increased amniotic pressure by a mean of 4.3 mm Hg (CI=3.4 to 5.3, $p<0.001$) and 2.7 z scores (CI 2.0 to 3.4, $p<0.001$) as shown in Figures 6.2 and 6.3 respectively. There was no significant correlation between Δ AP in z scores and gestational age, volume infused, the deepest pool

measurement or AFI. Δ AP was however, significantly negatively correlated with the preinfusion AP (y=0.94 - 1.0x where y=Δ AP z score and x=preinfusion AP z score, r=0.58, p<0.001, Figure 6.4) but not to post infusion AP. Multiple linear regression analysis indicated that this association remained significant (p<0.001) after accounting for volume, and post infusion z score.

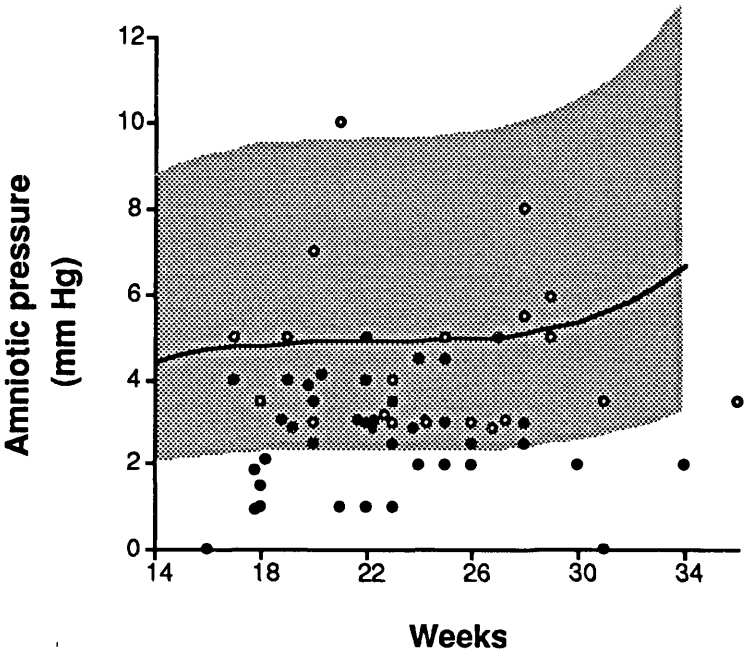

Figure 6.1: Amniotic pressure in pregnancies complicated by oligohydramnios shown against the reference range for pregnancies with normal amniotic fluid volume. Closed circles=severe oligohydramnios, open circles=mild/moderate oligohydramnios. Note that some coordinates have been altered slightly (by ≤0.2 weeks, ≤0.1 mm Hg) to avoid overlap.

There was no significant change in AP with infusion in those with mild oligohydramnios (mean Δ z score 0.6, CI -4.2 to +5.4, p=0.8), in contrast to the rise in those with moderate (3.4, CI 1.7 to 5.1, p=0.02) or severe oligohydramnios (2.8, CI 2.3 to 3.5, p<0.001). In pregnancies with severe oligohydramnios which underwent amnioinfusion, the Δ AP was significantly greater in the 15 with a preinfusion AP beneath the reference range (mean 3.8 z scores, CI 2.8 to 4.8) compared to those with a preinfusion AP within the reference range (2.3, CI 1.7 to 2.9, p=0.01). Nevertheless, the change in AP z scores in those with a normal preinfusion z score was still significantly different from zero (p<0.001).

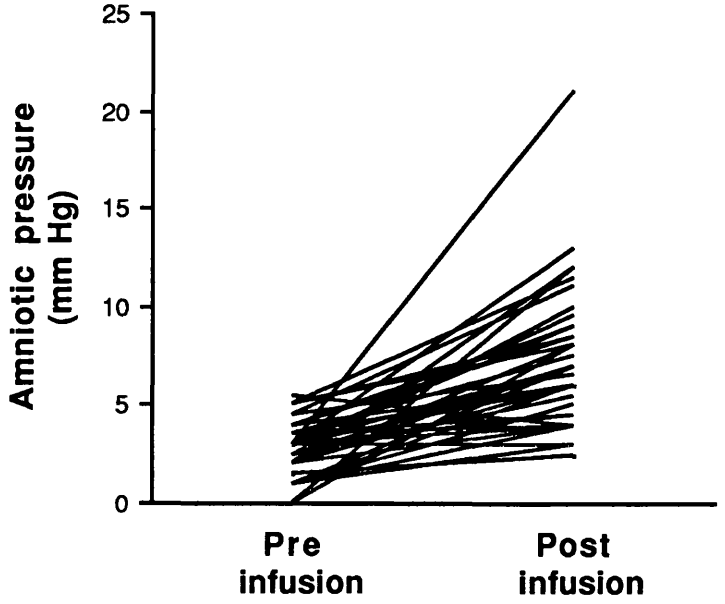

Figure 6.2: The effect of infusion of normal saline in pregnancies complicated by oligohydramnios on amniotic pressure in mm Hg. Mean Δ 4.3 mm Hg, CI 3.4 to 5.3, p<0.001.

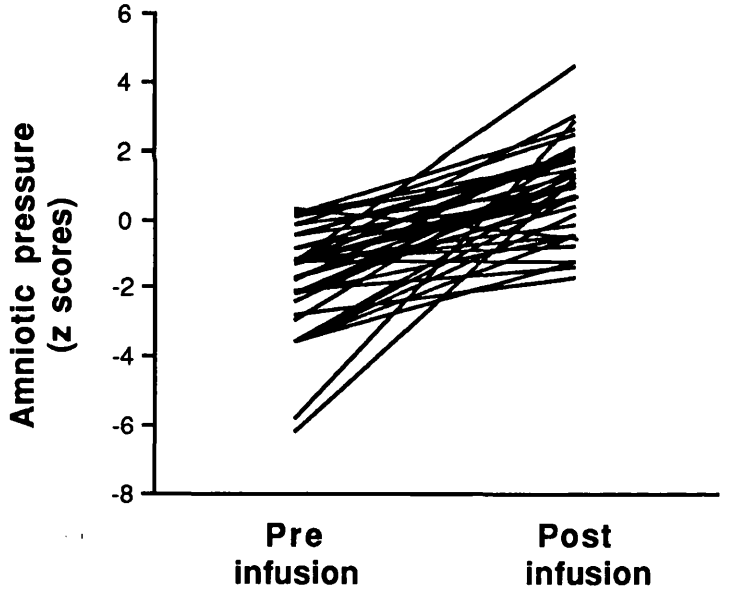

Figure 6.3: The effect of infusion of normal saline in pregnancies complicated by oligohydramnios on amniotic pressure in z scores. Mean Δ z score =2.7, CI 2.0 to 3.4, p<0.001.

Pregnancies with ruptured membranes (n=8) had similar basal APs to those with intact membranes (mean z score -1.3, CI=-1.9 to -0.7 and -1.8, CI -2.2 to -1.3 respectively), and with infusions of similar volume (median 248 ml, range 160-326, and 160, range 42-637, p=0.5) had similar Δ APs (mean Δ z score 2.4, CI 1.8 to 3.0 and 2.8, CI 1.9 to 3.6 respectively). In one pregnancy at 23 weeks gestation, infusion of 200 ml produced an unusually

high AP of 21.5 mm Hg (Figure 6.2); 60 ml was then withdrawn, resulting in a post-infusion AP of 10.5 mm Hg.

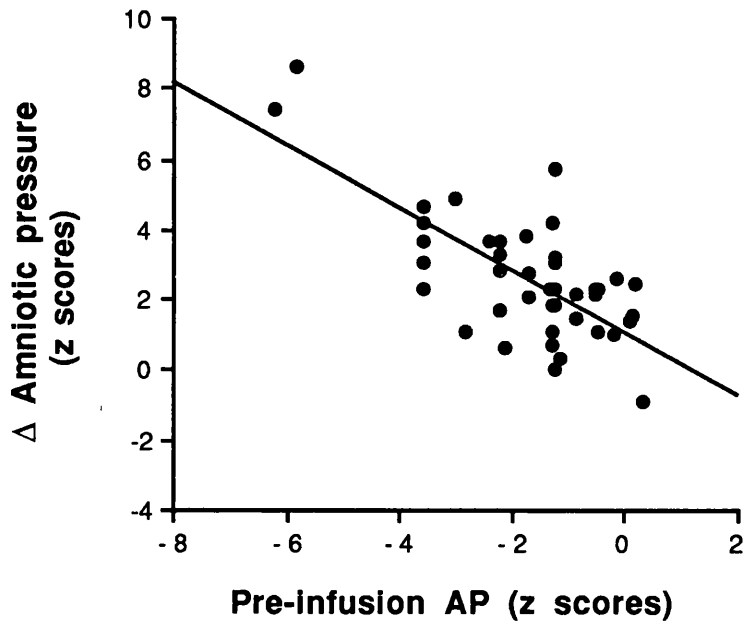

Figure 6.4: The relationship between the rise in amniotic pressure and basal pressure prior to infusion. (y=0.94 - 1.0x where y=Δ AP z score and x=preinfusion AP z score, r=0.58, p<0.001)

Fetal acidaemia (pH >2SDs beneath the reference mean) was present in 9, fetal hypercapnia (pCO_2 >2SDs beneath the reference mean for gestation) in 16, and fetal hypoxaemia (pO_2 >2SDs beneath the reference mean for gestation) in 36 of the 43 pregnancies with oligohydramnios investigated by fetal blood sampling in which sufficient blood was obtained for blood gas analysis. However, in contrast to the situation in polyhydramnios, there was no significant correlation between the initial AP and any of the fetal blood gas variables expressed in z scores. The same applied when only the 28 with severe oligohydramnios so investigated were considered.

The scatter of preinfusion AP recordings in those with serial readings as shown in Figure 6.5 is similar to that of the single readings in Figure 6.1. Analysis of variance on this data was not possible in view of the differing number of observations per patient. The same applies to the change in AP with serial infusions; AP rose acutely with 44 of the 46 serial infusions as shown in Figure 6.6.

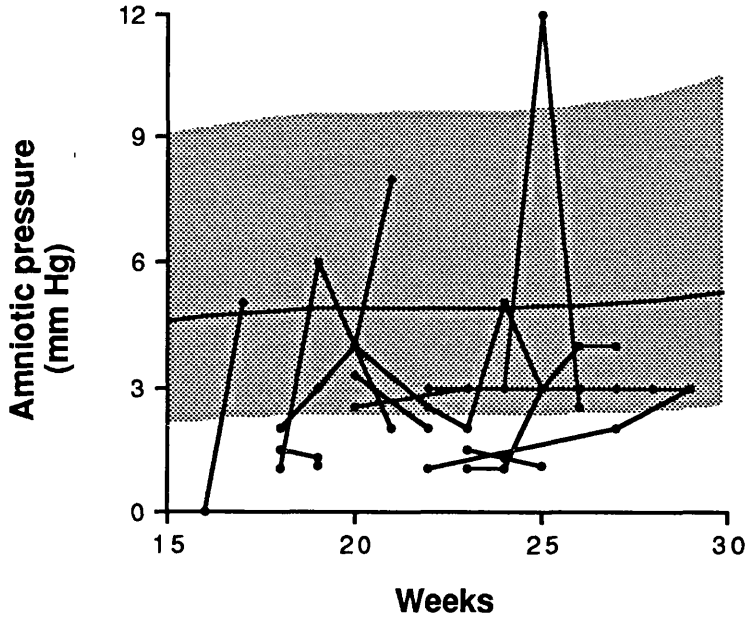

Figure 6.5: Serial amniotic pressure readings (dots) in 13 patients with oligohydramnios. Note that where amnioinfusion was performed, readings refer to pre-infusion AP. The shaded area represents the 95% reference range.

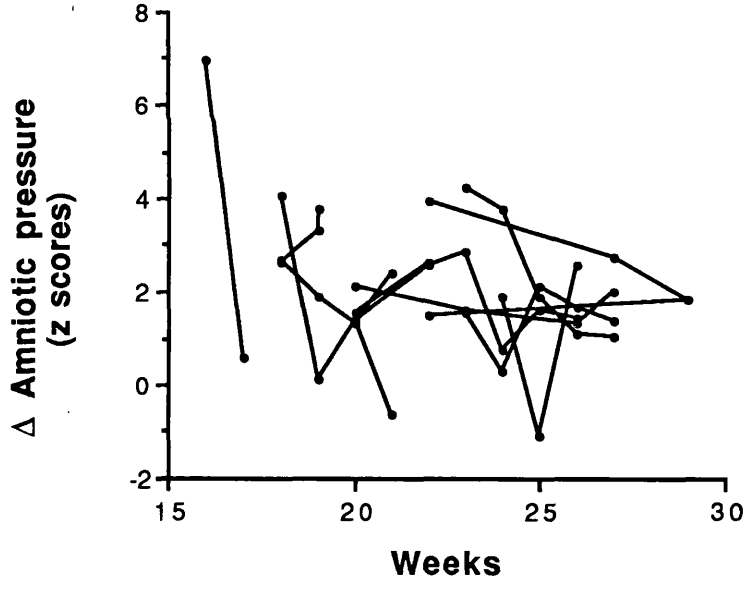

Figure 6.6: The change (Δ) in AP *z* scores with amnioinfusion (dots) in 12 patients in whom serial infusions were performed.

6.4 Discussion

This study demonstrates that amniotic pressure is low in oligohydramnios. Resting AP in human oligohydramnios has not previously been reported, although some descriptive data in labouring patients suggests that removal of 300-700 ml of amniotic fluid by amniocentesis results in a fall in basal AP (Csapo et al 1963). In a recent study in late gestation sheep (Harding et al

1990), AP fell by 1 mm Hg with 48 hours of amniotic (and allantoic) fluid drainage, and returned to baseline values with reinfusion of the drained volumes, although this change was not significant ($0.05<p<0.1$). The wide SDs (c1.5 mm Hg with n=8) in that study suggested considerable variation in response, and the degree of oligohydramnios achieved was unknown, as amniotic fluid volume was not measured. Severe oligohydramnios may not necessarily have been achieved, and it should be remembered that in the human pregnancies AP was only reduced in those with severe and not mild/moderate oligohydramnios.

It remains possible that confinement of low readings to those with severe oligohydramnios may have reflected technical difficulties in obtaining accurate readings in pregnancies with no visible and thus sizeable pool of amniotic fluid. However great care was taken with flushing the needle to ensure accurate pressure readings from within the amniotic cavity. Furthermore, the lack of correlation between AP and the deepest pool/AFI in those with severe oligohydramnios does not support the contention that technically inadequate readings in anhydramnios accounted for the significantly lower AP z scores in severe oligohydramnios compared to those with mild or moderate oligohydramnios, or to those with normal amniotic fluid volume.

This finding that AP is decreased with reduced amniotic fluid volume, together with the finding of increased AP with elevated volume (Chapter 4), is broadly consistent with hydrostatic principles governing contents within inelastic confines. Abnormal AP in oligohydramnios however, was confined to severe degrees of derangement in amniotic fluid volume, as it was in pregnancies complicated by polyhydramnios. This further supports the suggestion (Chapter 3.4) that the relationship between amniotic pressure and intrauterine volume is non-linear, presumably reflecting the different physical principles determining pressure within a musculoelastic structure like the human uterus, as discussed earlier (Chapter 3.4). The degree of abnormality in AP found in both severe oligohydramnios and polyhydramnios is far greater than would be expected if AP was simply determined by gestation-related changes in intrauterine volume: i.e. low AP in severe oligohydramnios at x weeks cannot be attributed to the lower intrauterine volume consequent upon reduced amniotic fluid approximating that found normally several weeks earlier in

gestation (Figure 3.2). From the limited serial data in both polyhydramnios (Chapter 4.3) and oligohydramnios, derangement in AP appears more a long-standing than acute manifestation of abnormal amniotic fluid volume.

Amnioinfusion in oligohydramnios pregnancies significantly increased AP. The rise in AP with infusion was greatest in those with severe oligohydramnios and those with a preinfusion AP beneath the reference range, consistent with preinfusion AP being the main variable determining this rise (Figure 6.4), and the suggestion, made earlier with reference to polyhdramnios (Chapter 4.4), that restoration of fluid volume restores AP towards normal. Nevertheless, a significant rise in AP also occurred in those with moderate oligohydramnios and those with severe oligohydramnios whose preinfusion AP was within the reference range, suggesting that fluid infusion per se may have an additional effect on AP. Indeed, the majority of the postinfusion AP z scores lay in the upper half of the reference range (0 to +1.96 z) as may be seen from Figure 6.3. Posner et al (1990) recently reported a significant increase in basal AP with intrapartum amnioinfusion in patients with an AFI <5 cm, but did not quantitate the rise or relate it to the degree of oligohydramnios. They also noted a pronounced pressure rise in one case, as occurred in one of the patients in this series, suggesting an occasional idiosyncratic response.

The clinical implications of low AP in oligohydramnios will be discussed in the next two chapters.

6.5 Summary

Amniotic pressure was reduced in 60 pregnancies with oligohydramnios (mean z score -1.4, CI -1.7 to -1.0, $p<0.001$), and was significantly correlated with semi-quantitative amniotic fluid volume (deepest pool and AFI). Only in those with severe oligohydramnios, was AP significantly lower than with normal amniotic fluid volume. Intra-amniotic infusion of saline (mean 204 ml, CI 171 to 237) increased AP by a mean of 2.7 z scores (CI 2.0 to 3.4, $p<0.001$), with Δ AP being significantly related to the pre-infusion AP z score ($y=0.94 - 1.0x$, $r=0.58$, $p<0.001$), but not to the volume infused. AP did not change with infusion in those with mild oligohydramnios, in contrast to the significant rise in those with moderate (mean Δ AP 3.4 z scores, CI 1.7 to 5.1, $p=0.02$) or severe

oligohydramnios (mean Δ AP 2.8 z scores, CI 2.3 to 3.5, $p<0.001$). In pregnancies with severe oligohydramnios, Δ AP was significantly greater in those with a preinfusion AP beneath the reference range (mean 3.8 z scores, CI 2.8 to 4.8) compared to those with a preinfusion AP within the reference range (mean 2.3 z scores, CI 1.7 to 2.9, $p=0.01$). These data suggest that AP is low in oligohydramnios, and rises with restoration of amniotic fluid volume. This work compliments that in polyhydramnios (Chapter 4), suggesting that abnormal AP occurs only in the presence of gross derangement in amniotic fluid volume, and that pressure changes with restoration of amniotic fluid volume, at least in part, reflect restoration of AP towards normal.

CHAPTER 7: LOW AMNIOTIC PRESSURE CONTRADICTS THE CONCEPT OF FETAL "COMPRESSION" IN OLIGOHYDRAMNIOS

7.1 Background

The fetus in prolonged oligohydramnios is widely held to be compressed, as discussed in Chapter 1.6.vi., on the basis of its characteristic flattened facies and postural limb deformities. In addition, the umbilical cord has also been assumed to be compressed on the basis of an increased incidence of FHR decelerations (Chapter 1.6.v). The demonstration that the amniotic pressure surrounding the fetus is low is not consistent this concept of fetal "compression" in oligohydramnios. Although not all pregnancies with oligohydramnios had reduced AP (Chapter 6), there was certainly no evidence that AP was elevated (Figure 6.1). Furthermore, low AP was confined to pregnancies with severe oligohydramnios, the ones documented to have the highest incidence of soft tissue sequelae considered indicative of fetal compression (Thibeault et al 1985).

The mechanism for oligohydramnios-related PH is not understood. As compression by intrathoracic space occupying lesions is known to cause lung hypoplasia, many authors have attributed oligohydramnios-related PH to extrathoracic compression of the developing lung, as discussed in Chapter 1.6.vi.. Since inhibition of FBM also results in PH, it has further been suggested that the mechanism by which extrathoracic compression impairs fetal lung development is inhibition of FBM (Gruenwald 1957, Wigglesworth et al 1977). In this regard Blott et al (1987, 1990, Blott & Greenough 1988) recently noted in human pregnancies with prolonged oligohydramnios that FBM were absent in fetuses with, and present in those without, lethal PH. These findings however were disputed by the work of another group (Fox & Moessinger 1985, Moessinger et al 1987). The propriety of various definitions of FBM used in human studies remains controversial (Greenough et al 1988, Pillai & James 1990), with one of the 2 groups attributing this disparity in findings to the differing criteria used for defining FBM epochs (Greenough et al 1988). Restoration of amniotic fluid volume in animals with oligohydramnios has been shown to have significant beneficial effects on fetal lung development (Harrison et al 1982b, Nakayama et al 1983). If FBM are reduced as a result of chest

"compression" in oligohydramnios, restitution of amniotic fluid volume should be accompanied by an increase in FBM.

As discussed in Chapter 1.6.v., the increased incidence in oligohydramnios of fetal distress, meconium staining of the amniotic fluid, and variable FHR decelerations has been attributed by many authors to umbilical cord compression. Intrapartum amnioinfusion has been shown in several studies of patients with oligohydramnios to reduce the frequency of variable decelerations in labour and ameliorate fetal condition at birth (Nageotte et al 1985, Strong et al 1990a). Antepartum, there have been only anecdotal reports of the effect of restoration of amniotic fluid volume on fetal condition, leading in one case to the disappearance of FHR decelerations (Imanaka et al 1989), and in another to normalization of the umbilical artery Doppler waveform, with return of the abnormality when oligohydramnios recurred (Van der Wjingaard et al 1987).

7.2 Aims

The aim of this section was to investigate in human pregnancies with severe oligohydramnios the effect on various fetal biophysical variables of relief of presumed fetal "compression" by restoring amniotic fluid volume as performed for diagnostic or therapeutic purposes. Specifically:

i. to determine the acute effect of restoration of amniotic fluid volume on FBM using both definitions of FBM epochs employed in the studies mentioned
ii. to determine the acute effect of restoration of amniotic fluid volume on fetal umbilical artery Doppler waveforms
iii. to compare the above effects with those seen in patients with oligohydramnios undergoing amnioinfusion in whom immediate leakage of the infused fluid vaginally prevented restoration of amniotic fluid volume (fortuitous controls).

These experiments were necessarily limited by the need for an underlying clinical indication for amnioinfusion.

7.3 Relief of presumed "compression"- effect on fetal breathing.

7.3.i. Methods

Sixteen women with severe oligohydramnios in singleton pregnancies scheduled to undergo clinically-indicated diagnostic

amnioinfusion between 17-31 weeks (median 23) gave written informed consent to inclusion in this study, approved by the institutional ethics committee. Transabdominal amnioinfusion with 150-350 ml warmed normal saline was performed without sedation under ultrasound control as previously described (Chapter 6.2). Fetal blood sampling was also performed in 13 cases for rapid karyotyping.

Using high resolution ultrasound (Acuson, Mountain View, California), the deepest vertical pool and AFI were measured before and immediately after infusion, as was AP, as described earlier. Two ml samples of amniotic fluid, collected in 7 cases before and after amnioinfusion, were immediately spun down, and stored frozen (-70°C). After thawing, they were assayed for prostaglandin E_2 and $F_{2\alpha}$ by Dr M Sullivan of the Department of Obstetrics & Gynaecology at the Royal Postgraduate Medical School, Hammersmith Hospital using reverse phase high performance liquid chromatography (Rose et al 1987). End-diastolic frequencies were observed in the umbilical artery Doppler waveforms of all fetuses, both before and after the procedure, excluding significant fetal hypoxaemia and acidaemia (Nicolini et al 1990). The aetiology of oligohydramnios was classified after infusion as follows: PPROM in 8, bilateral fetal renal pathology in 2, IUGR in 3, and idiopathic in 3.

FBM were recorded continuously for 40 minutes before and 40 minutes immediately after amnioinfusion. No patient smoked or consumed alcohol within one hour of recording. Glucose loading was not performed in view of (i) glucose loading not having an effect on FBM until ≥30 weeks (Trudinger & Knight 1980, Natale et al 1988) and (ii) the difficulty standardising the pre and post-infusion recordings for maternal glucose. With the mother lying semi-recumbent, a realtime ultrasound transducer (model 4000 S/L, ADR, Arizona) was used to visualize the fetal thorax and abdomen in a longitudinal plane. Care was taken to avoid undue compressive force on the fetal thorax by supporting the operator's arm during the recording on pillows level with the vertical height of the fundus. Each fetal breathing movement, defined as inward movement of the thorax with paradoxical outward movement of the abdominal wall, was recorded by means of a manually operated tone generator onto audiotape. All recordings were made by the same person (the candidate) using the same apparatus.

Prior to infusion, the deepest pool and AFI were ≤1.2 and ≤2.3 cm respectively, and mean AP was -1.5 z scores (CI -3.0 to 0.0). Vaginal leakage was noted during the procedure in 6 of the 16 patients. No patient in the fluid retained group leaked within 4 hours of infusion, whereas further vaginal leakage was noted during the post-infusion recording in 4 of 6 in the leakage group. Amnioinfusion significantly increased amniotic fluid volume (mean Δ AFI=10.0 cm, CI 8.7 to 11.2) in 10 women in whom fluid was retained. In contrast, there was only minimal increase in 6 patients, in whom vaginal leakage of fluid was noted during the procedure (Δ AFI=1.8 cm, CI 0.2 to 3.4). The deepest pool and AFI post-infusion were both significantly greater in the fluid retained (median 4.0 [range 2.9-4.8] and 10.6 [9.1-13.4] respectively) than the fluid leakage group (median 1.2 [0.6-2.9], $p<0.01$ and 2.1 [0.6-5.9], $p<0.01$).

The audiotape was replayed into the input port of a BBC microcomputer, which quanititated the intervals between initiation of tone busts at 0.5 second intervals. Digitized data were then analysed for total breaths, as well as incidence of FBM using the two definitions (Greenough et al 1988), as shown in Figure 7.1. As per convention, an episode of FBM was only considered to have occurred when several movements were noted within a minimum time-frame or epoch; this was done to exclude isolated diaphragmatic movements and hiccoughs (Pillai & James 1990). Under definition A, an epoch of fetal breathing comprised continuous breathing movements for ≥ 60 seconds in the absence of any apnoeic interval >6 seconds (Blott & Greenough 1988). With definition B, an epoch began whenever 3 breaths occurred within a 6 second window, and ended whenever there were <3 breaths within any subsequent 6 second window (Moessinger et al 1987). The incidence of FBM was thus defined as the percentage of time spent in FBM epochs. Prior to commencing the pre- and post-infusion recordings, 6 AGA pregnancies with normal amniotic fluid volume were studied on 1-3 occasions to test the ability of the recording technique to document FBM (Figure 7.2).

Five of these women underwent serial infusions (3 had one subsequent infusion, 1 had three, and another five) at 1-2 weekly intervals as prophylaxis to promote lung development. Fetal blood sampling was not performed with any subsequent infusion, and leakage occurred after 3. Data were similarly collected and FBM

recordings made as for single infusions.

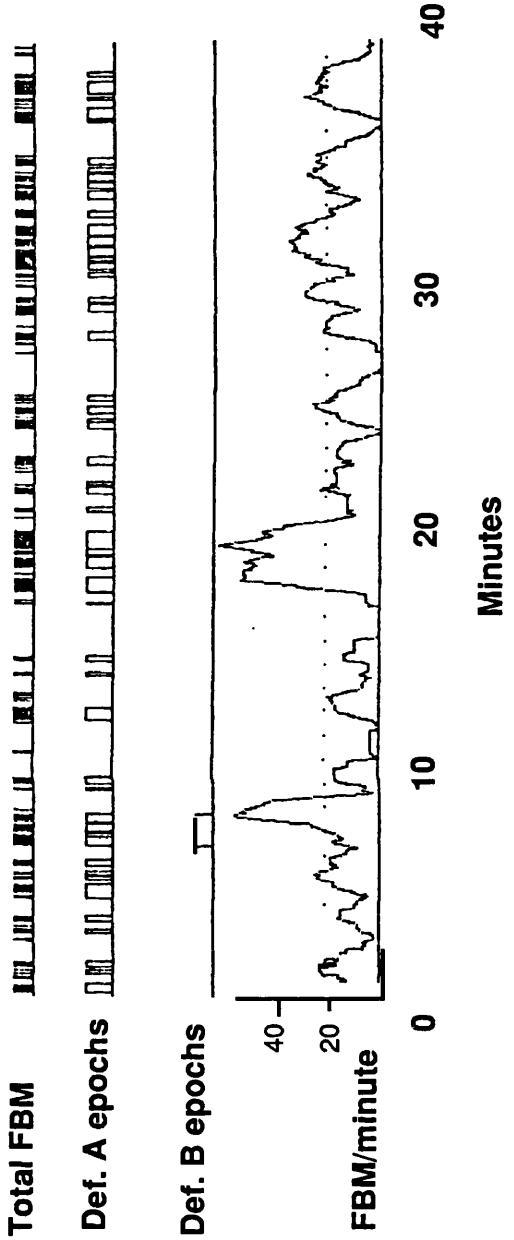

Figure 7.1: Representative computer analysis of 40 minute FBM recording, showing the total number of breathing movements, the number and duration of epochs using both definition A & B, and the frequency of breathing movements. This recording illustrates the considerable effect of the different definitions on the number of epochs recorded.

Changes in variables with infusion were expressed as Δ variables (=post-infusion minus pre-infusion value). Non-parametric statistics were used when non-Gaussian distributions were evident on histograms. Relationships between variables were assessed by Pearson and Spearman correlation coefficients for

parametric and non-parametric data as appropriate. Two-tailed comparisons were by t testing (paired or unpaired) for parametric data and for non-parametric data by Wilcoxon or Mann-Whitney tests as appropriate. After analysis of data for single infusions, all infusions (first and subsequent) were then re-examined as pooled data. Although pooled analysis is statistically less desirable, it was still considered appropriate in that each infusion represented a completely new occurrence in terms of degree of oligohydramnios, gestational age, volume infused, Δ AP and Δ AFI.

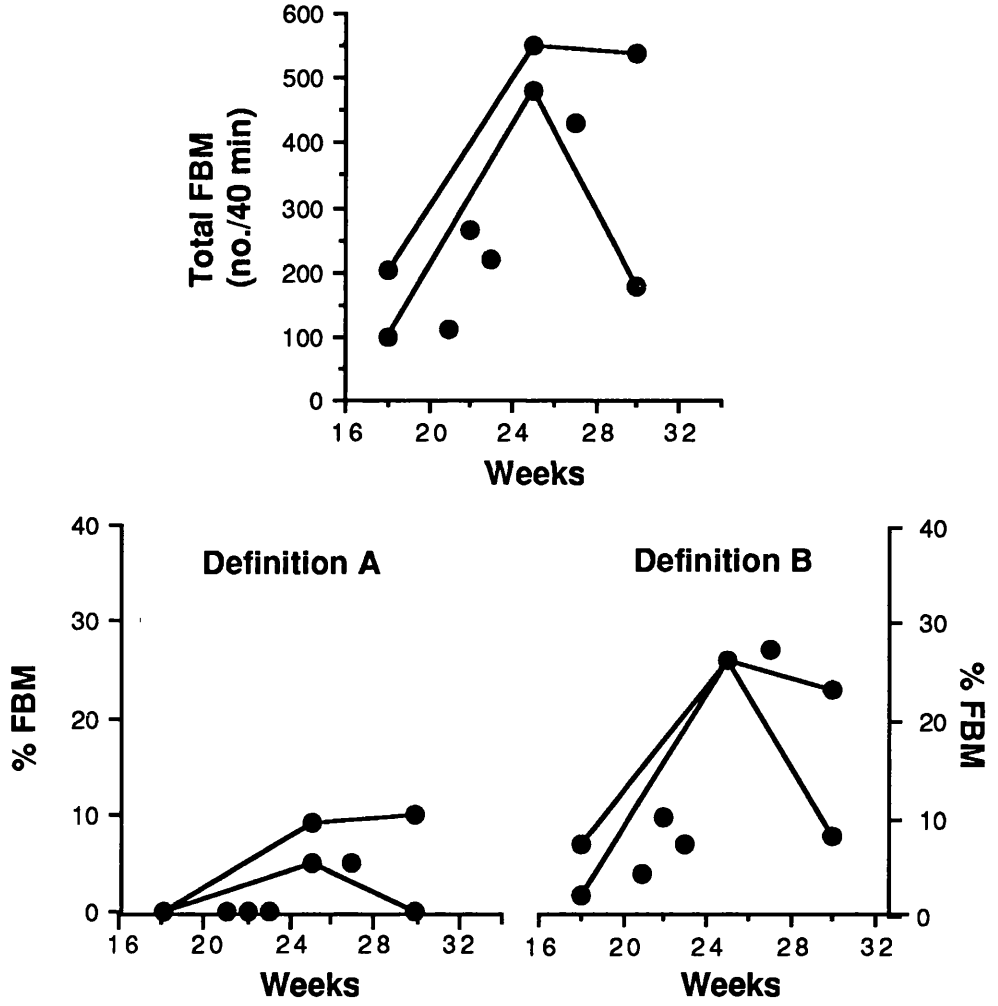

Figure 7.2: Pilot analysis of 10 control FBM recordings made in the presence of normal amniotic fluid volume, analysed for total breathing movements, and incidence of Definition A & B epochs. Note lines join the serial observations.

7.3.ii. Results

Single infusions: The total number of FBM per recording did not change significantly with amnioinfusion, either in the fluid retained or the fluid leakage group, as shown in Figure 7.3. Most

fetuses had no Definition A FBM epochs detected either before (n=10) or after infusion (n=12). All 6 with Definition A epochs present pre-infusion had no epochs present post-infusion, while all 4 with definition A epochs present after infusion did not have epochs present prior to infusion. In contrast, all but one fetus had Definition B FBM epochs present before, and all but 2 after infusion (Figure 7.4). There was no significant change in incidence of FBM with infusion in either the fluid retained or leakage group, using Definition A and Definition B (Table 7.1). There was similarly no significant change in duration of Definition B epochs in the fluid retained (median Δ -1s, range -15 to +10) or fluid leakage groups (median Δ 4s, -22.5 to +20.5). A comparable analysis with definition A was not possible, as most fetuses did not demonstrate any such epochs.

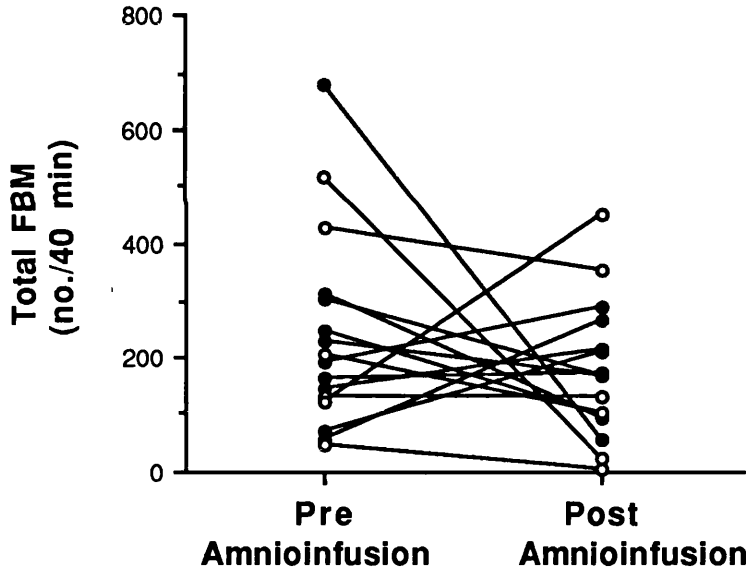

Figure 7.3: The number of FBM before and after amnioinfusion in those who leaked (open circles) and retained (closed circles) infused fluid.

In the fluid retained group, there was no significant correlation between Δ incidence FBM (Definition B) and gestational age, Δ AFI, Δ deepest pool, or volume infused. However, Δ incidence (Definition B) increased significantly with increasing Δ amniotic pressure expressed in mm Hg ($y=-25.0 + 8.8x$, $r=0.97$, $p<0.001$, where y=Δ % FBM and x= Δ amniotic pressure in mm Hg, Figure 7.5), but not in z scores ($r=0.3$, $p=0.4$). Similar correlations were observed with Δ total FBMs and epoch duration (Definition B); however only that with Δ incidence (Definition B)

remained significant when all 3 FBM variables were analysed by multiple linear regression (p=0.047). Multiple regression also indicated that Δ % FBM (Definition B) was highly significantly correlated (p<0.001) with both pre and post infusion AP in mm Hg. However the incidence of FBM (Definition B) did not correlate with baseline amniotic pressure, either before or after amnioinfusion. There was no significant correlation between Δ amniotic pressure in mm Hg and gestational age, volume infused, AFI or deepest pool, either as the pre, post or Δ value. When gestational age was added to a linear regression model of Δ incidence (Definition B) on Δ AP in mm Hg, the negative effect of gestation fell just short of statistical significance (p=0.053), suggesting that the above relationship may in part be attributable to gestation: this would explain the lack of significant correlation with AP in z scores. In the fluid leakage group, there was no significant correlation between Δ incidence (Definition B) and AP, expressed in either units.

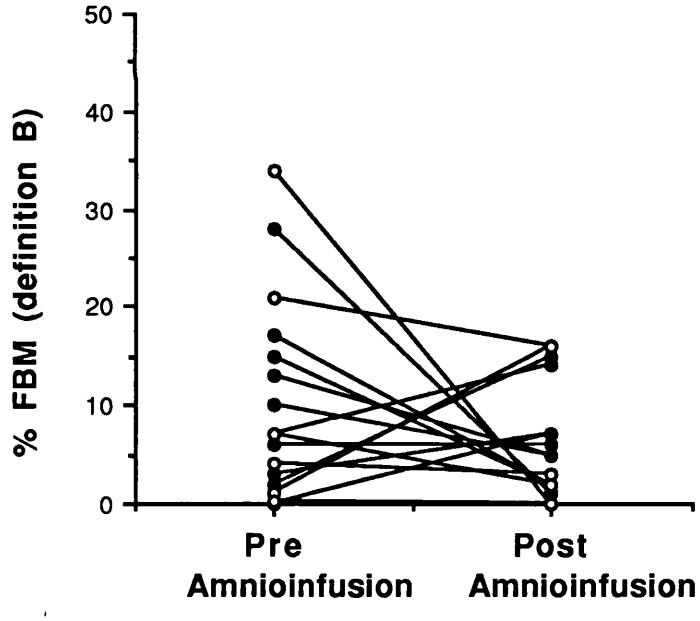

Figure 7.4: The incidence of FBM using Definition B (Moessinger et al 1987) before and after amnioinfusion in those who leaked (open circles) and retained (closed circles) infused fluid.

Amniotic fluid prostaglandin (PG) E_2 and $F_{2\alpha}$ concentrations fell significantly with amnioinfusion (mean Δ PGE_2=-64.2%, CI -82.0 to -46.4, p<0.001, and Δ $PGF_{2\alpha}$=-78.9%, CI -92.6 to -65.2, p<0.001).

Table 7.1: The effect of amnioinfusion on the number and incidence of FBM using two different definitions (A=Blott & Greenough 1988, B=Moessinger et al 1987). NS= no significant difference between fluid retained and fluid leaked groups. *NS*= not significantly different from zero.

	Fluid retained (n=10)	**Fluid leaked** (n=6)	**Significance** (retained vs leaked)
Total FBM (no.) (mean & CIs)			
Preinfusion	238 (129 to 347)	241 (91 to 391)	NS (unpaired t test)
Postinfusion	173 (127 to 219)	178 (32 to 323)	NS (unpaired t test)
Δ FBM	-72 (-218 to +74)	-64 (-273 to +145)	NS (unpaired t test)
	NS (paired t test)	*NS (paired t test)*	
Definition A (%) (median & range)			
Preinfusion	0 (0 to 7)	0 (0 to 11)	NS (Mann Whitney)
Postinfusion	0 (0 to 3)	0 (0 to 12)	NS (Mann Whitney)
Δ FBM	0 (-7 to +3)	0 (-11 to +12)	NS (Mann Whitney)
	NS (Wilcoxon)	*NS (Wilcoxon)*	
Definition B (%) (median & range)			
Preinfusion	8.5 (0 to 28)	5.5 (0.3 to 34)	NS (Mann Whitney)
Postinfusion	5.5 (1 to 15)	2.5 (0 to 16)	NS (Mann Whitney)
Δ FBM	-2.5 (-27 to +13)	-4.5 (-34 to +15)	NS (Mann Whitney)
	NS (Wilcoxon)	*NS (Wilcoxon)*	

Pooled infusions: When the single and subsequent infusions were considered together, there was no significant difference in Δ total FBM or Δ % FBM (Definition B) between infusions in which fetal blood sampling was performed and those in which it was not, both

overall and in the fluid retained group. With the pooled infusions, there was still no significant change in total FBM, or incidence using either definition. However, FBM (Definition A) were present more often than in the single infusions, presumably due to the later gestation of subsequent infusions (Figure 7.6).

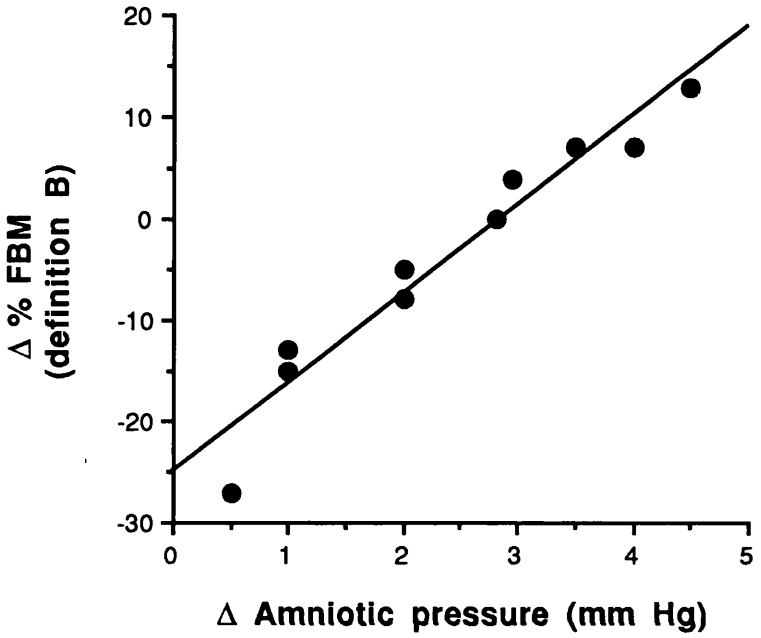

Figure 7.5: The association between the change in FBM incidence (Definition B) and the rise in AP with amnioinfusion (y=-25.0 + 8.8x, r=0.97, p<0.001, where y=Δ FBM (definition B) and x=Δ AP in mm Hg.

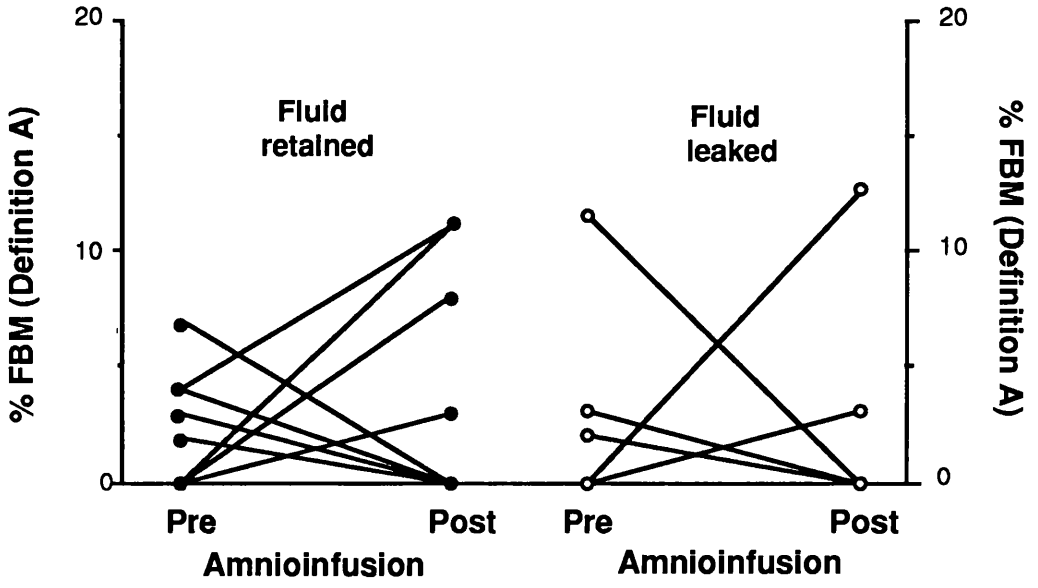

Figure 7.6: Comparison of the incidence of FBM using Definition A (Blott & Greenough 1988a) between all infusions in which fluid was retained (closed circles) and those in which it leaked out (open circles).

7.3.iii. Discussion

This study indicates that restitution of amniotic fluid volume in human pregnancies complicated by severe oligohydramnios does not acutely alter the incidence of FBM. There was similarly no change in pregnancies in which leakage of infused fluid prevented restoration of amniotic fluid volume. The latter pregnancies served as controls for the amnioinfusion procedure without amniotic fluid volume being restored.

The acute effect was studied because (i) this standardized for confounding variables by minimising the interval between recordings, and (ii) most of the subjects were outpatients referred long distances to the institution. It is possible that some aspect of the procedure other than restitution of amniotic fluid volume may acutely have prevented any increase in FBM, which instead might have been detectable later. Prostaglandins are known to inhibit FBM (Kitterman et al 1983), and transgressing the uterus with a needle or puncturing the fetal umbilical vein for blood sampling may theoretically have precipitated local prostaglandin release. However amniocentesis has no immediate effect on the incidence of FBM (Hill et al 1979), and Δ FBM in our study was similar whether or not fetal blood sampling was performed. Allowing for the diluent effect of saline, the fall in amniotic fluid prostaglandin concentrations with amnioinfusion does not support this procedure being associated with a major release of prostaglandins.

Observation periods of 30-100 minutes have previously been used to study human FBM (Roberts et al 1980, Pillai & James 1990). In this study, a relatively short interval of 40 minutes was chosen, out of concern for maternal tolerance of two recordings and an invasive procedure in the one session. The considerable variance in FBM incidence noted here has also been reported by others using longer recording periods (Pillai & James 1990), suggesting that increasing the observation period would be unlikely to exert a major influence on the results.

Great care was taken to minimize variability in FBM recordings, by ensuring that all recordings were made by the same person using the same apparatus. The need for a dedicated scanner unfortunately necessitated the use of an older machine, whose lack of appropriate output port precluded video recordings, by which intra-observer reproducibility could have been checked. Nevertheless, the data obtained in terms of incidence of FBM, in

the controls with normal amniotic fluid volume as well as in those with oligohydramnios, were broadly similar to that found in Pillai and James's recent longitudinal study (1990) involving over 400 hours of recording in normal fetuses. To minimize any effect of gestation, paired comparisons were used for pre- and post-infusion data .

These data support an increasing literature suggesting that impairment of fetal breathing is not the mechanism for oligohydramnios-related PH. Fetal breathing movements continue during acute (Moessinger et al 1985) and chronic oligohydramnios (Harding et al 1990) in fetal sheep. Indeed, Dickson & Harding (1991) have recently shown that FBM are not reduced throughout oligohydramnios of sufficient duration to produce PH. Experimental inhibition of FBM in addition to oligohydramnios produces more severe PH than oligohydramnios alone (Adzick et al 1984). Extrathoracic compression impairing FBM is considered unlikely as the major component of FBM is mediated by diaphragmatic rather than intercostal muscles (Dawes et al 1980) and experimentally intra-abdominal diaphragmatic compression has no effect on lung development (Sauer et al 1987). In fact, FBM are paradoxical in that the chest wall moves inwards during diaphragmatic contraction (Liggins 1984), and it is difficult to envisage how external compression could prevent this inward movement.

The group which claimed FBM were "absent' in fetuses with PH secondary to prolonged ruptured membranes (Blott et al 1987), later elaborated that this meant continuous epochs of FBM lasted <60 seconds (Greenough et al 1988), a definition used in the third trimester (Roberts et al 1980, Vintzileos et al 1983). The incidence of FBM however, is considerably lower in the mid-trimester, when the risk of oligohydramnios-related pulmonary hypoplasia is greatest (Wigglesworth & Desai 1981). Using their definition, FBM were "absent" in most fetuses in this study, both before and after infusion, in keeping with the pilot observations in mid-trimester fetuses with normal amniotic fluid volume (Figure 7.2). Pillai and James (1990) found that more than 75% of normal fetuses ≤28 weeks did not breathe in epochs of ≥ 60 seconds. The 60 second definition is thus inappropriate in the mid-trimester, the gestational period of the majority of fetuses in this study, and the gestational age at which most fetuses at risk of PH are likely to

be evaluated. Even with definition B, the median incidence of FBM prior to 26 weeks is normally only 1.4%, increasing to 20% between 26-30 weeks (Pillai & James 1990). The fact that the incidence of FBM is normally relatively low during the cannalicular period of lung development may explain the clinical observation that PH due to chronic inhibition of FBM is not always fatal, whereas that due to prolonged oligohydramnios, such as in renal agenesis, is.

The factors determining fetal breathing remain poorly understood. Using the more appropriate definition B, the incidence of FBM decreased in this experiment when the rise in AP was ≤ 3 mm Hg, but otherwise increased slightly (Figure 7.5). This may reflect some as yet uncharacterized fetal response to the amnioinfusion-induced rise in AP, since FBM incidence, either before or after infusion, did not appear determined by AP. However, the fact that no such association was found when Δ AP was expressed in z scores, suggests that instead this correlation may simply reflect a confounding effect of gestational age.

Analysis of the power of this study was limited by the non-parametric nature of the data, the inability to define population variability with definition A, and the inability to log-transform data which includes zero. Nevertheless, an approximation was obtained after log transformation of total FBM, such that with a one sided analysis with α=0.05 and 1-β=0.8, this study only had sufficient power to exclude a rise of 90%. Although this study indicates that "absent" FBM in oligohydramnios do not become "present" as a result of restoration of amniotic fluid volume, it remains possible that smaller alterations in FBM may be present in oligohydramnios.

7.4 Relief of presumed "compression"-effect on fetal Doppler waveforms

7.4.i. Methods

Sixteen women with severe oligohydramnios in singleton pregnancies scheduled to undergo clinically-indicated amnioinfusion between 17-28 weeks (median 22.5) were enrolled in this study. Warmed 0.9% NaCl solution (50-350 ml) was infused transabdominally as described earlier in this chapter. The deepest vertical pool and AFI were measured before and immediately after infusion, as was AP. The aetiology of oligohydramnios was classified

after infusion as follows: PPROM in 7, bilateral fetal renal pathology in 4, IUGR in 2, and idiopathic in 3. Three had a fetal abdominal circumference measurement <5th centile, two in the IUGR group and one in the renal pathology group. Fetal blood sampling for rapid karyotyping was performed in 8 cases; if sufficient blood was obtained, blood gas analysis was performed as described previously and results compared to reference ranges (Chapter 5.3.i).

Umbilical artery Doppler waveforms were obtained before and immediately after amnioinfusion. Recordings were made with the mother lying semi-recumbent in approximately 15° lateral tilt, using a continuous wave machine (Vasoflo 4, Oxford Sonicaid, Oxford), as described in Chapter 5.4.i. Waveforms were recognized as being from the umbilical artery by all of the following: (i) asynchrony with the maternal pulse (ii) their characteristic appearance (iii) demonstration of a non-pulsatile venous waveform in the opposite channel. The Doppler index of downstream resistance used was the pulsatility index (PI=[x-y]/z where x=peak systolic velocity, y=end-diastolic velocity, and z=mean velocity). Each reading was obtained during fetal apnoea by "freezing" the image when the coefficient of variation of the PI values in 5 consecutive waveforms was <5%. The mean of each of 3 means obtained from 5 consecutive waveforms was then used for statistical analysis. Fetal heart rate (FHR) was similarly calculated on line, from the time interval between 5 consecutive waveforms. PI values were compared to a published cross-sectionally derived reference range based on large numbers (c.500), the upper limit of which fell from 1.60 to 1.45 over the gestational period of patients in this study (Ferrazzi et al 1990).

Prior to infusion, the deepest pool and AFI were ≤1.0 and ≤2.3 cm respectively, and mean AP was -1.2 z scores (CI -1.8 to -0.6). Vaginal leakage occurred during the procedure in 5 patients. Amnioinfusion significantly increased amniotic fluid volume (mean Δ AFI=9.8 cm, CI 7.9 to 11.7) in 11 women in whom fluid was retained. In contrast, there was only minimal increase in 5 patients, in whom vaginal leakage of fluid was noted during the procedure (Δ AFI=1.0 cm CI 0.2 to 1.8). The deepest pool and AFI post-infusion were both significantly greater in the fluid retained (mean 3.7, CI 3.1 to 4.4 and 10.2, CI 8.4 to 12.0 respectively) compared to the fluid leakage group (mean 0.9, CI 0.7 to 1.2, $p<0.001$ and 1.5, CI 0.8 to 2.2, $p<0.001$).

Changes in variables with infusion were expressed as Δ variables (=postinfusion-preinfusion value). Parametric statistics were used only where Gaussian distributions were evident on histograms. Comparisons were made using paired or unpaired t-testing as appropriate.

7.4.ii. Results

Amnioinfusion had no significant effect on PI in either the fluid retained or fluid leakage groups (Table 7.2), and Δ PI was similar in the both groups. In addition, there was no significant change in FHR. There was no significant relationship in either group between Δ PI and Δ FHR.

Table 7.2: The effect of amnioinfusion on umbilical artery PI and fetal heart rate, in those that leaked and those that retained the infused fluid. NS= no significant difference between fluid retained and fluid leaked groups. *NS= not significantly different from zero.*

(mean & CIs)	**Fluid retained** (n=11)	**Fluid leaked** (n=5)	**Significance** (retained vs leaked)
Pulsatility index			
Δ PI	-0.07 (-0.17 to +0.07)	-0.11 (-0.31 to +0.09)	NS (unpaired t test)
	NS (paired t test)	*NS (paired t test)*	
Fetal heart rate			
Δ FHR	-4.9 (-10.2 to +0.4)	-7.7 (-21.7 to +6.3)	NS (unpaired t test)
	NS (paired t test)	*NS (paired t test)*	

Three pregnancies had raised umbilical artery PI values; all had IUGR, and all were in the fluid retained group (Figure 7.7). Of these, one had absent, and another reverse end-diastolic frequencies, and both had fetal pH and pO_2 values beneath the reference range. The mean PI in these 3 fell significantly with amnioinfusion (-0.18, CI -2.4 to -1.2, p=0.03), whereas in those whose preinfusion PI was within the reference range, Δ PI was not significantly different from zero (mean -0.03, CI -0.13 to +0.10). To exclude the result in those with raised values being due to their greater variation around the mean, the change in PI was also expressed as a % of the preinfusion value. However, when so

expressed, the finding of a significant fall in those with an initially raised value (-8%, CI -10 to -6, p=0.02) but not in those with a normal value (-2%, CI -16 to +11), remained. The difference in Δ PI however, expressed either as the raw figure or as a percentage, was not significantly different between the two groups. FHR did not change significantly with infusion in the the 3 with raised PI values (mean Δ FHR 10.9/min, CI 2.3 to 19.5, p=0.13).

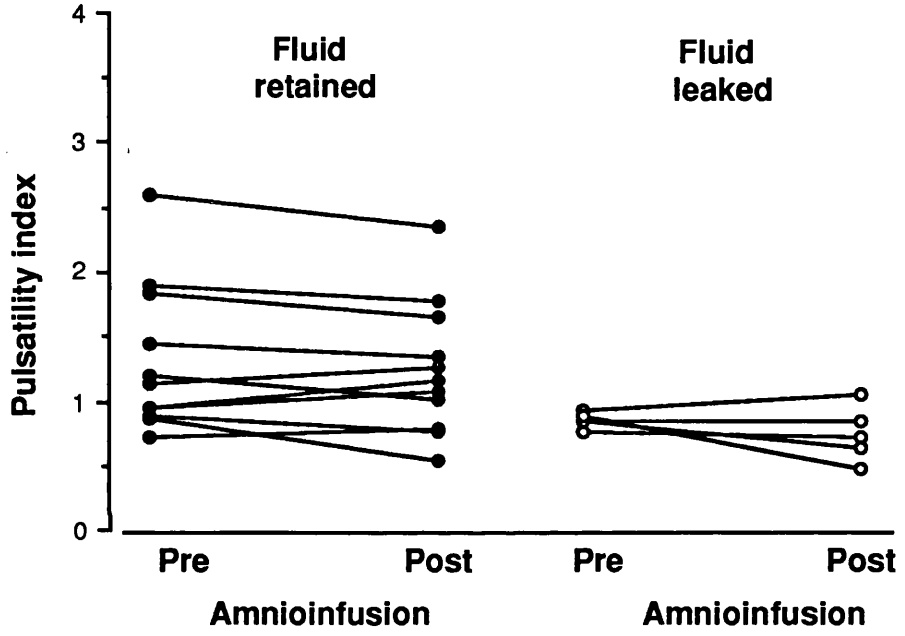

Figure 7.7: Umbilical artery PI values before and after amnioinfusion in pregnancies in which fluid was (filled circles) and wasn't retained (open circles).

In the fluid retained group, there was no significant difference in Δ PI, either as the raw value or as a percentage of the preinfusion value, between fetuses which underwent blood sampling at the time of amnioinfusion and those which did not. There was no significant correlation between Δ PI, either as the raw value or as a percentage of the preinfusion value, and gestational age, volume infused, Δ FHR, Δ AFI, Δ AP in mm Hg, or Δ AP in z scores.

7.4.iii. Discussion

This study suggests that the majority of fetuses with severe oligohydramnios have normal umbilical artery Doppler waveforms. Only 3 of 16 had a raised PI value, and each was SGA, suggesting that abnormal umbilical artery Doppler waveforms in

oligohydramnios may reflect increased downstream resistance as part of the underlying placental pathphysiology of IUGR (Giles et al 1985). Consistent with this, Cruz et al (1988) noted in 43 patients with oligohydramnios that raised umbilical artery systolic/diastolic ratios were identified significantly less frequently in those with ruptured compared to intact membranes, the latter having a greater frequency of SGA fetuses. In addition, Lombardi et al (1989) found elevated systolic/diastolic ratios in 9 of 21 patients with oligohydramnios and intact membranes, each of whom had a birth weight <10th centile. Although Hackett et al (1987) studied fetal aortic and not umbilical artery waveforms, they similarly found that all 14 oligohydramnios pregnancies with abnormal waveforms were SGA, in contrast to the 18 with normal waveforms which were AGA.

These results do not support the suggestion that cord "compression" alters the umbilical artery Doppler waveform in pregnancies with oligohydramnios (Van den Wijngaard et al 1987). Not only was there no change in PI with restoration of amniotic fluid volume, but the fall in PI which did occur with amnioinfusion in the 3 with a raised initial value was of <10%. This contrasts with a fall of 35% reported by Van den Wjingaard et al (1987). They however did not give details of FHR, and it remains possible that their results could be attributable, at least in part, to fetal tachycardia induced by some aspect of the procedure such as fetal blood sampling or the infusate not being at body temperature. FHR has been shown in several studies to be negatively correlated with Doppler indices of downstream resistance (Mires et al 1987, Kofinas et al 1989), one study suggesting that an increase of 40/minute would result in a 40% fall in systolic/diastolic ratio (Brar et al 1989). In this study, amnioinfusion had no significant effect on FHR.

Any change that occurred in PI with infusion was small and confined to those with initially raised values; in this regard it should be noted that the preinfusion PI in Van Den Wjingaard et al's case report was only 1.4, a figure still within the reference range used herein.

Although most of the data used to implicate cord "compression" in pregnancies with oligohydramnios is from cardiotocographic studies in late pregnancy, Doppler assessment of the umbilical artery was used in this study in view of (i) the

earlier gestation of patients undergoing antepartum amnioinfusion, rendering cardiotocography impractical, and (ii) the need to reproduce the findings of Van Den Wjingaard et al (1987). The pulsatility index was chosen as the index of downstream resistance for study, as it is still yields a figure when end-diastolic frequencies are absent or reversed. As with the FBM study earlier in this chapter, pregnancies in which the infused fluid leaked out prior to the postinfusion Doppler served as controls for the amnioinfusion procedure without amniotic fluid volume being restored. Again the acute effect only was studied as outlined in Chapter 7.3.iii.

7.5 Discussion

In these studies of pregnancies with oligohydramnios, restoration of amniotic fluid volume did not alter the umbilical artery PI or the incidence of FBM, providing no support for the suggestion that fetal 'compression" in oligohydramnios impairs fetal breathing or fetoplacental blood flow. Similarly, the incidence of gross fetal body movements has recently been shown not be reduced in the presence of oligohydramnios, nor, anecdotally, to increase with restoration of amniotic fluid volume (Sival et al 1990).

As discussed in Chapters 1.6.vi. and 7.1, the assumption that the fetus is compressed in oligohydramnios, and that compression is the mechanism for the pulmonary, soft tissue and FHR sequelae, was not based on any scientific data. The demonstration that the pressure surrounding the fetus in oligohydramnios is low, together with the lack of effect of restoration of amniotic fluid volume on the fetal biophysical variables discussed above, suggests that reappraisal of this concept of fetal compression is now warranted.

This finding of low AP in oligohydramnios is not surprising if the law of Laplace is considered (Figure 7.8). A decrease in the quantity of amniotic fluid sufficient to cause persistent contact between fetal parts and the uterine wall isolates pockets of amniotic fluid. As the theoretical radius of these amniotic fluid pockets increases with decreasing amniotic fluid volume, AP should thus fall.

Nevertheless, the finding in oligohydramnios of low AP in the presence of soft tissue manifestations considered indicative of compression (talipes, flattened facies, arthrogryposis etc) appears paradoxical. However, as illustrated in Figure 7.8, amniotic fluid no

longer completely surrounds the fetus in severe oligohydramnios. Thus with decreasing amniotic fluid volume, the entire tension of the uterine wall becomes exerted over some fetal parts (head, extremities) that happen to be the internal "pillars" of the uterus. Fetal compression by the surrounding uterine wall could thus still be mediated through these pillars, even though pressure in the amniotic fluid pockets is low. However, Harding et al (1990) have recently shown in sheep that pressures within fetal body cavities (i.e. pleural, peritoneal) do not change with removal and then restoration of amniotic fluid volume, which suggests that direct transmission of uterine tone to the fetal trunk via splinted extremities does not occur.

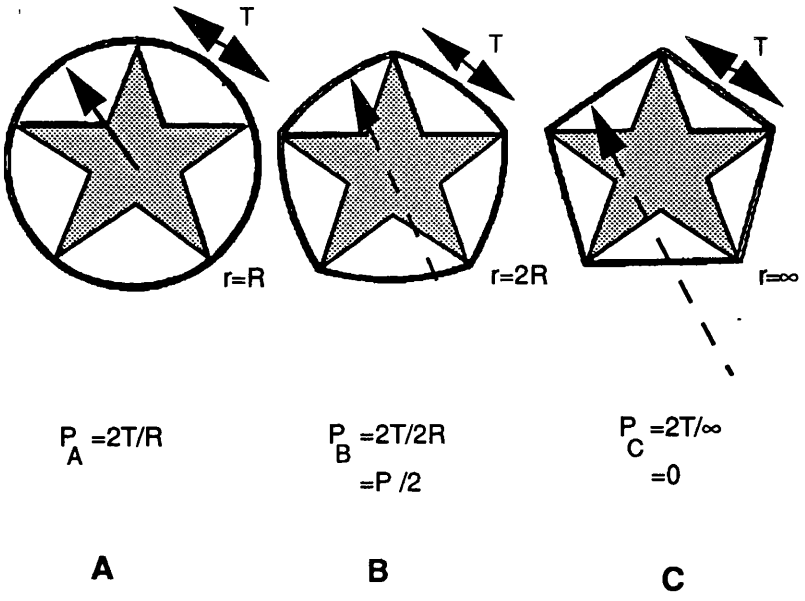

Figure 7.8: Diagrammatic representation of changes in fluid pressure within the amniotic cavity with progressive reduction of amniotic fluid volume, according to the law of Laplace. A: Normal amniotic fluid volume. If the uterus is considered a sphere of radius (R) and wall tension (T), the pressure exerted on the amniotic fluid is $P_A=2T/R$. The fetus is represented as a star object that almost touches the inner surface of the uterus. B: Mild oligohydramnios. The radius of curvature of the uterine wall theoretically increases with reduction in fluid. In the example, when the radius increases to 2R, the pressure in the pocket decreases to half P_A. C: Severe oligohydramnios. With further reduction of fluid, some segments of uterine wall become flattened and r becomes infinite. Fluid pressure is now 0 for any value of T.

Accordingly, fetal soft tissue manifestations of oligohydramnios may instead be due to restriction of movement by the uterine wall. Indeed several authors have speculated that immobility of the fetal extremities is implicated in the aetiology of

these sequelae (DeMyer & Baird 1969, Thibeault et al 1985, Pringle 1986). Fetal immobility is known to produce arthrogryposis in a variety of experimental animals (Drachman & Coulombre 1962, Swinyard 1982). Moessinger (1983) demonstrated in rats that fetal curarization produced in addition to arthrogryposes, bilateral talipes and flattened facies, features similar to those reported after oligohydramnios.

7.5 Summary

Fetal breathing movements and umbilical artery Doppler waveforms were recorded before and immediately after amnioinfusion in pregnancies with severe oligohydramnios. There was no significant difference in the change in total breathing movements or in the change in incidence of FBM between the 10 in which amniotic fluid volume was restored, and 6 in which amniotic fluid volume was not restored due to vaginal leakage. In both groups, there was no significant change with infusion in number (mean Δ = -72, CI -218 to +74 and -64, CI -273 to +145 in the fluid retained and leakage groups respectively) and incidence of FBM (median Δ= -2.5%, range -27 to +10 and -4.5%, range -34 to +15 respectively). Similarly, there was no significant difference in the change in umbilical artery pulsatility index between 11 in which amniotic fluid volume was restored, and 5 in which it was not. Again, there was no change with infusion in PI in the fluid retained group (mean Δ PI = -0.07, CI -0.17 to +0.07) or the leakage group (-0.11, CI -0.31 to +0.09). These studies suggest that restitution of amniotic fluid volume in human pregnancies complicated by severe oligohydramnios does not acutely alter the incidence of FBM, or the umbilical artery PI. Together with the finding of low amniotic pressure in oligohydramnios (Chapter 6), they challenge the concept of fetal compression in oligohydramnios that has become widely accepted in the literature.

CHAPTER 8: LOW AMNIOTIC PRESSURE AND LUNG DEVELOPMENT IN OLIGOHYDRAMNIOS

8.1 Background

Much of our current understanding of fetal lung development is based on experimental work in sheep. The lungs of late-gestation fetal lambs contain approximately 30 ml / kg body weight of lung liquid (Normand et al 1971, Scarpelli et al 1975), which is produced in the developing airways at a rate of 4.5 ml/kg body weight/hour (Mescher et al 1975). Lung liquid travels from the alveolae along the upper airways, from where it is either swallowed or drains into the amniotic cavity, the direction of net flow being away from the lungs both during fetal breathing and during apnoea (Harding et al 1984a, Harding et al 1986a). Little if any amniotic fluid enters the lungs in non-acidaemic fetuses (Adams et al 1967, Block et al 1981, Harding et al 1986a, Harding et al 1986b).

The volume of lung liquid within the airways determines the degree of lung expansion, and seems a gross determinant of lung growth. Tracheal ligation in both fetal sheep and rabbits leads to an increase in lung weight and volume (Carmel et al 1965, Alcorn et al 1977), and ligation of a main stem bronchus in fetal lambs produces ipsilateral lung hyperplasia, as evidenced by increased weight and DNA content (Moessinger et al 1990). Conversely, chronic lung liquid drainage in fetal sheep reduces lung:body weight ratios by 30-40% (Alcorn et al 1977, Fewell et al 1983). It has thus been suggested that lung liquid acts as an internal stent around which the lung grows (Alcorn et al 1977, Adzick et al 1984).

Normally, lung liquid escapes from the upper airways at a rate approximately 3-8 times as great during FBM as during apnoea (Harding et al 1984a, Harding et al 1986a). As its secretion rate is unaltered by the presence or absence of FBM (Fewell et al 1981), episodes of FBM lower lung volume, which is then restored during intervening episodes of apnoea (Dickson et al 1987). There is a standing supra-amniotic pressure within the fetal trachea of 1.5-3.0 mm Hg (Vilos & Liggins 1982, Fewell & Johnson 1983, Moessinger et al 1985), considered by most workers to result from the resistance to egress of lung fluid provided during apnoea by an active laryngeal retentive mechanism (Adams et al 1967, Harding et al 1980).

This resistance plays a pivotal role in maintaining lung liquid

volume in utero, as when it is bypassed in fetal tracheostomy experiments, the normal tracheal-amniotic pressure gradient is eliminated (Fewell & Johnson 1983), lung liquid loss increases (Harding et al 1986a), and lung development is impaired (Alcorn et al 1977, Fewell et al 1983). Although the exact mechanism of oligohydramnios-related PH is not known, it involves loss of lung liquid from the airways via the larynx. In fetal sheep exposed to chronic oligohydramnios, the volume of lung liquid within the airways is reduced by up to 65%; contemporaneous measurement of tracheal flow rates indicated that this could not be accounted for solely by the reduction in secretion rate, which was proportionately smaller (Dickson & Harding 1989). Tracheal occlusion in both humans and experimental animals appears to prevent the adverse pulmonary effects of oligohydramnios. In rabbit experiments in which oligohydramnios was created by draining amniotic fluid into the maternal peritoneal cavity, Adzick et al (1984) found lower lung weight and DNA content compared to controls, but no reduction in these parameters in the presence of oligohydramnios when the fetal trachea was ligated. This is further supported by anecdotal reports of human fetuses with laryngeal atresia whose lungs, following prolonged oligohydramnios secondary to renal agenesis / dysgenesis, did not become hypoplastic (Wigglesworth et al 1987, Scurry et al 1989).

As discussed in Chapter 7.3, an accumulating literature now suggests that cessation of FBM is unlikely to be the mechanism for oligohydramnios-related PH. Nevertheless, Roberts et al (1991) have recently shown in women with PPROM that FBM are reduced in incidence by 60%, but only for the first 2 weeks after amniorrhexis. Although the study of Δ FBM with amnioinfusion in the last chapter suggested that severe oligohydramnios does not result in "absent" FBM which return with restoration of amniotic fluid volume, it was of insufficient power to exclude an association between oligohydramnios and reduced FBM. It remains possible that a reduction in FBM during oligohydramnios may represent an attempt by the fetal central nervous system to restore lung liquid volume, as the rate of lung liquid escape during FBM exceeds that during apnoea (Harding et al 1984a, Harding et al 1986a). In this respect, Dickson & Harding found the incidence of FBM in sheep to be significantly less (by c.20%) in the first hour after acute reduction in lung volume, facilitating restoration of lung liquid

volume by passive means. FBM during chronic lung liquid drainage leading to PH have not been reported. Thus any reduction in FBM in oligohydramnios could represent a fetal central nervous system response to chronic lung liquid loss to limit fluid escape, rather than part of the underlying pathogenic mechanism of oligohydramnios-related PH.

The net outflow of lung liquid along the trachea must adhere to general principles of fluid dynamics (intra-alveolar pressure must exceed AP). Therefore, the increased escape of lung liquid in oligohydramnios must be due to an increase in the alveolar-amniotic pressure gradient, either secondary to an increase in alveolar pressure, such as might occur if the thorax was compressed, or to a reduction in AP. Since external compression is now considered unlikely, as discussed in Chapter 7.5, the role of low AP in the pathogenesis of oligohydramnios-related PH warrants consideration.

Harding et al (1984a) found a mean outflow of fetal sheep lung liquid of 7.8 ml/hour in fetal sheep. During apnoea, the larynx was normally closed and leakage outflow was 2.5 ml/hour; thus fluid was accumulating at a rate of approximately 5 ml/hour. During fetal breathing, outflow increased to 12.9 ml/hour, releasing the accumulated fluid. In the normal fetus, this apnoeic leakage occurs at a tracheal-amniotic pressure difference of approximately 2 mm Hg. If AP were reduced by approximately 3 mm Hg, as found in severe oligohydramnios (Chapter 6), the pressure gradient drawing the fluid through the closed larynx becomes 5 (=2 + 3) mm Hg, and the flow rate should increase to 2.5 x 5/2 or 6.3 ml/hour. This flow rate is greater than the rate at which lung fluid is being produced, so the volume of lung liquid within the airways should decrease.

Accordingly, a novel hypothesis for the mechanism of PH in oligohydramnios is suggested by the findings of Chapter 6: a reduction in AP disturbs the normal tracheal-amniotic pressure gradient thereby increasing lung liquid escape and impairs lung development.

8.2 Aims

The aims of this section were as follows:

i. to confirm in fetal sheep the presence, and to determine the magnitude, of pressure gradients normally present within the upper airways

ii. to determine in fetal sheep the effect on lung development of eliminating the tracheal-amniotic pressure gradient

iii. to determine the effect of ii. above on FBM

iv. to develop a model for mimicking the effect on the upper fetal airways of low AP, in the presence of normal amniotic fluid volume

v. to determine the effect on fetal lung development of mimicking low AP at the upper airway without creating oligohydramnios.

8.3 Pressure gradients in the upper airways

8.3.i. Methods

Chronically-instrumented fetal sheep were prepared as described in Chapter 5.5.i. Briefly, general anaesthesia was induced in 3 cross-bred ewes at 118-120 days gestation, one horn of the gravid uterus was exposed at laparotomy and the fetal head delivered through a uterine incision. Polyvinyl catheters (ID 1.0 mm, OD 2.0 mm) were implanted into a fetal carotid artery and a fetal jugular vein. Additional polyvinyl catheters for pressure measurement were fenestrated at one end and implanted into the amniotic cavity, the fetal trachea, and the fetal pharynx. The pharyngeal catheter was positioned in the posterior aspect of the oropharynx approximately 2 cm above the larynx and secured with sutures to the buccal membrane lining the cheek to avoid dislodgment. The tracheal catheter was positioned below the cricoid cartilage and above the carina, while the amniotic catheter was fixed 2 cm from its end to the skin over the fetal praecordium. The fetus was replaced, the uterus closed in 2 layers, and all catheters exteriorized through the ewe's right flank. Five days were allowed for postoperative recovery, during which time antibiotics were administered to ewe and fetus.

In each animal, pressure recordings were made between 123-127 days gestation with the ewe in the standing position. After fetal pH and blood gas values had been shown to be normal (mean $\pm$ SE pH 7.31 $\pm$ 0.01, pCO_2 40.9 $\pm$ 1.6 mm Hg, pO_2 21.7 $\pm$ 1.7 mm Hg), pressures were measured with standard biomedical transducers attached to fluid filled lines and electronically subtracted pharyngeal-amniotic (P-A) and tracheal-amniotic (T-A) pressures recorded on a polygraph run at either 1.25 or 1.5

cm/min. Prior to each recording, all lines and transducers were flushed with saline (0.9% sodium chloride) to ensure the absence of bubbles. The transducers were then zeroed to within 0.5 mm Hg, and the amplifier gains checked by elevating a fluid-filled line connected to the manometer vertically in 6.8 cm (corresponding to 5 mm Hg) increments, similar to the calibration procedure described for human recordings (Chapter 2.1.iii). This procedure was repeated after 10 minutes of a 60 minute recording period: if any drift in gain and/or zero was >0.5 mm Hg, the trace was abandoned, the amplifiers settings corrected and a new recording period started. At the conclusion of the experiments, animals were killed with pentobarbitone (4g intravenously), and the position of the catheters confirmed at necropsy.

Each minute of pressure trace was examined for negative pressure fluctuations consistent with fetal breathing, gasping or swallowing activity, and such minutes (range 12 to 28 min/h) excluded from further analysis. Point estimates of T-A and P-A pressures to the nearest 0.5 mm Hg were then obtained manually at the midpoint of each stable minute, and a mean per hour calculated. A pooled mean was calculated from the individual means per animal. The data were analysed was by standard parametric statistics using paired or non-paired t-testing as appropriate. Within-recording variability was calculated from individual SDs from each of the 9 one hour traces, and between-recording variability from the mean SD from each animal's 3 recordings.

8.3.ii. Results

Mean values for resting T-A and P-A pressures in the 3 animals are shown in Figure 8.1. There were significant pressure gradients between the trachea, pharynx and amniotic cavity. Both T-A pressure (mean +1.5 $\pm$ 0.2 [SE] mm Hg) and P-A pressure (mean +0.7 $\pm$ 0.1 mm Hg) were significantly positive, i.e. greater than AP ($p=0.008$, and $p=0.011$ respectively, one tailed t test). and tracheal pressure was significantly greater than pharyngeal when both were referenced to AP ($p=0.005$, 2 tailed test).

Within-recording variability was similar for T-A (mean SD 1.2 $\pm$ 0.2 mm Hg) and P-A pressure (mean SD 0.9 $\pm$ 0.1 mm Hg), and not significantly different from between-recording variability (0.8 $\pm$ 0.1, and 0.9 $\pm$ 0.1 respectively).

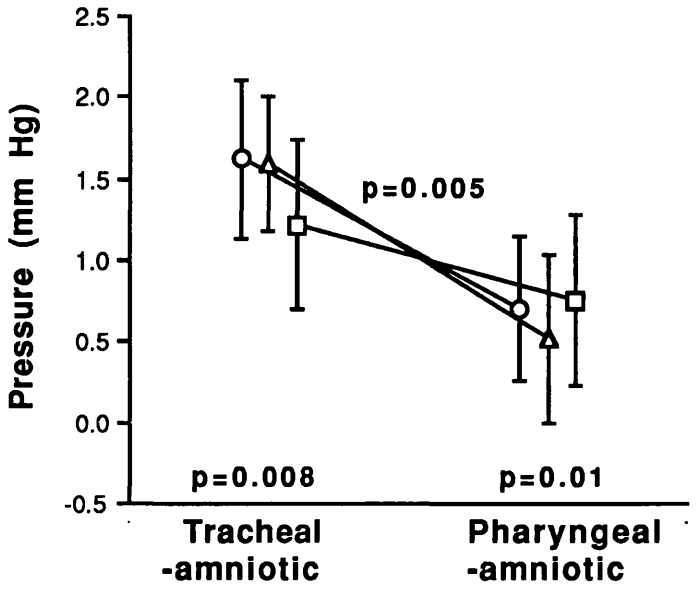

Figure 8.1: Normal tracheal-amniotic and pharyngeal-amniotic pressure gradients in 3 late-gestation fetal sheep. Each symbol represents each animal's mean ± SE from 3 recordings on consecutive days. Both T-A and P-A pressures are significantly different from zero (i.e.amniotic, $p \leq 0.01$), and T-A pressure (mean 1.5 ± 0.2 mm Hg) is significantly greater than P-A pressure (0.7 ± 0.1, $p=0.005$).

8.3.iii. Discussion

These data confirm in late gestation fetal sheep the existence of a small tracheal-amniotic pressure gradient. Measurements were made using simple fluid filled lines attached to standard transducers, similar to those used in the human work (Chapters 2-6). As fetal breathing, gasping, hiccoughing and swallowing movements all produce negative fluctuations in intrathoracic pressure, great care was taken to make readings only from stable minutes of the T-A or P-A pressure trace. The variability found in these recordings seems acceptable when one considers the limit of resolution of the technique, the long duration of each trace and the small number of animals studied. Vilos & Liggins (1982) and Moessinger et al (1985) found slightly smaller between-trace SDs (0.3 to 0.6 mm Hg), although these may in part be attributed to the use of only a few point estimates (1-3) and larger numbers of animals. Standard deviations rather than coefficients of variation have been chosen to express variability in this chapter, as they were in other studies, because the small means engendered by T-A and P-A being subtraction measurements close to zero renders coefficients of variation implausibly large.

The standing tracheal pressure of 1.5 mm Hg found in this study is consistent with previous studies in late-gestation sheep, Moessinger et al (1985) finding a mean T-A pressure of 1.2 mm Hg and Vilos & Liggins (1982) a mean of 1.8 mm Hg. Another study (Fewell & Johnson 1983) reported that T-A pressure during apnoea ranged from 1.5 to 3.0 mm Hg, but this was based on a descriptive rather than statistical analysis. This resistance to egress of lung fluid during apnoea, which can be abolished by tracheostomy (Fewell & Johnson 1983), is considered by most workers to be due to an active laryngeal retentive mechanism. Adams et al (1967) observed that laryngeal constriction prevented outflow of radio-opaque dye instilled into the trachea of exteriorized fetal lambs, and were the first to suggest that the fetal larynx regulates lung volume by controlling the outflow of lung liquid. In this regard, lung liquid efflux during apnoea increases when the upper respiratory tract is bypassed in fetal sheep (Harding et al 1986a). Furthermore, during episodes of apnoea, the laryngeal adductor muscles have been shown to be tonically active (Harding et al 1980), and when their activity is halted by recurrent laryngeal nerve denervation, the standing tracheal pressure is eliminated and lung fluid efflux increases (Harding et al 1984a, Harding et al 1986b).

In contrast, Fewell & Johnson (1983) have claimed that the major resistance to lung liquid egress occurs not at the larynx, but in the buccal cavity. They found supra-amniotic pressures in the oropharynx of fetal sheep similar to those in the trachea, although again these data were descriptive and thus must be interpreted with caution. Furthermore, the standing tracheal pressure could be eliminated by inserting a tube from the pharynx into the amniotic cavity, although this was based on observation of 2 sheep only. They nevertheless suggested that the standing tracheal pressure during apnoea results from the formation of lung fluid either into a closed compliant pharyngeal/buccal cavity or against a high oral or nasal resistance. The existence of such a pharyngeal resistance seems plausible, given that the fetal mouth appears normally to be closed, based both on direct observation (Rigatto 1984), and on the inability to aspirate amniotic fluid from the pharynx (Harding et al 1986b).

The pressure results in this section suggest that there does exist a degree of resistance to lung liquid efflux in the pharynx,

although to a lesser extent than in the larynx. Harding et al (1986b) similarly found positive supralaryngeal resistances to fluid outflow in 3 of 6 fetal sheep, although mean laryngeal resistance remained significantly greater than mean supralaryngeal resistance. However, only laryngeal and not supralaryngeal resistance paralleled laryngeal adductor electromyographic activity (Harding et al 1986b), suggesting that the small positive P-A pressure may instead be determined by other motor activities influencing oropharyngeal volume, such as swallowing and mouth opening.

The methodology used herein yielded results for upper airway pressures comparable to those in the literature, and was thus considered suitable for use in controlled experiments to determine the effect on fetal lung development of altering upper airway-amniotic pressure gradients.

8.4 Effect of eliminating tracheal-amniotic pressure gradient on fetal lung development

8.4.i. Methods

Experimental protocol: Eight cross-bred ewes with twin pregnancies at 112-113 days gestation were prepared as described earlier. One fetus was chronically instrumented to determine the effect on fetal pulmonary development of bypassing the tracheal resistance by chronic lung liquid drainage, while its uninstrumented twin acted as a control.

Under general anaesthesia, one horn of the gravid uterus was exposed at laparotomy and a fetal head delivered through a uterine incision. Catheters for vascular access (ID 1.0 mm, OD 2.0 mm) were implanted into a fetal carotid artery and a fetal jugular vein. Bipolar electrodes (Cooner Wire Ltd, California) were placed in the fetal diaphragm to measure electromyographic activity (EMG). The upper trachea was ligated below the cricoid cartilage, while the lower end was cannulated for chronic drainage with a polyvinyl tube (ID 4.5 mm, OD 6.0 mm, 180 cm long). Catheters for pressure measurement were fenestrated at one end and implanted into the trachea (ID 1.0 mm, OD 2.0 mm) alongside the tracheostomy tube, and the amniotic cavity (ID 2.0 mm, OD 3.0 mm) positioned as in Chapter 8.3.i. The experimental fetus was replaced, and the uterus closed in 2 layers. In each pregnancy, only one twin was catheterized, allowing the other to serve as a control. All catheters were exteriorized through the ewe's right

flank. A drainage bag (SE4 Closed System, Svend-Anderson, Denmark) was attached to the distal end of the tracheostomy tube and positioned on the floor, approximately 40 cm beneath the lowermost point of the ewe's abdomen. Five days were allowed for postoperative recovery, during which time antibiotics were administered to ewe and fetus. Fetal arterial pH and blood gas values were then demonstrated to be normal (mean ± SE pH 7.33 ± 0.01, pCO_2 46.9 ± 2.1 mm Hg, pO_2 21.3 ± 1.7 mm Hg).

The drainage bag was emptied every 2-3 days and the equivalent volume of normal saline (0.9% NaCl) warmed to 38-39°C infused intra-amniotically to avoid causing oligohydramnios. Samples of lung liquid were frozen for later analysis (Chapter 8.5.ii). One hour recordings of T-A pressures were measured on two separate days during the drainage period as described earlier.

Lung specimens: On Day 21 of drainage (133-134 days gestation), the ewes were killed with pentobarbitone (4g intravenously). At necropsy, both twins were delivered, towel dried and weighed. The fetal tracheas were transected 0.5 cm above the carina, and lung liquid allowed to drain passively. The lungs were were then removed, dissected and weighed separately. In order to control for any difference in lung expansion in utero between the tracheally-drained and the control lungs, specimens were inflated and fixed under constant pressure. The left main bronchus from each twin was cannulated and the left lung inflated with buffered formalin under constant pressure (25 cm formalin for 30 minutes followed by 15 cm for 11.5 hours) (Figure 8.2). The main bronchus was then tied off beneath the cannula, the lung removed and reweighed in both air and water. Based on water displacement, the lung's volume thus equalled its weight in air minus its weight in water, allowing calculation of its specific gravity (weight in air/volume).

Five representative blocks approximately 2 mm thick were obtained from both lobes and were routinely processed and embedded in araldite. Morphometric studies were performed on toluidine-blue stained semithin sections. Sections in which staining was suboptimal or in which large vascular or airway structures predominated, were excluded. Five fields of view per section were examined microscopically (x40) from each of 5 representative blocks. A fixed area of each field of view, measuring approximately 1 cm^2 was projected onto a monitor, and the inner

surface of each airspace traced by light pen on a morphometry tablet. The data were then fed on-line into a personal computer, programmed to calculate for each morphometry field the number of airspaces, the cross-sectional area of each airspace, and the percentage of lung tissue occupied by airspaces (={Σ [airspace area]/total area). The first two of these were then expressed quantitatively, by incorporating into the above calculations the square of the magnification factor (based on the mean of 4 light pen measurements on the tablet of a standard 0.1mm length (Leitz micrometer slide, Wetzlar, Germany). The mean number of airspaces counted per field was 55.8 ± 2.2. Morphometric reproducibility was determined by measuring 5 fields of view three times from each of 3 specimens while blinded to the results. The coefficients of variation were 2.0%, 4.8%, and 1.7% for airspace number, cross-sectional airspace area, and percentage of lung occupied by airspaces respectively.

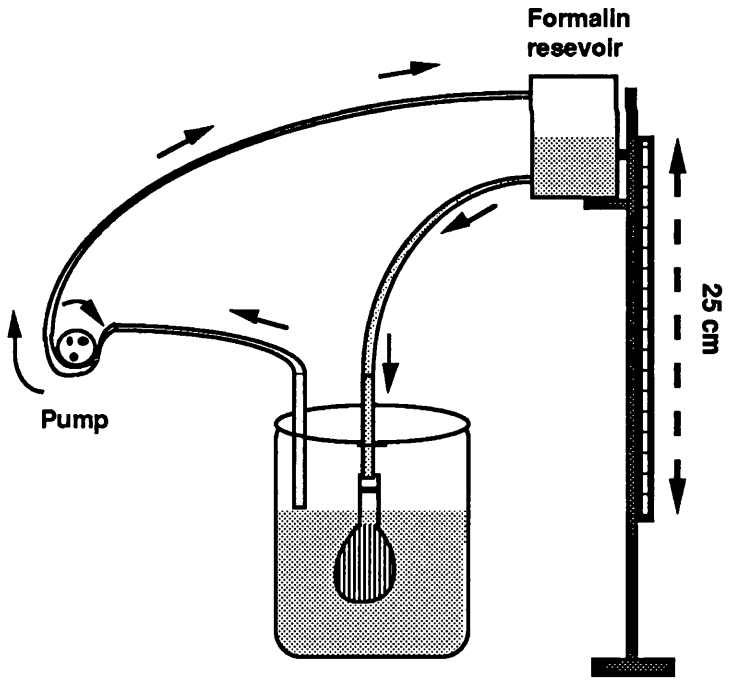

Figure 8.2: Inflation apparatus for fetal lamb lungs. The main bronchus is cannulated, the lung (denoted by the vertically striped structure above) inflated manually with formalin and the bronchus secured in place on the end of the infusion tubing with sutures. The lung is then suspended freely in formalin and the reservoir elevated to a height of 25 cm. Any leakage that occurs, either from an inadequate seal at the main bronchus, or from minor external trauma during specimen preparation, leads to an increase in the fluid level around the lung; this is corrected by the scavenger device, which returns the leaked volume to the reservoir, maintaining a constant inflation pressure.

Portions of right lung approximately 2 cm in diameter were harvested from each lobe, weighed (range 10-18 g in total), and stored at -20°C for 3-9 months until subsequent fluorescent microassay for DNA (Kapuscinski & Skoczylas 1977). After thawing, samples were homogenized in an equivalent weight of Hepes buffer (5 mM containing 12 μM NaCl, pH 7) using a Polytron PCU-2 for 5 minutes, then diluted 1:4 in Hepes, and sonicated (MSE ultrasonicator, amplitude 6 μ) for 6 minutes, demonstrated in preliminary experiments as necessary for maximal DNA extraction. Homogenates were further diluted 1:40. Standards were prepared from DNA stock solution (calf thymus) in 7 concentrations ranging from 0 to 800 ng/ml. The fluorescent dye 4,6-diamidino-2-phenylindole-2HCl (DAPI), which binds to DNA to form a fluorescent complex, was added as a 40 μg/ml (114 μM) in a 1:2 ratio to both the lung homogenates and the DNA standards, and the solutions diluted 9:1 in Hepes. Flourimetric readings were made with a spectrophotometer (Perkin-Elmer model LS-3B) with maximal excitation and emission wavelengths set at 372 and 454 nm respectively. All samples were prepared and tested in duplicate. The calibration curve derived from the standards (Figure 8.3) was applied to the average of the two results in the lung homogenates to estimate their DNA concentration, and whole lung DNA contents was calculated on the basis of the proportion by weight of right lung homogenized. The dilutions used herein were chosen from the results of preliminary experiments to ensure (i) that the concentrations in the homogenates lay within the range of concentrations in the standards, and (ii) that the calibration curve was linear (Kapuscinski & Skoczylas 1977).

Analysis: Pressure records were analysed as described in Chapter 8.3.i. Laboratory specimens were coded to ensure that the DNA analysis and lung morphometry were performed with the operator (the candidate) unaware of whether the specimen was from a drained or control fetus.

Parametric statistics were used only where histograms confirmed a normal distribution; this necessitated logarithmic transformation for airspace cross-sectional area. Pulmonary variables in the tracheostomized and control groups were expressed for comparison as means of the mean value in each fetus; to indicate the degree of difference between the two groups,

they were also expressed as the mean of each tracheostomized fetus' value as a percentage of its control co-twin. Unpaired t-testing was used for comparison of pulmonary variables; paired statistics were considered less desirable in view of the small degrees of freedom so engendered, the fact that the comparison group, although co-twins, were separate fetuses, and that only the experimental fetuses were insulted surgically.

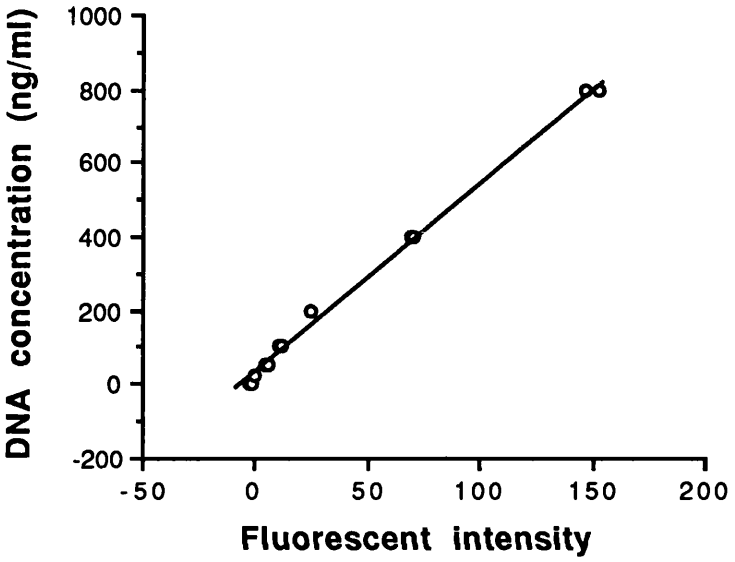

Figure 8.3: Calibration curve for DNA estimation based on spectrophotometric results in solutions of DNA standards. y=36.2 + 5.2x, where y=DNA concentration in ng/ml and x=fluorescent intensity relative to Perkin-Elmer spectrophotometric standard (Kapuscinski & Skoczylas 1977), r=0.98, p<0.001.

8.4.ii. Results

Only four experimental preparations lasted the 21 day drainage period, and were suitable for analysis; of the remainder, the tracheostomy tube became blocked in 2 (nil drainage) which were killed on Day 5-7, and in the other two, one or both fetuses died in utero on Days 13 and 15.

In the four successful preparations, the volume of lung liquid drained ranged from 206-296 ml/day (mean 245.7 $\pm$ 18.8). Mean T-A pressure in the 4 tracheostomized fetuses (-2.1 $\pm$ 1.3 mm Hg) was significantly lower than in the control animals in Chapter 8.3.ii (p=0.03). Mean T-A pressure in all 8 recordings was negative, although the large SD (2.5 mm Hg) meant that the mean of each animals mean above was not significantly different from zero.

Table 8.1: The effect of chronic lung liquid loss on fetal lung anatomy and DNA. NS = not significant

Parameter Mean ± SE	Control fetuses	Tracheostomy fetuses	Tracheostomy as % of control	Significance p value
Body weight (g)	3605 ± 191.5	3127 ± 76.3	87.5 ±4.9	NS
Left lung weight (g)	39.8 ± 2.7	21.5 ± 2.8	53.9 ± 6.2	0.003
Right lung weight (g)	59.7 ± 4.9	31.3 ± 4.1	52.8 ± 6.4	0.004
Total lung weight (g)	99.5 ± 7.6	52.8 ± 6.9	53.3 ± 6.3	0.004
Lung:body weight ratio	0.0275 ± 0.0012	0.0169 ± 0.0021	60.1 ± 5.2	0.004
Left lung weight post-inflation (g)	90.8 ± 5.2	48.0 ± 5.0	53.2 ± 5.6	0.001
Left lung post-inflation:body weight ratio	0.0251 ± 0.0006	0.0153 ± 0.0013	60.51 ± 4.1	0.001
Left lung volume (ml)	88.5 ± 5.0	47.0 ± 4.9	53.4 ± 5.5	0.001
Left lung volume: body weight ratio	0.0245 ± 0.0006	0.0150 ± 0.0013	60.8 ± 4.0	<0.001
Specific gravity	1.0257 ± 0.0016	1.0212 ± 0.0038	99.6 ± 0.3	NS
Right lung DNA conc. (mg/g)	12.8 ± 0.8	16.1 ± 1.2	126.4 ± 9.0	NS
Right lung total DNA (mg)	755 ± 34.6	493 ± 41.5	65.1 ± 3.5	0.003
Right lung DNA: body weight ratio	212.6 ± 14.7	157.4 ± 11.9	74.0 ± 1.5	<0.001
% lung occupied by airspaces	75.8 ± 0.9	56.5 ± 7.4	74.3 ± 9.0	0.04
Cross-sectional airspace area (μ^2)	508.2 ± 29.4	528.3 ± 53.9	106.0 ± 14.2	NS
Airspace number (/mm^2)	48.2 ± 1.3	42.2 ± 2.7	87.8 ± 6.5	NS

Elimination of the positive tracheal-amniotic pressure gradient produced significantly lower lung volume, wet weight,

and DNA content in relation to controls, both outright and when standardized for body weight (Table 8.1).

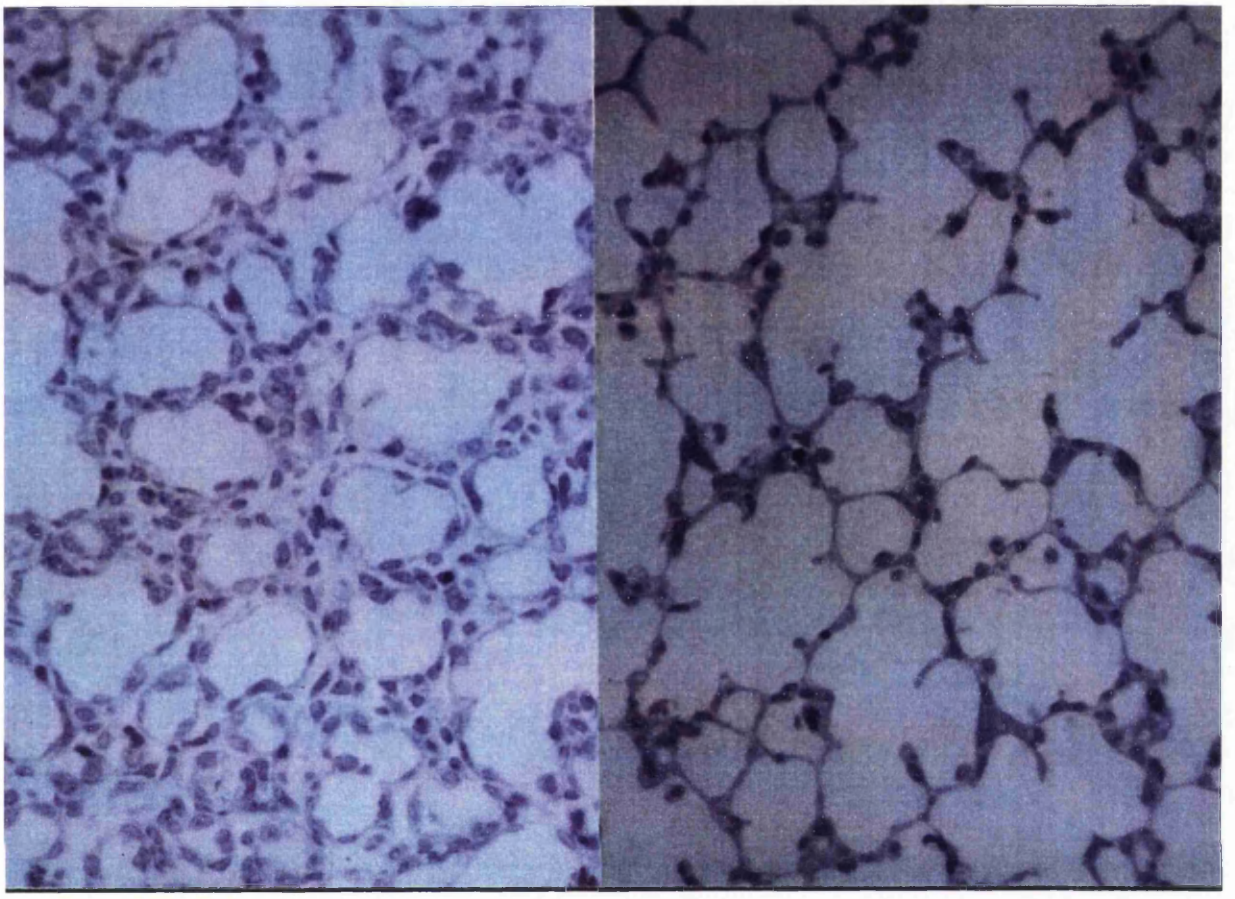

Figure 8.4: Light micrograph (x40) of left lung of chronic lung liquid drainage fetus (left) compared with that of twin control (right). Semithin section stained with toluidine blue. Note the poorer alveolar development, and the thicker alveolar walls in the lung from the tracheostomized fetus.

8.4.iii. Discussion

Eliminating the positive tracheal-amniotic pressure gradient by fetal tracheostomy with chronic drainage to the exterior produced pulmonary hypoplasia. Although tracheostomy, with drainage to the exterior (Alcorn et al 1977) or the amniotic cavity (Fewell et al 1983), has previously been shown to impair lung development, these studies did not document its effect on T-A pressure. Theoretically, insertion of a tube into the trachea lowers the reference component of the T-A pressure gradient such that the gradient temporarily increases; as a result flow out of the trachea increases in the absence of any resistance to egress, until the two pressures equilibrate and T-A approaches zero. The aim of this experiment was simply to eliminate the positive T-A pressure gradient, this being achieved by drainage to the exterior, rather

than to ensure equilibration of the two pressures, which could have been achieved more simply by drainage into the amniotic cavity. The more complex design was chosen for the following reasons: (i) to achieve comparability with experiments involving manipulation of the pharyngeal-amniotic pressure gradient in Chapter 8.6 and (ii) to provide lung liquid samples for analysis, especially for comparison with those in Chapter 8.6.ii. The small magnitude of the T-A pressure reduction achieved with this design was surprising considering the size of the gravitational component. There are several explanations. Firstly, the collection system used was neither an open system, nor a closed system in a rigid tube, and positive pressures may have been generated within the tubing by either the entry valve or by the bag itself when filled. Secondly, despite great care to ensure watertight seals during surgery, pockets of air were inevitably found in the tubing during the drainage period, reducing or eliminating the gravitational pressure gradient between the fetal trachea and the drainage bag. Finally, the fetus was continuously introducing more fluid into the system.

The volume of lung liquid drained was similar to that reported by Alcorn et al (1977). In contrast to that study however, the drained volume in this study was replaced intra-amniotically to ensure that oligohydramnios did not contribute to the pulmonary changes.

The 40% reduction in lung:body weight ratio achieved in this study by 3 weeks drainage was greater than the 29% reduction reported by Fewell et al (1983) who commenced a similar period of drainage later at 117-122 days, and less than the 59% reported by Alcorn et al (1977) with drainage of similar duration but begun slightly earlier at 105-110 days. This suggests that the earlier the insult, the greater the degree of impairment in lung development achieved. Although desirable for drainage to commence as early in the canalicular phase (80-125 days (Pringle 1986)) as possible, experience in this laboratory indicates that chronically-instrumented fetal sheep preparations are less likely to be successful when surgery is performed prior to 110-120 days.

In addition, this study confirmed that chronic tracheal drainage reduces lung volume, and the percentage of lung occupied by airspaces. A feature of hypoplastic lungs is that growth of both epithelial and interstitial components is impaired similarly. Unlike other studies in which the degree of reduction in lung

volume was considerably greater than that in weight (Alcorn et al 1977, Moessinger et al 1985), the technique of lung inflation, used routinely in human studies, demonstrated that the reduction in wet lung:body weight ratio achieved with drainage was almost identical to that in post inflation lung volume:body weight ratio (39.9 and 39.2% respectively). The specific gravity of inflated lungs was thus similar in the drained and non-drained fetuses.

As in previous animal (Fewell et al 1981, Adzick et al 1984) and human studies (Wigglesworth & Desai 1981), the differences found in total lung DNA reflect differences in lung weight rather than in DNA concentration, consistent with DNA being an index of cell number (Enesco & LeBlond 1962). Similarly, Fewell et al (1981) found the same lung DNA concentrations in fetal sheep after phrenic nerve section compared to controls, although total lung DNA was lower. However in their tracheostomy study (Fewell et al 1983), they not only found a significant increase in DNA concentration in the diaphragmatic lobe, and a significant decrease in the apical lobe of drained lungs compared to controls, but also that DNA concentration in the control lungs was almost twice as high in the apical as the diaphragmatic lobe. Here morphological or morphometric parameters from individual lobes were not examined and therefore no comment can be made as to whether chronic lung drainage effects individual lobes differentially.

The controls did not undergo sham operation in this study for the following reasons: (i) to ensure comparability with experiments involving manipulation of the P-A pressure gradient in Chapter 8.6 and (ii) to avoid the high loss rates encountered in this laboratory in related pilot experiments and in work by others, when both fetal sheep in twin pregnancies were instrumented. Nevertheless, similar effects on lung development were found to those reported by Fewell et al (1983), who did perform sham operations on control co-twins.

8.5 Effect of chronic lung liquid loss on FBM

8.5.i. Methods

In order to assess the effect of chronic lung liquid loss on FBM, recordings of diaphragmatic EMG and electronically subtracted T-A pressure were made in the tracheally-drained twins described in Chapter 8.4. Continuous recordings of FBM

were made from Day 5 to 21 on chart recorders run at either 2.5 or 3.0 mm/min.

Figure 8.5: Polygraph recording in a tracheostomized fetus in which lung liquid was drained via a closed system to the exterior. Note episodic FBM, as indicated by diaphragmatic EMG activity, are still reflected in the T-A pressure trace.

Fetal breathing was defined as episodes of continuous diaphragmatic EMG activity of ≥1 minute duration in the presence of repeated negative deflections in the tracheal-amniotic pressure

trace. Although tracheal drainage into the amniotic cavity is known to attenuate the amplitude of negative tracheal pressure changes during FBM (Fewell & Johnson 1983), pilot work indicated that with drainage via a closed system to the exterior, fluctuations in tracheal-amniotic pressure could still be readily discerned (Figure 8.5). FBM recordings from every third day were analysed over a 24 hour period, in view of possible diurnal variation (Boddy et al 1973).

FBM recordings in tracheostomized fetuses were compared with those of 4 gestational age-matched control pregnancies (3 singleton, 1 twin), similarly instrumented except for the tracheostomy tube. These sheep were prepared by Dr MJ Parkes and used in experiments involving the effect of various pharmacological agents on FBM; no fetus or mother was administered any drug during, or in the 8 hours prior to, control recordings. FBM recordings were attained under the same conditions in the same laboratory with the same equipment as used herein. All recordings, both from control and tracheostomized fetuses, were analysed on the same day. Comparable 24-hour recordings from control fetuses were only available for gestations between 126-133 days.

Parametric statistics were used after confirming normal distributions on histograms; for duration of FBM epochs this necessitated $\log_e$ transformation. One and two-way analysis of variance (ANOVA) was used to detect differences in FBM as appropriate.

8.5.ii. Results

The incidence of FBM in tracheostomized fetuses declined with advancing gestation/ duration of drainage ($F=3.2$, $p=0.03$), although the number and duration of FBM epochs did not change significantly. As shown in Fig. 8.6, the incidence of FBM over the last 3 recording periods was significantly lower in the tracheostomized fetuses than in controls ($F=6.4$, $p=0.02$). The magnitude of the difference was 12.5, 2.8, and 8.0 in % incidence /24 hours at 126-7, 129-30 and 132-3 days respectively. Thus between 126-133 days the incidence of FBM in the tracheostomized fetuses was $77.2\pm10.3\%$ of that in controls. Although Fig 8.7 suggests a trend towards fewer and shorter FBM epochs in tracheostomized fetuses between 126-133 days, this was not statistically significant ($F=2.0$, $p=0.14$, and $F=3.2$, $p=0.08$

respectively).

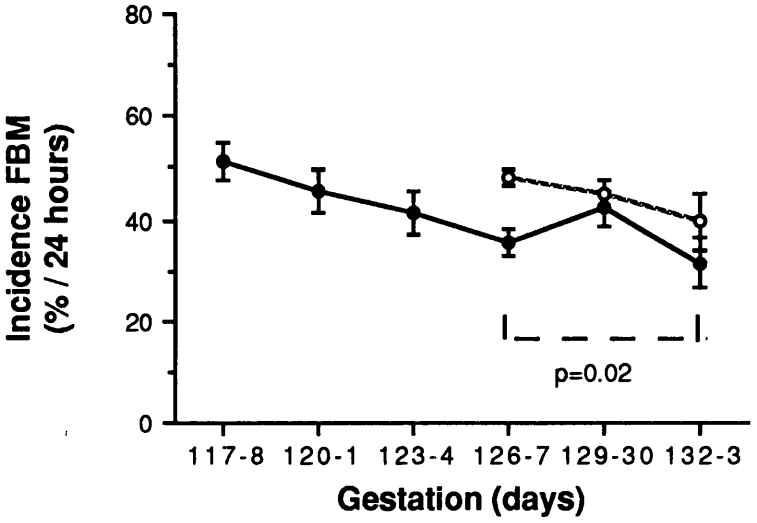

Figure 8.6: The mean incidence ± SE of FBM in tracheostomized fetuses (filled symbols) and control fetuses (open symbols).

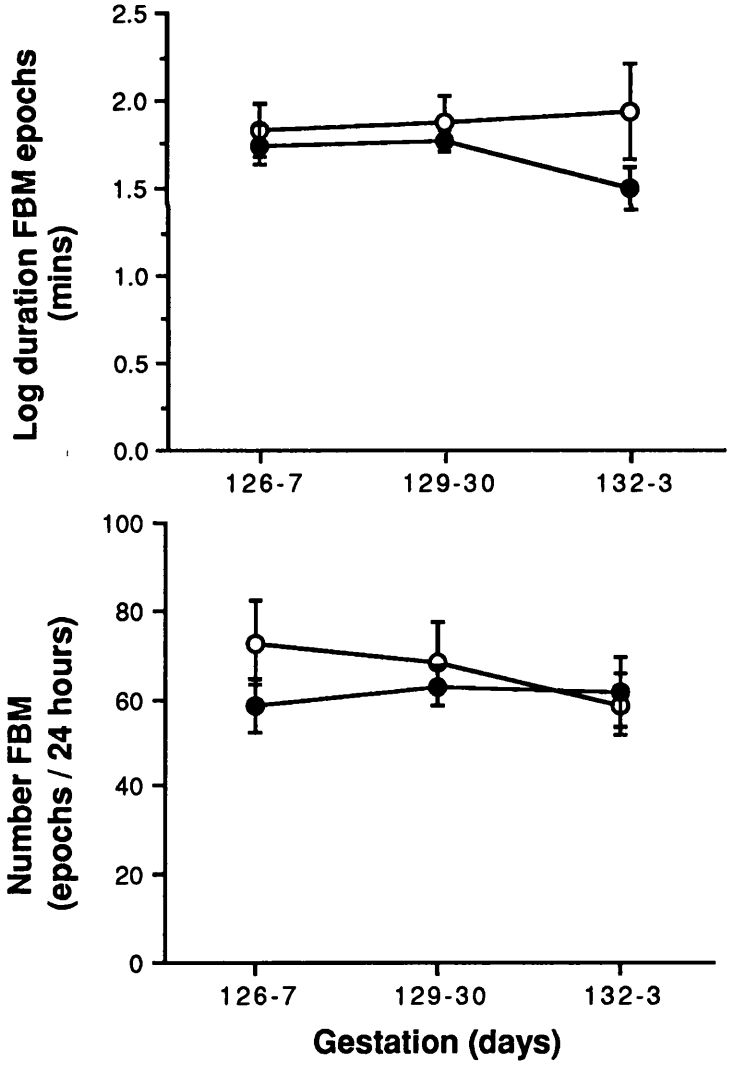

Figure 8.7: Comparison of the duration and number of FBM epochs in control (open symbols) and tracheostomized fetuses (filled symbols) between 126 and 133 days gestation.

8.5.iii. Discussion

This study indicates that FBM continue during chronic lung liquid loss. Since chronic lung liquid drainage appears an appropriate model for oligohydramnios-related PH, these data add support to other studies suggesting that inhibition of FBM is not the primary mechanism for impairment of lung growth in oligohydramnios, as discussed in Chapter 7.3.iii.

The incidence of FBM was marginally less (by 23%) in tracheostomized fetuses than controls between 126-133 days. Overestimation of FBM in controls is considered unlikely as their incidence (44%) was similar to other reports using the same definition: 48% in a previous study in this laboratory (Moore et al 1989b), and 45% by another group (Bahoric & Chernick 1975). Dickson & Harding (1987) reduced lung liquid volume acutely in fetal lambs, and found that the incidence of FBM fell slightly, by 19%. Both studies thus suggest that the incidence of FBM may decline marginally during lung liquid loss. Given that the rate of escape of lung liquid from the upper respiratory tract is normally greater during FBM than during apnoea, any such reduction might facilitate restoration of lung volume. Dickson & Harding (1987) raised the possibility that this situation may be similar to the reduced respiratory frequency observed in postnatal lambs in response to negative airway pressure. It is not known whether this response, which is attributed to pulmonary slowly-adapting receptors and abolished by vagotomy (Harding 1980), is present in the fetus. However, before attributing any physiological significance to this reduction in FBM incidence, caution is advised in view of the small magnitude of difference found. Furthermore, it is difficult to see how a vagal response, which mediates respiratory frequency, could account for this reduction in FBM incidence, which was observed mainly to be a reduction in duration of FBM epochs.

The control FBM data used in this study were less than ideal, being neither contemporaneous nor covering the entire gestational age range of recordings in instrumented fetuses. Recordings were not made in control co-twins, for the reasons outlined in Chapter 8.4.iii. Previous control recordings from this laboratory were only available for the latter half of the drainage period, and the laboratories scientific and financial priorities at that time did not accommodate running chronic fetal sheep preparations simply to

record FBM in controls, when a large amount of normative data had previously been published. Accordingly caution is advised in the interpretation of the small reduction in FBM incidence found with chronic lung liquid drainage. Indeed, since completing this study, a report has been published showing in sheep that the incidence of FBM during chronic oligohydramnios leading to PH is no lower than in controls (Dickson & Harding 1991), although FBM in that study were observed in one hour windows only.

In tracheostomized fetuses, the incidence of fetal breathing declined significantly from 51 to 32% between 117-132 days. In fetal sheep with intact airways, Bowes et al (1981) reported a linear increase in percentage apnoea (and thus a decrease in FBM incidence) between 110-140 days. We are unable to comment on whether the decline in FBM we found in tracheostomized fetuses is an effect of drainage or of advancing gestation, in view of the lack of comparable control data over the same period. Nevertheless, as seen in Fig 8.6, the incidence of FBM appeared to decline similarly in both control and tracheostomized fetuses between 126-133 days, although in neither group was this fall significant based on three 24 hour recordings.

The small reduction in FBM observed during chronic lung liquid loss may explain at least in part the conflicting accuracies reported for predicting PH in human oligohydramnios by monitoring FBM, as discussed in Chapter 8.3.iii. The human studies which suggest that FBM are "absent' in oligohydramnios-related PH (Blott et al 1987, Blott et al 1990), defined fetal breathing as periods of FBM lasting ≥60 seconds. Figure 8.7 suggests that the reduced incidence of FBM found during chronic lung liquid loss in this study reflects more a reduction in epoch duration than number. Accordingly it remains possible the findings of Blott et al (1987, 1990) could be attributed to FBM continuing during oligohydramnios-related PH, but with epochs lasting <60 seconds.

8.6 Effect of mimicking low AP by chronic pharyngeal drainage

8.6.i. Pilot study

The aim was to design a catheter system, which when inserted at one end into the fetal mouth and draining at the other to the exterior, would reduce pharyngeal-amniotic pressure by approximately 3-4 mm Hg. This was for use in controlled

experiments to mimic the effect on the upper airway of a reduction in AP without alteration in amniotic fluid volume. It was initially planned to test its effect on lung development in twin pregnancies, one fetus of which would be catheterized as above to achieve the pressure reduction, while the other, as a control, underwent sham operation with catheterization into the amniotic cavity.

The following criteria were considered in designing the pharyngeal catheter: (i) it should occupy the fetal oropharynx (ii) it should be of as wide a bore as practicable to facilitate pressure reduction and (iii) it should be small enough to allow the fetal lips to be sealed around it. Three prototypes were developed, and inserted into the mouth of a fetal sheep cadaver to show that the lips could be successfully sealed around the catheter.

These were next tested in 4 chronically-instrumented fetal sheep with twin pregnancies prepared as described previously between 115-122 days gestation. Under general anaesthesia, the drainage catheter was fixed in the posterior aspect of the fetal oral cavity, and the lips and nares sewn closed and sealed with cyanoacrylate glue. The pharyngeal drainage tube was filled with normal saline (0.9% NaCl) and in one of the twins was connected to a drainage bag later positioned beneath the floor of the cage while in the other it was left free in the amniotic cavity. Catheters for pressure measurement were positioned in the amniotic cavity, and in the fetal oropharynx alongside the drainage apparatus. After a 5 day recovery period, one hour recordings of P-A pressure were made and analysed as described earlier.

The Mark I prototype comprised two hard plastic catheters, each inserted laterally into either side of the mouth behind the alveolar ridge; at their other end they were each connected by 10 cm of soft polyvinyl tubing to a common Y-piece in front of the fetal face, and then to 150 cm of polyvinyl tubing. This proved unduly bulky, it being extremely difficult to close the uterus around it. The ewe delivered both fetuses spontaneously on Day 5 before pressure measurements were made.

The Mark II prototype comprised six 70 cm long polyvinyl catheters in parallel (ID 2.0 mm OD 3.0 mm). The catheters were protected from occlusion by the alveolar ridges where they entered through the front of the mouth by coursing through a hard plastic cylinder (ID 10.5 mm OD 13.0 mm, 4 cm long), the spaces

between the catheters being sealed with silicone gel and araldite. At their distal end, these were connected outside the maternal abdomen to a common 90 cm wide-bore polyvinyl tube. P-A pressure remained positive (c. 2-3 mm Hg) in all 4 fetuses tested, irrespective of whether drainage was to the exterior or the amniotic cavity. In the two with an exterior drainage tube, it was not possible to achieve negative P-A pressures even after applying suction, suggesting blockage or obstruction of the catheters. In one pregnancy, a fetus died on Day 6, and in the other the ewe delivered both fetuses spontaneously on Day 9.

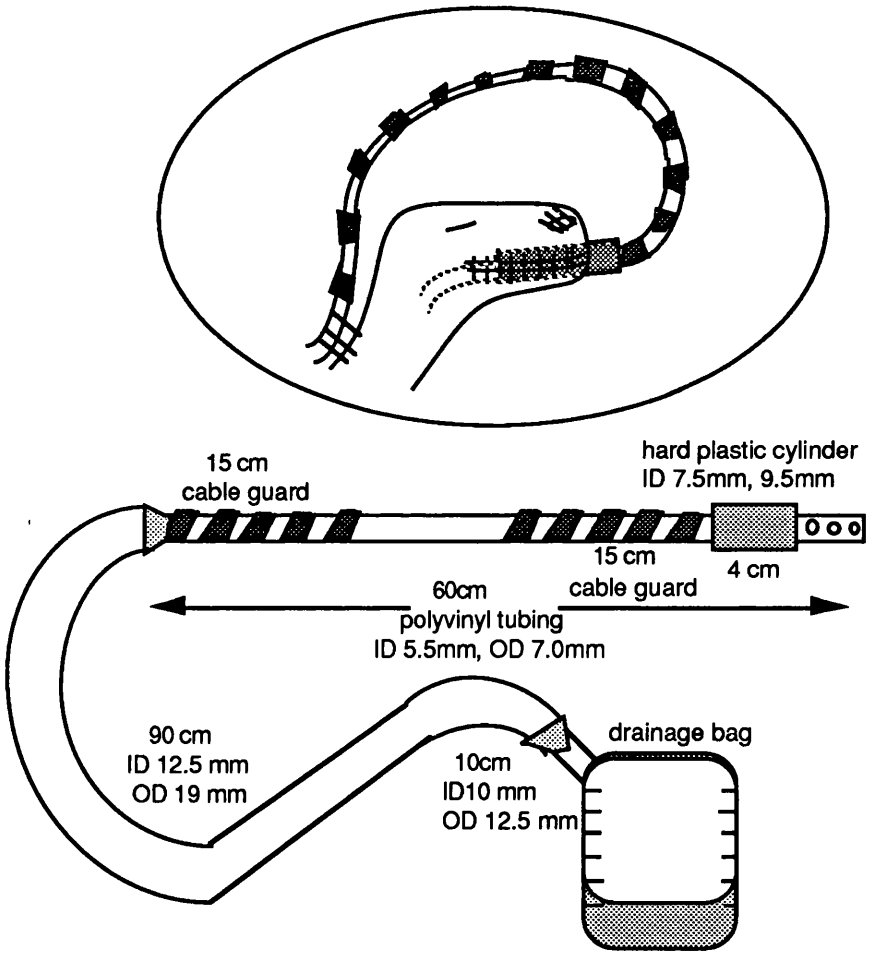

Figure 8.8: Fetal pharyngeal catheterization apparatus (Mark III) used to achieve sub-amniotic pharyngeal pressures. Note drawing not to scale.

The following modifications were therefore incorporated in the Mark III prototype (Figure 8.8): (i) an increase in ID of the narrowest segment of tubing to facilitate a greater pressure drop (ii) use of cable guard to avoid kinking of the soft tubing where it

left the mouth or its junction with connecting pieces (iii) careful positioning of tubing in a half coil over the back of the fetal head, again to avoid kinking. Mean P-A pressure in the fetus drained to the exterior (-3.0 $\pm$ 0.5 mm Hg) was significantly negative ($p<0.001$), in contrast to that in the fetus drained into the amniotic cavity (0.2 $\pm$ 0.2 mm Hg), which was not significantly different from zero. Both fetuses died in utero on Day 9.

The Mark III prototype was thus chosen for definitive study. In view of the high loss rates encountered when both twins were instrumented however, catheterization of the control twin was omitted in subsequent preparations.

8.6.ii. Methods

Experimental protocol: Chronically-instrumented fetal sheep were prepared as described previously. Briefly, anaesthesia was induced in 26 cross-bred ewes with twin pregnancies at 112-120 days gestation, one horn of the gravid uterus was exposed at laparotomy and the fetal head delivered through a uterine incision. Catheters (ID 1.0 mm, OD 2.0 mm) were implanted into one carotid artery and jugular vein and onto the fetal oropharynx. An additional catheter (ID 2.0 mm OD 3.0 mm) was fixed intra-amniotically to the fetal praecordium, as described earlier. In 8 fetuses, a catheter was also placed into the trachea. Multiple fenestrations were fashioned at one end of a 60 cm long polyvinyl tube which was then fixed in the fetal oral cavity (Figure 8.8). This catheter was placed on the dorsal surface of the tongue with its tip approximately 3.5 cm posterior to the central incisors and lying at the extreme posterior margin of the tongue so as to prevent the tongue obstructing the connection between the catheter's lumen and the oropharynx. Its diameter occupied most of the cavity at this point. The lips and nares were sewn closed and sealed with cyanoacrylate glue. The pharyngeal drainage tube was filled with normal saline (0.9% NaCl) and the integrity of the pharyngeal seal isolating the pharyngeal from the amniotic cavity confirmed. As leakage around the catheter developed subsequently in some, an additional seal was achieved in the last 8 preparations by enveloping the snout in a rubber glove tied around the drainage tube. The fetus was replaced, the uterus closed in 2 layers, and all catheters exteriorized through the ewe's right flank. A collection bag was connected to the distal end of the pharyngeal drainage apparatus (Figure 8.8) and positioned approximately 40 cm

beneath the floor of the cage. Free drainage was commenced immediately. At least 5 days were allowed for postoperative recovery before recordings were made, during which time antibiotics were administered to ewe and fetus.

After fetal arterial pH and blood gas values had been shown to be normal (mean ± SE pH 7.35 ± 0.01, pCO_2 43.1 ± 0.7 mm Hg , pO_2 21.2 ± 0.9 mm Hg), pressures were measured with standard biomedical transducers attached to fluid filled lines. Electronically subtracted P-A and T-A pressures were recorded for one hour every 2-3 days on a polygraph run at either 1.25 or 1.5 cm/min (Figure 8.9).

The drainage bag was emptied every 2-3 days and the equivalent volume of normal saline (0.9% NaCl) warmed to 38-39°C infused intra-amniotically. In addition, 2 ml indigo carmine dye diluted 1:20 was added to the saline, so that appearance of dye in the drainage bag gave an immediate indication of a break in the seal between the pharynx and the amniotic cavity. Dye was similarly instilled whenever the P-A pressure was >-0.5 mm Hg or when the volume of pharyngeal fluid in the drainage bag increased by >300 ml per day. Samples of the drained liquid were frozen until later analysis for electrolytes and viscosity.

Lung specimens: The experiment was terminated on Day 21 of drainage, when labour ensued, or when dye indicated leakage. Animals were killed with pentobarbitone (4g intravenously), necropsy was performed and both twins were delivered, towel dried and weighed. Pulmonary variables were analysed in those preparations with >10 days drainage, as detailed in Chapter 8.4.i., except that lung volume was measured in only 8 fetuses. Two preparations with light microscopic signs of autolysis were excluded from morphometric and DNA analysis. In the remainder, the mean number of airspaces analysed per field was 57.9 ± 3.7. The weight of the portions harvested from the right lung for DNA analysis ranged from 10-25 g. Laboratory specimens were again coded to ensure that the DNA analysis and lung morphometry were performed blind.

Figure 8.9: Pharygeal-amniotic and tracheal-amniotic pressure recording from a fetus undergoing pharyngeal drainage to the exterior. The repeated negative fluctuations in T-A pressure indicate fetal breathing. Note that during apnoea, P-A is negative and T-A positive.

Analysis: Each minute of pressure trace was examined for negative pressure fluctuations consistent with fetal breathing, gasping or swallowing activity. Such minutes were excluded and

point estimates of pressure were obtained at the midpoint of each stable minute, and a mean per hour calculated. After exclusion of recordings obtained within 24 hours of labour or detection of mouth leakage, mean P-A pressures per drained animal were derived from a median of 3 recordings (range 2-7), and a pooled mean calculated from the individual means per animal. Mean T-A pressures were similarly derived from a median of 3.5 recordings (range 2-6). Within trace variability in P-A pressure was calculated from individual SDs from 68 one hour traces, and between trace variability from the mean SD from each animal in which ≥ 3 recordings were made.

Viscosity was measured on a single specimen collected between day 8-10 of drainage from 10 fetuses undergoing pharyngeal drainage, and from 6 fetuses undergoing tracheal drainage (Chapter 8.4.i). Measurements were made at 37°C by Dr M Rampling in the Department of Physiology at St Mary's Hospital using a rotating cup viscometer.

Electrolytes were measured in specimens of tracheal and pharyngeal fluids collected throughout the drainage period. Sodium (Na^+) and potassium (K^+) assays were done by Ms H Watson of the Biochemistry Department, Queen Charlotte's & Chelsea Hospital using an automated flame photometer (Corning model 480), and chloride (Cl^-) by Mr J Beecham of the Biochemistry Department at Hammersmith Hospital using a chloride meter (Chemlab model CCMI). The coefficients of variation for the above are 0.4, 0.5, and 1.5%, according to manufacturers' specifications. Amylase was measured in a single sample from 10 fetuses undergoing pharyngeal drainage using a commercially available calorimetric assay (Amylase kit AY 891, Randox Laboratories) and adult serum used for internal assay controls.

Parametric statistics were used only after histograms confirmed a normal distribution, which necessitated logarithmic transformation for airspace cross-sectional area. Pulmonary variables in the pharyngeally-drained and control fetuses were expressed as means of the mean value in each fetus; to indicate the degree of difference between the two groups, each pharyngeally-drained fetus' value was also expressed as a percentage of its control co-twin and a mean calculated. In contrast to the tracheal drainage experiments, paired testing was chosen for comparison

of pulmonary variables, as the differing durations of drainage required that each experimental twin's lungs be compared directly with that of its control co-twin.

8.6.iii. Results

No pressure measurements were made in 9 preparations which lasted ≤ 5 days and only one recording was made in 2 which lasted ≤ 8 days: 4 ewes died, 2 laboured, in 4 the instrumented fetus died, and two were complicated by mouth leakage. These were excluded from further analysis.

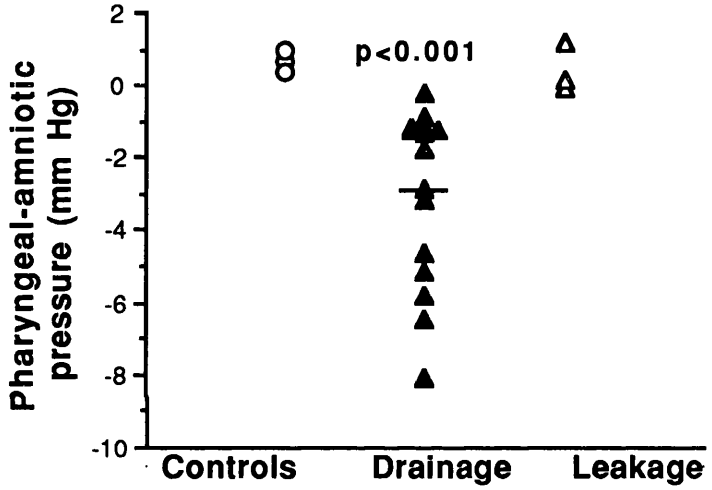

Figure 8.10: Pharyngeal-amniotic pressure in 15 fetuses which underwent chronic pharyngeal drainage (filled triangles, mean indicated by line), compared to that in 3 control fetuses (open circles). Also shown are values from 3 of the drained fetuses taken on the day mouth leakage was detected.

In the remaining 15 drained fetuses in which serial P-A recordings were made, negative P-A pressures were achieved in all, as shown in Figure 8.10. Mean P-A (-3.0 mm Hg ± 0.6 mm Hg) was significantly lower than zero ($p<0.001$) and significantly lower than that in the controls in Chapter 8.3.ii (0.7 ± 0.1 mm Hg, $p=0.02$). Mean within-trace variability in P-A was 2.2 ± 0.2 mm Hg, and mean between-trace variability 2.5 ± 0.5 mm Hg. These were both significantly greater ($p<0.001$) than found in Chapter 8.3.ii in control fetuses. Unlike in controls, P-A pressure traces in pharyngeally-drained fetuses were characterized by occasional large negative pressure excursions lasting approximately 1-3 minutes, an example of which is shown in Figure 8.11.

The experimental period lasted ≥ 10 days in 10 drained animals: two were put down on day 21, 5 went into labour and mouth leakage occurred in 3. Mean P-A pressure in these 10 was also significantly negative (-2.7 ± 0.6 mm Hg, p=0.001). In contrast, mean P-A on the day of detection of mouth leakage was not significantly different from zero (0.4 ± 0.4 mm Hg).

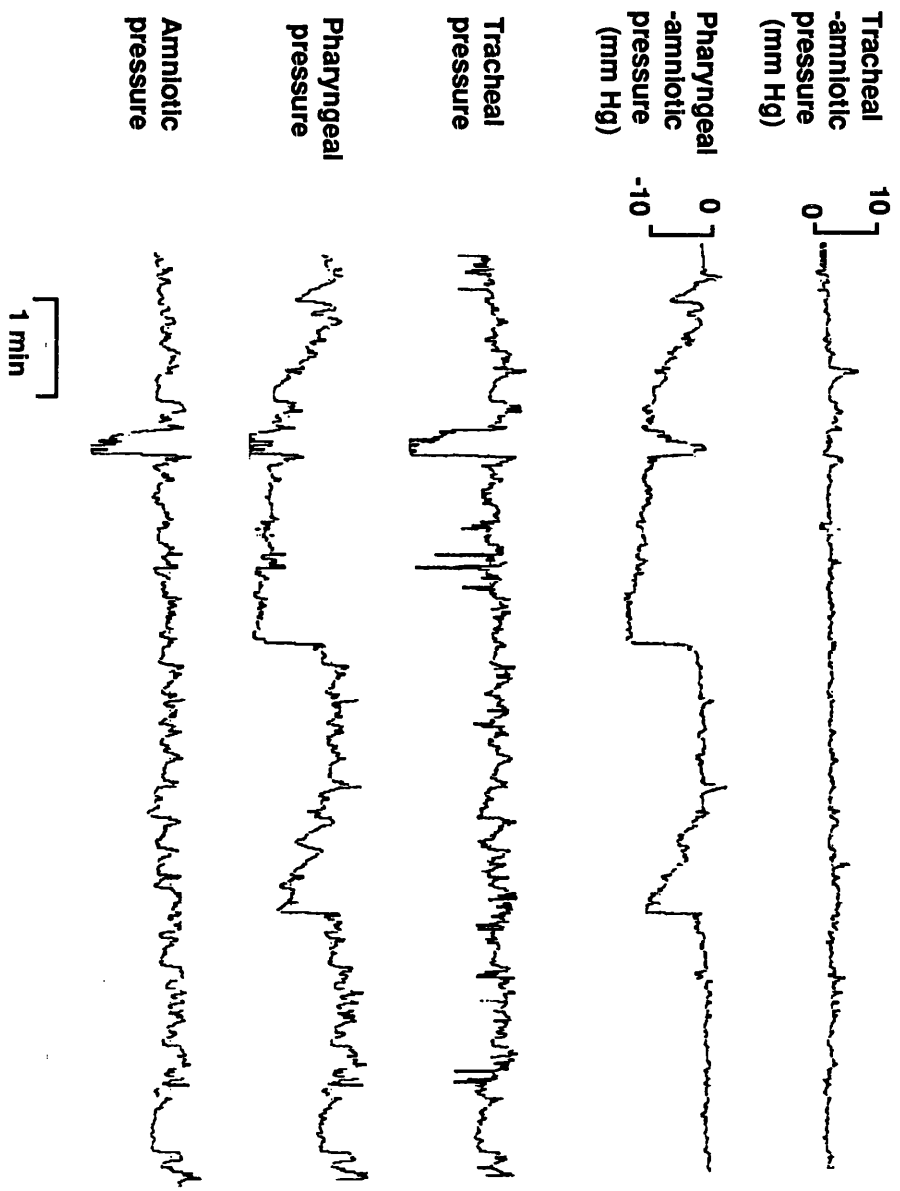

Figure 8.11: Pressure recording in a pharyngeally-drained fetus showing large negative pressure excursions in the pharyngeal-amniotic pressure trace. Note that these are reflected in the baseline pharyngeal trace, but not in the baseline amniotic trace.

The volume of fluid drained per day in the 10 preparations

lasting ≥10 days, excluding the last recording in the 3 with mouth leakage, was 237 ± 19 ml/day, similar to that in tracheally-drained fetuses, but significantly less than the final reading in those with mouth leakage (343 ± 19 ml/day, $p<0.05$). There were gross differences between the fluid drained from the trachea and that from the pharynx, which appeared to contain excessive mucus (Figure 8.12). Viscometry of the pharyngeal drainage fluid demonstrated readings which were non-Newtonian, i.e. varied with shear rate, as shown in Figure 8.13. These were considerably higher than those in the tracheal fluid (mean 0.75 ± 0.06 mPa), which were similar to water (0.7 mPa) and did not vary with shear rate. As non-linearity of the pharyngeal results hindered statistical comparison with those in tracheal fluid, an estimate was made (0.17 ± 0.1/s) of the biological shear rate operating in the pharynx in vivo, based on the inner diameter of the tubing, and the measured flow rate. As this was below the slowest operating speed of the viscometer, results were compared at the lowest shear rate (1.75/s), indicating that viscosity was significantly higher in the pharyngeal than in the tracheal fluids ($p<0.001$).

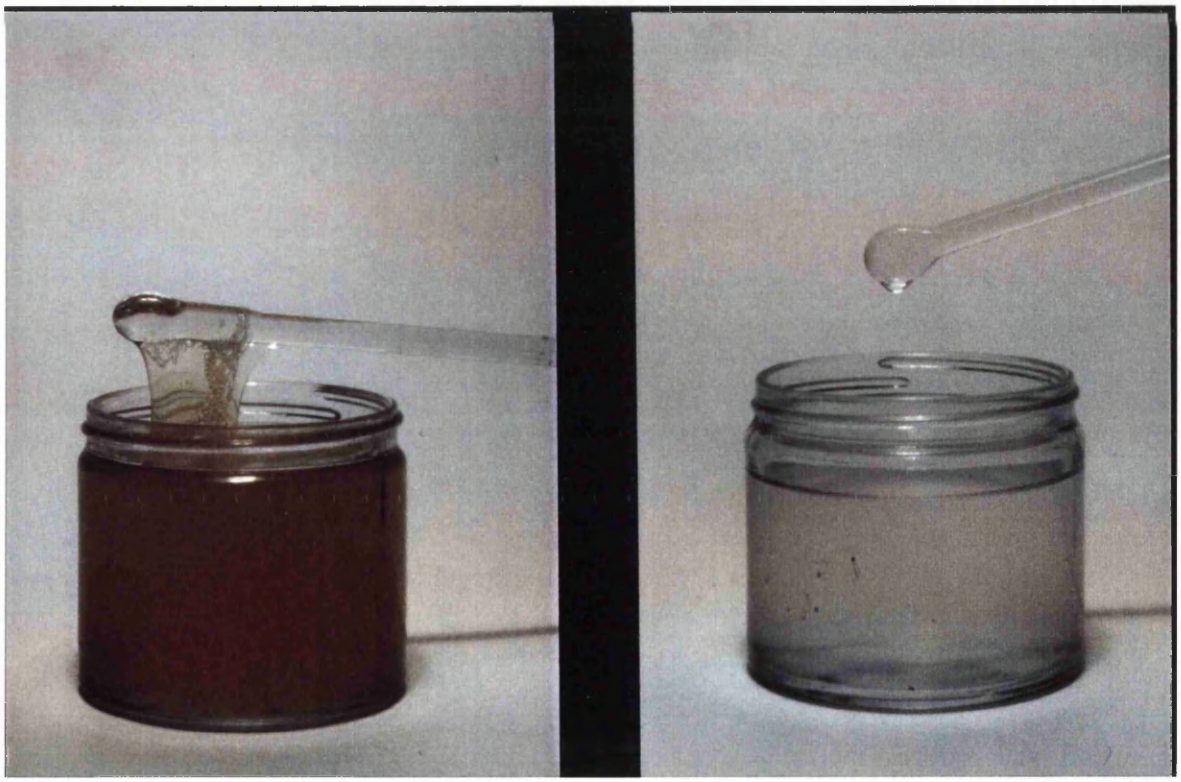

Figure 8.12: Gross difference between pharyngeal fluid on the left, which appeared to contain excessive mucus, and tracheal fluid on the right.

Table 8.2: The effect of chronic pharyngeal drainage on fetal lung anatomy and DNA (n=10 unless otherwise stated). NS=not significant.

Parameter Mean ± SE.	Control fetuses	Experimental fetuses	Experimental as % of control	Significance p value
Body weight (g)	3199 ± 303	2553 ± 204	81.8 ± 5.4	0.01
Left lung weight (g)	35.5 ± 2.8	27.6 ± 2.9	78.3 ± 7.1	0.02
Right lung weight (g)	51.6 ± 4.7	41.0 ± 4.3	81.4 ± 8.3	0.04
Total lung weight (g)	87.0 ± 7.5	68.6 ± 7.2	80.0 ± 7.7	0.03
Lung:body weight ratio	0.028 ± 0.001	0.027 ± 0.002	97.9 ± 7.2	NS
Left lung weight post inflation (g)	83.7 ± 7.4	67.5 ± 6.2	83.2 ± 7.7	0.05
Left lung post inflation:body weight ratio	-0.027 ± 0.001	0.027 ± 0.002	102.7 ± 7.8	NS
Left lung volume (ml) (n=4)	66.7 ± 5.3	72.3 ± 10.0	107.2 ± 7.0	NS
Left lung volume: bodyweight ratio(n=4)	0.027 ± 0.003	0.032 ± 0.003	123.0 ± 7.4	0.04
Right lung DNA conc. (mg/g) (n=8)	12.2 ± 0.9	12.0 ± 0.7	100.2 ± 5.0	NS
Right lung total DNA (mg) (n=8)	667.7 ± 68.9	495.3 ± 57.2	78.4 ± 12.1	NS
Right lung DNA:body weight ratio (n=8)	195.8 ± 13.8	187.5 ± 20.1	96.9 ± 10.5	NS
% lung occupied by airspaces (n=8)	65.7 ± 2.8	63.7 ± 2.5	97.5 ± 3.8	NS
Cross-sectional air-space area (μ^2) (n=8)	601.3 ± 40.1	646.9 ± 39.2	108.4 ± 4.3	NS
Airspace number (/mm^2) (n=8)	57.8 ± 2.2	58.1 ± 7.3	99.7 ± 10.7	NS

Amylase was not detectable in the pharyngeal fluids (<3 U/l), even after 1:10 dilution. In the tracheal fluids, concentrations of Na^+, K^+, and Cl^- did not change significantly over the drainage period (two way ANOVA F=2.3 p=0.1, F=2.8 p=0.1, and F=1.4,

p=0.3 respectively). A comparable ANOVA could not be used for the pharyngeal fluid results in view of the differing durations of drainage; however visual inspection of electrolyte concentrations plotted against drainage duration suggested similarly the absence of a significant association. Accordingly, mean electrolytes (mean of each animal's mean) in pharyngeal fluid are compared with those in tracheal fluid in Figure 8.14. Although Na^+ and K^+ were similar, Cl^- concentration was lower in pharyngeal fluids (119.6 ± 4.5 mEq/l) than in tracheal fluids (142.4 ± 7.3, p=0.02).

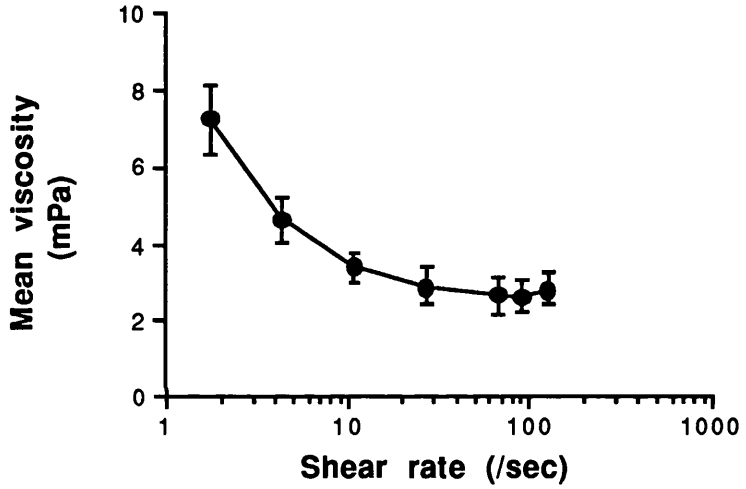

Figure 8.13: Viscosity of pharyngeal fluid collected on day 8-10 of drainage varies with shear rate, indicating its non-Newtonian nature. Symbols denote mean ± SE in milliPascals.

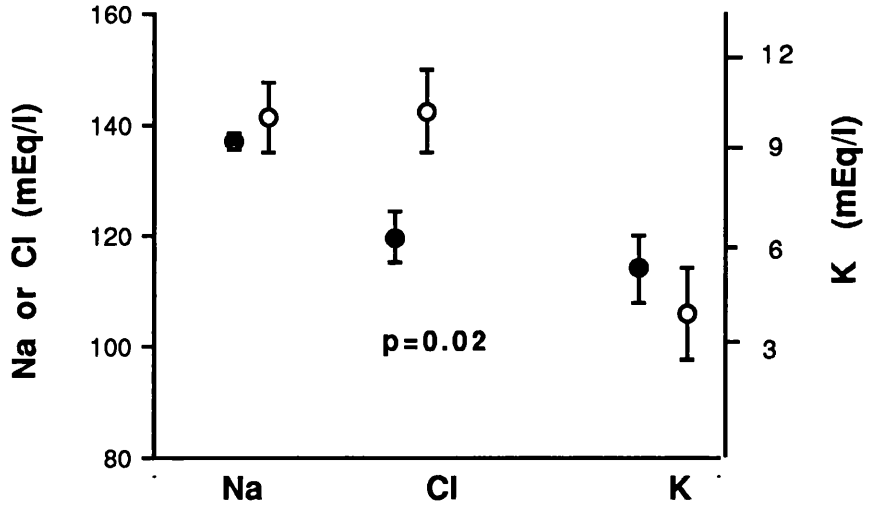

Figure 8.14: Comparison of electrolyte concentrations (mean ± SE) in pharyngeal fluid (filled symbols) compared to tracheal fluid (open symbols).

As often found in chronic fetal preparations, the body weights of chronically-instrumented fetuses were reduced (by 18%) compared to the uninstrumented controls. Accordingly, the lung weights were similarly reduced. However, relative to body weight, there was no significant difference between fetuses undergoing chronic pharyngeal drainage and their control co-twins in lung weight, either before or after inflation, or in lung DNA as shown in Table 8.2. In addition, on the basis of limited numbers, lung volume was no lower in pharyngeally-drained fetuses. Furthermore, lung morphometric variables were similar in control and experimental fetuses.

When expressed as a percentage of control, there was no significant correlation in the pharyngeally-drained fetuses between any variable indicative of PH, such as lung:body weight ratio, inflated lung:body weight ratio, cross-sectional airspace area, or lung DNA:body weight ratio (as validated in Chapter 8.4.ii), and the degree of P-A pressure reduction or the duration of drainage (an example is shown in Figure 8.15.

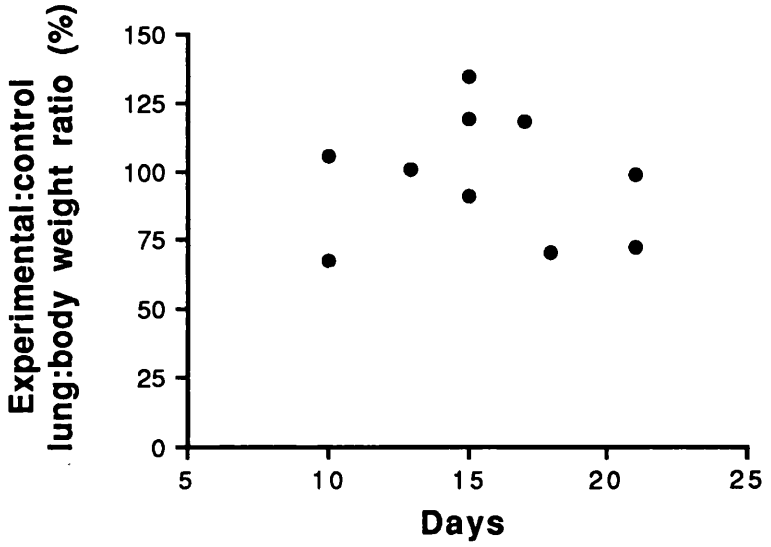

Figure 8.15: Lung:body weight ratio in the experimental twin as a percentage of that in the control twin shown as a function of duration of pharyngeal drainage.

Despite the negative P-A pressure, T-A pressure remained positive (1.7 ±0.9 mm Hg), similar to the control readings in Chapter 8.3.ii (1.5 ± 0.1 mm Hg). In pharyngeally-drained fetuses, T-A pressure was significantly greater than contemporaneous measurements of P-A pressure (-3.0 ± 0.9 mm Hg, p=0.002) . Thus

the increase in the tracheal-pharyngeal pressure gradient in drained fetuses (5.8 ±1.2 mm Hg) compared to controls (0.8 ±0.2 mm Hg, p=0.02) was attributable to the reduction in pharyngeal pressure, rather than any alteration in tracheal pressure.

8.6.iv. Discussion

This study demonstrates in fetal sheep that mimicking the effect of low amniotic pressure on the upper airway by reducing pharyngeal pressure does not impair fetal lung growth. The hypothesis addressed in this study was that a reduction in amniotic pressure would impair fetal lung growth by producing a net loss of lung fluid, since the rate of lung liquid escape would then exceed that at which lung liquid was produced. As calculated in Chapter 8.1, AP would need to be reduced by >2 mm Hg for this to occur. Chronic fetal pharyngeal drainage in these experiments led to significantly reduced P-A pressure compared to controls, and mimicked a mean drop in AP at the laryngeal outlet of 3.7 mm Hg (P-A in controls minus P-A in pharyngeally-drained fetuses). The degree of pressure reduction achieved was similar to that found in severe oligohydramnios in human pregnancies (Chapter 6) and slightly greater than that reported in experimental oligohydramnios in sheep (Harding et al 1990).

Testing this hypothesis necessitated a study design which did not produce oligohydramnios. Accordingly, the pharynx was isolated from the amniotic cavity, and care taken to replace the drained volume intra-amniotically.

The initial aim was to compare fetal lung parameters after 21 days drainage with that in their control co-twins. Lung liquid drainage of similar duration has been shown to produce PH, both in this work (Chapter 8.4) and in published reports by others (Alcorn et al 1977, Fewell et al 1983). Furthermore, a 21 day drainage period would have allowed comparison of the effects on lung development of pharyngeal drainage with those of tracheal drainage. However, only 2 of 26 ewes in which the Mark III prototype was inserted lasted the full 21 day drainage period, this high attrition being due to maternal and fetal death, preterm labour, and mouth leakage. The first three of these may in some cases be attributed to the size of the catheter apparatus used. Mouth leakage on the other hand appeared related to continued growth of the snout around the rigid catheter apparatus, and/or

loosening of the cyanoacrylate sealant in the surrounding fluid environment. Pharyngeal and amniotic pressures equilibrated in the presence of leakage, negating the point of the study. Experiments were thus terminated as soon as leakage was detected, usually within 24 hours of its occurrence, this complication being suggested by a large increase in drainage volume and confirmed with indicator dye.

Accordingly, a compromise was introduced into the study protocol, whereby a shorter period of drainage was chosen, 10 days, as the minimum necessary to produce detectable impairment in lung development. Fewell et al reported significantly reduced lung:body weight ratios following 10-22 days phrenic denervation in 6 fetal lambs, 3 of which were subjected to this insult for ≤15 days (Fewell et al 1981). The same lung processing techniques were used here as in the tracheal drainage experiments, in which 21 days chronic lung liquid loss resulted in a 40% reduction in lung:body weight ratio, both before and after inflation, and a 26% reduction in both lung DNA: body weight ratio and the percentage of lung occupied by airspaces. None of these parameters was affected by 10-21 days chronic pharyngeal drainage. Furthermore, none showed any correlation with increasing duration of drainage, which might be expected if the negative results were attributable to the minimum drainage period being too short.

The normal standing pressure in the fetal trachea is due to the resistance to egress of lung fluid provided during apnoea by an active laryngeal retentive mechanism (Harding et al 1984a, Harding et al 1986a, Harding et al 1986b). Bypassing this resistance in tracheostomy experiments allows free escape of fluid along the T-A pressure gradient, leading to equilibration of tracheal and amniotic pressures (Fewell & Johnson 1983). The lack of effect on lung growth compared to controls in this experiment may be explained by the failure of pharyngeal drainage to alter standing tracheal pressure, which remained positive and similar to values in control fetuses. It may be speculated that the negative pressure generated within the fetal pharynx did not impair laryngeal retention to increase lung fluid escape; thus there was no flow-related reduction in standing tracheal pressure.

The degree of pressure reduction achieved was smaller than would be predicted for a column of Newtonian fluid in a rigid tube in a closed system. Some reasons for this were discussed in

relation to the tracheal drainage experiments (Chapter 8.4.iii). Additional explanations in the pharyngeal drainage study are that (i) the fetal pharynx was non rigid and (ii) the fluid non-Newtonian (i.e. viscosity varied with shear rate) and highly viscous. The degree of P-A pressure drop with catheterization of the pharynx was however, only slightly greater than that in T-A pressure with tracheal catheterization. Notwithstanding the greater viscosity of the pharyngeal fluids, much wider tubing was needed in the pharyngeal drainage experiments to achieve the required gradient.

The viscous nature of the pharyngeal fluid may have created unphysiological conditions in the pharynx, possibly preventing any increase in lung liquid escape otherwise attributable to altered pressure gradients alone. The fetal pharynx is known to produce mucoid secretions, Harding et al (1984b) noting that fluid swallowed by fetal sheep had a higher viscosity than could be explained on the basis of simultaneous measurements in tracheal and amniotic fluid, and Brace (1986) collecting fluid of extremely high viscosity from around the mouth in fetal sheep which had undergone oesophageal and tracheal ligation. The concentration of mucus within the pharynx here may have been even greater than normally found, either in response to the presence of a foreign body in close proximity to mucous membranes, or by preventing dilution of secretions in amniotic fluid during mouth opening. The pronounced negative P-A pressure fluctuations encountered during pharyngeal drainage may represent fetal swallowing movements, although this remains speculation in the absence of oesophageal EMG recordings. It is unlikely that these fluctuations explain the greater variability in P-A pressure compared to that in control fetuses (Chapter 8.3.ii), since most minutes in which they occurred would have been classified as unstable and thus excluded from point pressure measurements.

As lung liquid secretion declines in chronic lung liquid loss (Dickson & Harding 1989), electrolyte concentrations were examined in the tracheal drainage experiments to determine whether they changed during the evolution of PH, especially those normally found in greater concentration in lung liquid than in serum, such as chloride and to a lesser extent sodium (Mescher et al 1975). However, lung liquid electrolytes did not change and were similar to those reported by others (Mescher et al 1975). Chloride concentration was significantly lower in pharyngeal fluid

than in tracheal fluid, consistent with the former not being entirely composed of lung liquid. Drainage volumes were similar in the pharyngeal and tracheal drainage experiments; pharyngeally-drained fetuses were still able to swallow, so that the volume swallowed was presumably made up by the volume of the mucus contribution to the drained fluid. Such speculation however, is further complicated by the fact that lung liquid secretion rate declines by up to 35% in the presence of chronic lung liquid loss (Dickson & Harding 1989).

As discussed in Chapter 8.3.iii, Fewell & Johnson (1983) dispute the larynx as the location of the normal upper airways resistance, and consider instead that it occurs in the buccal cavity, although this is based on tenuous data. The lack of effect observed in this study with chronic pharyngeal drainage at subamniotic pharyngeal pressures, both on tracheal pressure and on lung development, does not support their contention. In addition, if the major site of resistance to lung liquid egress was the buccal cavity rather than the larynx, the control procedures in the pilot study (pharyngeal catheterization into the amniotic cavity) should also have produced PH. This was a further reason for not performing sham operations on the control co-twins.

8.7 Summary

The aetiology of oligohydramnios-related pulmonary hypoplasia is not understood, but is known to involve chronic lung liquid loss. The hypothesis examined in this chapter was that low amniotic pressure in oligohydramnios disturbs the normal tracheal-amniotic pressure gradient to increase lung liquid loss and impair lung development. In 15 fetal sheep, pharyngeal catheterization was used to mimic reduced amniotic pressure at the upper airway in the presence of normal amniotic fluid volume. P-A pressures were negative in all drained fetuses (mean -3.0 $\pm$ 0.6 mm Hg, $p<0.001$), in contrast to positive pressures in controls (0.7 $\pm$ 0.1 mm Hg). In 10 fetuses which underwent chronic pharyngeal drainage for 10-21 days, there was no significant reduction in lung weight, volume or DNA relative to body weight, or in lung morphometry, compared to their control co-twins. In contrast, chronic tracheal drainage produced significant reductions in relation to body weight in lung weight (39.9 $\pm$ 5.2%, $p=0.004$), volume (39.2 + 4.0%, $p<0.001$), DNA (26.0 $\pm$ 1.5%, $p=0.003$), and the percentage of lung occupied by airspaces (25.7

± 9.0%, p=0.04) compared to controls. The failure of pharyngeal drainage to produce PH may be explained by its failure to alter T-A pressure (mean +1.7 ± 0.9 mm Hg), which remained similar to that in controls; in contrast tracheal drainage produced a significant drop in T-A pressure (mean -2.1 ± 1.3 mm Hg, p=0.03). These studies indicate that mimicking low amniotic pressure in the upper airway by chronic fetal pharyngeal drainage does not impair lung development in fetal sheep.

CHAPTER 9: CONCLUSIONS

9.1 Main findings

In this work, I have attempted to characterize basal amniotic pressure in human pregnancies with particular emphasis on those with abnormal amniotic fluid volume, and to determine the relationship between abnormal AP and some of the complications of oligohydramnios and polyhydramnios.

The main findings and conclusions are as follows:

i. In human pregnancies with normal amniotic fluid volume, AP increases significantly with gestational age and the sigmoid curve indicates that AP, unlike volume, reaches a plateau in the mid-trimester. Phenomena which affect amniotic fluid volume, such as multiple pregnancy, did not influence AP nor did semi-quantitative indices of the amount of amniotic fluid, suggesting that AP is not primarily determined by intrauterine volume over the normal range.

ii. AP was significantly elevated in human pregnancies with increased amniotic fluid volume, and reduced in pregnancies with decreased amniotic fluid volume, compared to those with normal amniotic fluid volume. The degree of derangement in AP correlated with the severity of the abnormality in amniotic fluid volume. With removal of fluid in polyhydramnios and infusion in oligohydramnios, AP changed significantly, regressing towards the mean in pregnancies with normal amniotic fluid volume. It is concluded that AP is influenced by volume only in extreme degrees of derangement in amniotic fluid volume.

iii. In human pregnancies complicated by polyhydramnios, elevated AP correlated with low fetal pO_2 and pH, consistent with the hypothesis that raised AP in polyhydramnios impairs uteroplacental perfusion. However, no relationship was found between the degree of elevation in AP and Doppler indices of downstream resistance in the uteroplacental circulation. In addition, acutely elevating AP in sheep by amnioinfusion did not affect fetal blood gas status. It is concluded that, although abnormal fetal blood gas status in human polyhydramnios is associated with elevated AP, the failure to reproduce this effect in sheep suggests that this may not be due to compressive effects of raised AP on the uteroplacental

circulation.

iv. Restoration of amniotic fluid volume in human pregnancies complicated by severe oligohydramnios did not produce any change in the number or incidence of fetal breathing movements or the number of epochs, which is inconsistent with literature suggesting that FBM are absent in fetuses with oligohydramnios-related pulmonary hypoplasia. Similarly, restoration of amniotic fluid volume did not alter indices of downstream resistance in the umbilical artery Doppler waveform. Together with the demonstration of low amniotic pressure in oligohydramnios, these findings challenge the concept of fetal compression in oligohydramnios that has become widely accepted in the literature.

v. As fetal compression and/or inhibition of FBM are therefore unlikely mechanisms for oligohydramnios-related PH, it was hypothesized that low AP in oligohydramnios disturbs the normal tracheal-amniotic pressure gradient, facilitating lung liquid loss and thereby impairing fetal lung development. Mimicking the effect of low AP on the fetal upper airway by chronic pharyngeal drainage in fetal sheep however, failed to alter tracheal pressure and had no effect on fetal lung development. In contrast, chronic tracheal drainage ablated the standing tracheal pressure and produced pulmonary hypoplasia. This work also indicated that the resistance to lung liquid egress essential for normal lung development is not located within the fetal pharynx.

9.2 Limitations

The studies on AP in human pregnancies were necessarily limited by (i) the need to confine measurements largely to ongoing pregnancies undergoing clinically-indicated invasive procedures, (ii) the reliance on clinical availability in the study of pregnancies with abnormal amniotic fluid volume, (iii) the need to restrict studying the effects of manipulating amniotic fluid volume to clinically-indicated amnioinfusion and drainage procedures and (iv) the lack of an accurate ethically-acceptable method for determining amniotic fluid volume in ongoing pregnancies. Ethical constraints do not allow scientific evaluation of exact pressure/volume relationships with in the human uterus by incremental fluid infusion and removal experiments.

Although the sheep experiments allowed greater scientific

rigour in their design, they were also limited, by (i) their expense (ii) the high loss rates associated with chronic fetal catheterization, especially with preparations lasting >7 days, and (iii) the lack of direct comparability with human fetal pathophysiology. A non-human primate like the baboon or rhesus monkey would have been a more applicable animal model for human pregnancy, although I had no access to such a facility. Non-human primates have not been used widely in the study of fetal physiology in view of their even greater expense, their relative lack of availability, the greater ethical and practical difficulties with their care, and the even lower fetal survival rates following chronic catheterization procedures. The difference in uteroplacental physiology between sheep and man made the interpretation of the negative findings of the ovine experiments in which the effect of elevating AP on fetal blood gas status was assessed, difficult. On the other hand with reference to the investigation of the effect of low AP on lung development, many studies have vindicated the choice of fetal sheep as an appropriate model for oligohydramnios-related pulmonary hypoplasia.

In addition to the problems outlined above, the chief weaknesses of the work in this thesis were (i) the lack of systematic approach to the volumes removed and drained in human pregnancies, making it difficult to exclude volume as a confounding variable in determining the factors influencing Δ AP, (ii) the low numbers enrolled in the Doppler studies of the fetoplacental and uteroplacental circulation in oligohydramnios and polyhydramnios respectively, rendering them susceptible to type II error, (iii) the suboptimal control FBM data in the tracheal drainage experiments, which were neither contemporaneous nor comparable over the entire drainage period, and (iv) the short and variable study period in the pharyngeal drainage experiments engendered by high loss and leakage rates, jeopardizing, at least in part, the analysis of lung development, and precluding strict comparison with the tracheal drainage experiments. The reasons for each of the above are addressed in the Discussion of the relevant section.

9.3 Suggestions for future work

Further work is needed in singleton pregnancies to establish the pattern of AP development after 34 weeks, which was not addressed in this study in view of the paucity of clinical indications

for late pregnancy amniocentesis; this may need to be done outside the United Kingdom. In any study seeking to confirm the normal pregnancy findings, ultrasound planimetric determination of uterine and amniotic dimensions would lead to better delineation of the relationship between amniotic pressure and intrauterine volume. Study of greater numbers of twins and triplets would indicate whether the reference range in singletons was similarly applicable to multiple pregnancies.

There is urgent clinical need to determine the relationship between raised AP in pregnancies with polyhydramnios and the main complications leading to perinatal morbidity and mortality, preterm labour and rupture of the membranes. Such a study would need to be done without resorting to therapeutic interventions, and may raise ethical difficulties. The effect of therapeutic manoeuvres such as serial drainage and/or maternal indomethacin therapy on AP and its duration similarly warrants investigation.

A larger study is needed of uteroplacental Doppler waveforms in patients with gross polyhydramnios undergoing fetal blood gas analysis, so as better to determine the relationship between uteroplacental downstream resistance and impaired fetal blood gas status, although difficulties remain in recruitment as discussed. The experiment examining the effect on fetal blood gas status of acutely elevating AP would benefit from repetition in an animal model whose uterine and placental anatomy more closely resembled that of humans, preferably with the addition of flow probes to monitor uteroplacental perfusion.

Arising from the study of the effects of restitution of amniotic fluid volume on umbilical artery Doppler waveforms, it is now important to confirm the absence of compressive effects on the fetal circulation by using the human amnioinfusion model to study the effect of severe oligohydramnios on fetal thoracic aortic and intracerebral waveforms.

Despite the experiments manipulating pressure gradients in this work, the mechanism for oligohydramnios-related pulmonary hypoplasia remains elusive. Investigative attention should focus on what factors facilitate lung liquid loss in the presence of oligohydramnios. In this regard, a priority would be to investigate the recent suggestion by Harding et al (1990), that fetal chest compression does occur in oligohydramnios, but only during non-labour uterine contractions.

REFERENCES

Abramovich DR (1968). The volume of amniotic fluid in early pregnancy. **J Obstet Gynaec Brit C'wealth** 75: 728-731.

Abramovich DR (1970) Fetal factors influencing the volume and composition of liquor amnii. **J Obstet Gynaec Brit C'wealth** 77: 865-877.

Abramovich DR, Page KR, Jandial L (1976). Bulk flows through human fetal membranes. **Gynecol Invest** 7: 157-164.

Abramovich DR, Garden A, Jandial L, Page KR (1978). Fetal swallowing and voiding in relation to hydramnios. **Obstet Gynecol** 54: 15-20.

Achiron R, Rosen N, Zakut H (1987). Pathophysiologic mechanism of hydramnios development in twin transfusion syndrome. **J Reprod Med** 32: 305-308.

Adams FH, Desilets DT, Towers B (1967). Control of flow of fetal lung fluid at the laryngeal outlet. **Resp Physiol** 2: 302-309.

Adzick NS, Harrison MR, Glick PL, Villa RL, Finkbeiner W (1984). Experimental pulmonary hypoplasia and oligohydramnios: relative contributions of lung fluid and fetal breathing movements. **J Pediatr Surg** 19: 658-665.

Adzick NS, Harrison MR, Glick PL, Nakayama DK, Manning FA, deLorimier AA (1985). Diaphragmatic hernia in the fetus: prenatal diagnosis and outcome in 94 cases. **J Pediatr Surg** 20: 357-361.

Alcorn D, Adamson TM, Lambert TF, Maloney JE, Ritchie BC, Robinson PM (1977). Morphological effects of chronic tracheal ligation and drainage in the fetal lamb lung. **J Anat** 123: 649-660.

Alcorn D, Adamson TM, Maloney JE, Robinson PM (1980). Morphological effects of chronic bilateral phrenectomy or vagotomy in the fetal lamb lung. **J Anat** 130: 683-695.

Alexander ES, Spitz HB, Clark RA (1982). Sonography of polyhydramnios. **AJR** 138: 343-346.

Alvarez H, Caldeyro R (1950). Contractility of the human uterus recorded by new methods. **Surg Gynecol Obstet** 91:1-13.

Anderson ABM, Turnbull AC, Murray AM (1967). The relationship between amniotic fluid pressure and uterine wall tension in pregnancy. **Am J Obstet Gynecol** 97: 992-997.

Ang MS, Thorp JA, Parisi VM (1990). Maternal lithium therapy and polyhydramnios. **Obstet Gynecol** 76: 517-519.

Ardiuni D, Rizzo G (1991). Fetal renal artery velocity waveforms and amniotic fluid volume in growth-retarded and post-term fetuses. **Obstet Gynecol** 77: 370-373.

Ardiuni D, Rizzo G, Romanini C, Mancuso S (1987). Fetal blood flow velocity waveforms as predictors of growth retardation. **Obstet Gynecol** 70: 7-10.

Askenazi SS, Perlman M (1979). Pulmonary hypoplasia: lung weight and radial alveolar count as criteria of diagnosis. **Arch Dis Child** 54: 614-618.

Assali NS, Holm LW, Sehgal N (1962). Hemodynamic changes in fetal lamb in utero in response to asphyxia, hypoxia and hypercapnia. **Circ Res** 11: 423-430.

Bahoric A, Chernick V (1975). Electrical activity of phrenic nerve and diaphragm in utero. **J Appl Physiol** 39: 513-518.

Bang J (1985). Intrauterine needle diagnosis. In Holm HA, Kristensen JK (eds) **Interventional Ultrasound.** Munksgaard, Copenhagen, pp122-128.

Barkin SZ, Pretorius DH, Beckett MK, Manchester DK, Nelson TR, Manco-Johnson ML (1987). Severe polyhydramnios: incidence of anomalies. **AJR** 148: 155-159.

Barry AP (1953). Hydramnios. A survey of 100 cases. **Irish J Med Sci** 331: 257-264.

Barry AP (1958). Hydramnios. **Obstet Gynecol** 11: 667-675.

Barss V, Benacerraf B, Frigoletto F (1984). Second trimester oligohydramnios: a predictor of poor fetal outcome. **Obstet Gynecol** 64: 608-10.

Basso A, Fernandez A, Aldabe O, Sabini G, Piriz H, Belitzky R (1977). Passage of mannitol from mother to amniotic fluid and fetus. **Obstet Gynecol** 49: 628-631.

Battaglia F, Prystowsky H, Smisson C, Hellegers A, Bruns P (1960). Fetal blood studies XIII. The effect of the administration of fluids intravenously to mothers upon the concentrations of water and electrolytes in plasma of human fetuses. **Pediatrics** 25: 2-10.

Baumgarten K, Moser S (1986). The technique of fibrin adhesion for premature rupture of the membranes during pregnancy. **J Perinat Med** 14: 43-49.

Bebbington MW, Wittman BK (1989). Fetal transfusion syndrome: antenatal factors predicting outcome. **Am J Obstet Gynecol** 160: 913-915.

Beischer NA, Brown JB, Townsend L (1969). Studies in prolonged pregnancy III: Amniocentesis in prolonged pregnancy. **Am J Obstet Gynecol** 103: 496-503.

Bell RJ, Congiu M, Hardy KJ, Wintour EM (1984). Gestation-dependant aspects of the response of the ovine fetus to the osmotic stress induced by maternal water deprivation. **Q J Exp Physiol** 69: 187-195.

Benacerraf BR, Frigoletto FD, Wilson M (1986). Successful mid-trimester thoracocentesis with analysis of the lymphocyte subpopulation in the pleural effusion. **Am J Obstet Gynecol** 155: 398-399.

Benacerraf BR (1990). Examination of the second-trimester fetus with severe oligohydramnios using transvaginal scanning. **Obstet Gynecol** 75: 491-493.

Bengtson JM, Van Marter LJ, Barss VA, Greene MF, Tuomala RE, Epstein MF (1989). Pregnancy outcome after premature rupture of the membranes at or before 26 weeks gestation. **Obstet Gynecol** 73: 921-926.

Bengtsson LP, Csapo AI (1962). Oxytocin response, withdrawal and reinforcement of defence mechanism of the human uterus at midpregnancy. **Am J Obstet Gynecol** 83: 1083-1093.

Benzie R, Doran TA, Harkins JL, Owen VMJ, Porter CJ (1974). Composition of the amniotic fluid and maternal serum in pregnancy. **Am J Obstet Gynecol** 119: 798-810.

Bewley S, Campbell S, Cooper D (1989). Uteroplacental Doppler flow velocity waveforms in the second trimester: a complex circulation. **Br J Obstet Gynaecol** 96: 1040-1046.

Beydoun SN, Yasin SY (1986). Premature rupture of the membranes before 28 weeks: conservative management. **Am J Obstet Gynecol** 155: 471-479.

Bhutani VK, Abbashi S, Weiner S (1986). Neonatal pulmonary manifestations due to prolonged amniotic leak. **Am J Perinatol** 3: 225-230.

Bilardo CM, Nicolaides KH, Campbell S (1990). Doppler measurements of fetal and uteroplacental circulations: relationship with umbilical venous blood gases measured at cordocentesis. **Am J Obstet Gynecol** 162: 115-120.

Block MF, Kallenberger DA, Kern JD, Nepveux RD (1981). In utero meconium aspiration by the baboon fetus. **Obstet Gynecol** 57: 37-40.

Blott M, Greenough A, Nicolaides KH, Moscoso G, Gibb D, Campbell S (1987). Fetal breathing movements as predictor of favourable pregnancy outcome after oligohydramnios due to membrane rupture in second trimester. **Lancet** ii: 129-131.

Blott M, Greenough A (1988). Neonatal outcome after prolonged rupture of the membranes starting in the second trimester. **Arch Dis Child** 63: 1146-1150.

Blott M, Greenough A, Nicolaides K H, Campbell S (1990). The ultrasonographic assessment of the fetal thorax and fetal breathing movements in the prediction of pulmonary hypoplasia. **Early Hum Dev** 21: 143-151.

Boddy K, Dawes GS, Robinson JS (1973). A 24-hour rhythym in the foetus. In: **Foetal and Neonatal Physiology, Proceedings of the Sir Joseph J Barcroft Centenary Symposium**. Cambridge University Press, Cambridge, pp 63-66.

Bottoms SF, Welch RA, Zador IE, Sokol RJ (1986). Limitations of using maximal vertical pocket and other sonographic evaluations of amniotic fluid volume to predict fetal growth: technical or physiologic? **Am J Obstet Gynecol** 155: 154-158.

Bowes G, Adamson TM, Ritchie BC, Dowling M, Wilkinson MH, Maloney JE (1981). Development of patterns of respiratory activity in unanesthetized fetal sheep in utero. **J Appl Physiol** 50: 693-700.

Boylan P, Parisi V (1986). An overview of hydramnios. **Semin Perinatol** 10: 136-141.

Brace RA (1986). Amniotic fluid balance and its relationship to fetal fluid balance: review of experimental data. **Semin Perinatol** 10: 103-112.

Brace RA (1989). Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long term intravascular infusions of saline. **Am J Obstet Gynecol** 161: 1049-1054.

Brace RA, Wolf EJ (1989). Normal amniotic fluid volume changes throughout pregnancy. **Am J Obstet Gynecol** 161: 382-388.

Brandt AJ, Bates JS (1961). Transabdominal amniocentesis in hydramnios. **Obstet Gynecol** 17: 392-394.

Brar HS, Platt LD, DeVore G, Horenstein J, Medearis AL (1988). Qualitative assessment of maternal uterine and fetal umbilical artery blood flow and resistance in laboring patients by Doppler velocimetry. **Am J Obstet Gynecol** 158: 952-956.

Brar HS, Medearis AL, Platt L (1989). Relationship of systolic/diastolic ratios from umbilical velocimetry to fetal heart rate. **Am J Obstet Gynecol** 160: 188-191.

Brock DJH, Sutcliffe RG (1972). Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. **Lancet** ii: 197-199.

Brown DG, Benson CB, Driscoll SG, Doubilet PM (1989). Twin-twin transfusion syndrome: sonographic findings. **Radiology** 170: 61-63.

Brown GR (1958). Acute hydramnios treated by abdominal paracentesis. **J Obstet Gynaecol Br Emp** 65: 61-63.

Brown GR, Macaskill S (1961). Acute hydramnios, with twins, successfully treated by abdominal paracentesis. **Br Med J** 1: 1739.

Burri PH (1984). Fetal and postnatal development of the lung. **Ann Rev Physiol** 46: 617-628.

Cabrera-Ramirez L, Harris RE (1976). Controlled removal of amniotic fluid in hydramnios. **South Med J** 69: 239-240.

Cabrol D, Landesman R, Muller J, Uzan M, Sureau C, Saxena BB (1987). Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin). **Am J Obstet Gynecol** 157: 422-426.

Caldeyro R, Alvarez H, Reynolds SRM (1950). A better understanding of uterine contractility through simultaneous recording with an internal and a seven channel external method. **Surg Gynecol Obstet** 91: 641-650.

Caldeyro-Barcia R, Alvarez H (1952) Abnormal uterine action in labour. **J Obstet Gynaec Brit C'wealth** 59: 646-656.

Caldeyro-Barcia R, Pose SV, Alvarez H (1957). Uterine contractility in polyhydramnios and the effects of withdrawal of the excess of amniotic fluid. **Am J Obstet Gynecol** 73: 1238-1254.

Campanale RP, Rowland RH (1955). Hypoplasia of the lung associated with congenital diaphragmatic hernia. **Ann Surg** 142: 176-189.

Campbell S, Wladimiroff JW, Dewhurst CJ (1973). The antenatal measurement of fetal urine production. **Br J Obstet Gynaecol** 80: 680-686.

Campbell S, Pearce JM, Hackett G, Cohen-Overbeek T, Hernandez C (1986). Qualitative assessment of uteroplacental blood flow: early screening test for high-risk pregnancies. **Obstet Gynecol** 68: 649-653.

Campbell S, Vyas S, Bewley S (1988). Doppler uteroplacental waveforms. **Lancet** i: 1287-1288.

Cardwell MS (1987). Polyhydramnios: a review. **Obstet Gynecol Surv** 42: 612-617.

Carlson DE, Platt LD, Medearis AL, Horenstein J (1990). Quantifiable polyhydramnios : diagnosis and management. **Obstet Gynecol** 75: 989-993.

Carmel JA, Friedman F, Adams FH (1965). Fetal tracheal ligation and lung development. **Am J Dis Child** 109: 452-456.

Castillo RA, Devoe LD, Falls G, Holzman GB, Hadi HA, Fadel HE (1987). Pleural effusions and pulmonary hypoplasia. **Am J Obstet Gynecol** 157: 1252-1255.

Chamberlain PF, Manning FA, Morrison I, Harman CR, Lange IR (1984b). Ultrasound evaluation of amniotic fluid volume II. The relationship of increased amniotic fluid volume to perinatal outcome. **Am J Obstet Gynecol** 150: 250-254.

Chamberlain PF, Manning FA, Morrison I, Harman CR, Lange IR (1984c). Ultrasound evaluation of amniotic fluid volume I. The relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. **Am J Obstet Gynecol** 150: 245-249.

Chamberlain PF, Manning FA, Morrison I, Lange IR (1984a). Circadian rhythm in bladder volumes in the term human fetus. **Obstet Gynecol** 64: 657-660.

Charles D, Jacoby HE (1966). Preliminary data on the use of sodium aminohippurate to determine amniotic fluid volumes. **Am J Obstet Gynecol** 95: 266-269.

Chescheir NC, Seeds JW (1988). Polyhydramnios and oligohydramnios in twin gestations. **Obstet Gynecol** 71: 882-884.

Cibils LA (1978). Clinical significance of fetal heart rate patterns during labour. V. variable decelerations. **Am J Obstet Gynecol** 132: 791-805.

Cifuentes RF, Olley PM, Balfe JW, Radde IC, Soldin SJ. (1979). Indomethacin and renal function in premature infants with persistent patent ductus arteriosus. **J Pediatr** 95: 583-587.

Clifford SH (1954). Postmaturity -with placental dysfunction. Clinical syndrome and pathologic findings. **J Pediatr** 44: 1-13.

Coetzer JAW, Barnard BJH (1977). Hydrops amnii in sheep associated with hydranencephaly and arthrogryposis with Wesselsbron disease and Rift Valley fever viruses as aetiological agents. **Onderstepoort J Vet Res** 44: 119-126.

Cohen HE, Sacks EJ, Hejman MA, Rudolph AM (1974). Cardiovascular response to hypoxemia and acidemia in fetal lambs. **Am J Obstet Gynecol** 120: 817-824.

Cohn HE, Sacks E, Heyman MA, Rudolph AM (1974). Cardiovascular responses to hypoxemia and acidemia in fetal lambs. **Am J Obstet Gynecol** 120: 817-824.

Collins MH, Moessinger AC, Kleinerman J, James LS, Blanc WA (1986). Morphometry of hypoplastic fetal guinea pig lungs following amniotic fluid leak. **Pediatr Res** 20: 955-960.

Cooney TP, Thurlbeck WM (1982). The radial alveolar count method of Emery and Mithal: a reappraisal. 2-Intrauterine and early postnatal lung growth. **Thorax** 37: 580-583.

Coren RL, Csapo AI (1963). The intra-amniotic pressure. **Am J Obstet Gynecol** 85: 470-483.

Cowan F. Indomethacin, patent ductus arteriosus, and cerebral blood flow. **J Pediatr** 109: 341-344.

Crowley P (1980). Non-quantitative measurement of amniotic fluid volume in prolonged pregnancy. **J Perinat Med** 8: 249-251.

Crowley P, O'Herlihy C, Boylan P (1984). The value of ultrasound measurement of amniotic fluid volume in the management of prolonged pregnancies. **Br J Obstet Gynaecol** 91: 444-448.

Cruz AC, Frentzen BH, Gomez KJ, Allen G, Tyson-Thomas M (1988) Continuous-wave Doppler ultrasound and decreased amniotic fluid volume in pregnant women with intact and ruptured membranes. **Am J Obstet Gynecol** 159: 708-714.

Csapo A (1962) Smooth as a contractile unit. **Phys Rev** 42: 7-33.

Csapo A, Takeda H, Wood C (1963). Volume and activity of the parturient rabbit uterus. **Am J Obstet Gynecol** 85: 813-818.

Csapo A, Sauvage J (1968) The evolution of uterine activity during human pregnancy. **Acta Obstet Gynecol Scand** 47: 181-212.

Csapo A (1969) The luteo-placental shift, the guardian of prenatal life. **Postgrad Med J** 45: 57-64.

Csapo A (1970). The diagnostic significance of the intrauterine pressure. **Obstet Gynecol Surv** 25: 403-543.

Cunningham M, Stocks J (1978). Werdnig-Hoffman disease. **Arch Dis Child** 53: 921-925.

Daffos F, Capella-Pavlovsky M, Forestier F (1985). Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. **Am J Obstet Gynecol** 153: 655-660.

Danziger RW (1948). Twin pregnancy with acute hydramnios treated by paracentesis uteri. **Br Med J** 2: 205-206.

Dawes GS, Gardener WN, Johnston BM, Walker DW (1980). Activity of intercostal muscles in relation to breathing movements, electrocortical activity and gestational age in fetal lambs. **J Physiol** 307: 47-48P.

DeMyer W, Baird I (1969). Mortality and skeletal malformations from amniocentesis and oligohydramnios in rats: cleft palate, club foot, microstomia, and adalactyly. **Teratology** 2: 33-38.

Desmedt EJ, Henry OA, Beischer NA (1990a). Polyhydramnios and associated maternal and fetal complications in singleton pregnancies. **Br J Obstet Gynecol** 97: 1115-1122.

Desmedt E, Henry OA, Steinberg LH, Beischer NA (1990b). Acute and subacute polyhydramnios in singleton pregnancies. **Aust NZ J Obstet Gynecol** 30: 191-195.

Dickson KA, Harding R (1987). Restoration of lung liquid volume following its acute alteration in fetal sheep. **J Physiol** 385: 531-543.

Dickson KA, Harding R (1989). Decline in lung liquid volume and secretion rate during oligohydramnios in fetal sheep. **J Appl Physiol** 67: 2401-2407.

Dickson KA, Harding R (1991). Fetal breathing and pressures in the trachea and amniotic sac during oligohydramnios in sheep. **J Appl Physiol** 70: 293-299.

Dickson KA, Maloney JE, Berger PJ (1987). State-related changes in lung liquid secretion and tracheal flow rate in fetal lambs. **J Appl Physiol** 62: 34-38.

Dornan JC, Ritchie JWK, Meban C (1984). Fetal breathing movements and lung maturation in the congenitally abnormal human fetus. **J Dev Physiol** 6: 367-375.

Drachman DB, Coulombre AJ (1962). Experimental club-foot and arthrogryposis multiplex congenita. **Lancet** ii: 523.

Dyer SN, Burton BK, Nelson LH (1987). Elevated maternal serum α-fetoprotein levels and oligohydramnios: poor prognosis for pregnancy outcome. **Am J Obstet Gynecol** 157: 336-339.

Elliott JP, Urig MA, Clewell WH (1991). Aggressive therapeutic amniocentesis for treatment of twin-twin transfusion syndrome. **Obstet Gynecol** 77: 537-540.

Elliott PM, Inman WHW (1961). Volume of liquor amnii in normal and abnormal pregnancy. **Lancet** ii: 835-840.

Emery JL, Mithal A (1960). The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. **Arch Dis Child** 35: 544-547.

Enesco M, LeBlond CP (1962). Increase in cell number as a factor in the growth of the organs and tissues of the young male rat. **J Embryol Exp Morphol** 10: 530-562.

Erkkola RU, Pirhonen JP (1990). Flow velocity waveforms in uterine and umbilical arteries during the angiotensin II sensitivity test. **Am J Obstet Gynecol** 162: 1193-1197.

Erskine JP (1944). A case of acute hydramnios successfully treated by abdominal paracentesis. **J Obstet Gynaecol Br Emp** 51: 549-551.

Fantel AG, Shepard TH (1975). Potter syndrome: nonrenal features induced by oligoamnios. **Am J Dis Child** 129: 1346-1347.

Feingold M, Cetrulo CL, Newton ER, Weiss J, Shakr C, Shmoys S (1986). Serial amniocenteses in the treatment of twin to twin transfusion complicated with acute polyhydramnios. **Acta Genet Med Gemellol** 35: 107-113.

Ferrazzi E, Gementi P, Bellotti M, Rodolfi M, Peruta D, Barbera A, Pardi G (1990). Doppler velocimetry: critical analysis of umbilical, cerebral and aortic reference values. **Eur J Obstet Gynecol Reprod Biol** 38: 189-196.

Fewell JE, Lee CC, Kitterman JA (1981). Effects of phrenic nerve section on the respiratory system of fetal lambs. **J Appl Physiol** 51: 293-297.

Fewell JE, Johnson PJ (1983). Upper airway dynamics during breathing and during apnoea in fetal lambs. **J Physiol** 339: 495-504.

Fewell JE, Hislop AA, Kitterman JA, Johnson P (1983). Effect of tracheostomy on lung development in fetal lambs. **J Appl Physiol** 55: 1103-1108.

Fisk NM (1988). Modifications to selective conservative management in preterm premature rupture of the membranes. **Obstet Gynecol Surv** 43: 328-334.

Fisk NM, Borrell A, Hubinont C, Tannirandorn Y, Nicolini U, Rodeck CH (1990). Fetofetal transfusion syndrome: do the neonatal criteria apply in utero? **Arch Dis Child** 65: 657-661.

Fisk NM, Rodeck CH (1992) Antenatal diagnosis and fetal medicine. In Roberton NC: **Textbook of Neonatology** 2nd ed, Churchill Livingstone, Edinburgh, in press.

Fisk NM, Ronderos-Dumit D, Soliani A, Nicolini U, Vaughan J, Rodeck CH (1991). Diagnostic and therapeutic transabdominal amnioinfusion in oligohydramnios. **Obstet Gynecol** 78: 270-278.

Fleischer A, Anyaegbunam AA, Schulman H, Farmakides G, Randolph G (1987). Uterine and umbilical artery velocimetry during normal labor. **Am J Obstet Gynecol** 157: 40-43.

Fox H E, Moessinger AC (1985). Fetal breathing movements and lung hypoplasia: Preliminary human observations. **Am J Obstet Gynecol** 151: 531-533.

Gabbe SG, Ettinger BB, Freeman RK, Martin CB (1976). Umbilical cord compression associated with amniotomy; laboratory observations. **Am J Obstet Gynecol** 126: 353-355.

Galea P, Scott JM, Goel KM (1982). Feto-fetal transfusion syndrome. **Arch Dis Child** 57: 781-783.

Gembruch U, Hansmann M (1988). Artificial instillation of amniotic fluid as a new technique for the diagnostic evaluation of cases of oligohydramnios. **Prenat Diagn** 8: 33-45.

Ghaly RG, Flynn RJ, Moore J (1988). Isoflurane as an alternative to halothane for Caesarean section. **Anaesthesia** 43: 5-7.

Gilbert WM, Brace RA (1988). Increase in fetal hydration during long-term intraamniotic isotonic saline infusion. **Am J Obstet Gynecol** 159: 1413-1417.

Gilbert WM, Brace RA (1989). The missing link in amniotic fluid volume regulation: intramembranous absorption. **Obstet Gynecol** 74: 748-754.

Giles WB, Trudinger BJ, Baird PJ (1985). Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. **Br J Obstet Gynaecol** 92: 31-38.

Gitlin D, Kumate J, Morales C, Noriega L, Arevalo N (1972). The turnover of amniotic fluid protein in the human conceptus. **Am J Obstet Gynecol** 113: 632-645.

Glick PL, Harrison MR, Golbus MS, et al (1985). Management of the fetus with congenital hydronephrosis II: Prognostic criteria and selection for treatment. **J Pediatr Surg** 20: 376-387.

Gluck L, Kulovich M, Borer RCJ, Brenner PH, Anderson GG, Spellacy WN (1971). Diagnosis of the respiratory distress syndrome by amniocentesis. **Am J Obstet Gynecol** 109: 440-445.

Gohari P, Berkowitz RL, Hobbins J (1977). Prediction of intrauterine growth retardation by determination of total intrauterine volume. **Am J Obstet Gynecol** 127: 255-260.

Golbus MS, Simpson TJ, Koresawa M, Appelman Z, Alpers CE (1988). The prenatal determination of glucose 6 phosphatase activity by fetal liver biopsy. **Prenat Diagn** 8: 401-404.

Goldkrand JW, Speichinger JP (1975). "Mixed cord compression", fetal heart rate pattern and its relation to abnormal cord position. **Am J Obstet Gynecol** 122: 144-150.

Goldstein JD, Reid LM (1980). Pulmonary hypoplasia resulting from phrenic nerve agenesis and diphragmatic amyoplasia. **J Pediatr** 97: 282-287.

Goldstein RB, Filly RA (1988). Sonographic estimation of amniotic fluid volume-subjective assessment versus pocket measurements. **J Ultrasound Med** 7: 363-369.

Gonik B, Bottoms SF, Cotton DB (1985). Amniotic fluid volume as a risk factor in preterm premature rupture of the membranes. **Obstet Gynecol** 65: 456-459.

Goodlin RC, Anderson JC, Gallagher TF (1983). Relationship between amniotic fluid volume and maternal volume expansion. **Am J Obstet Gynecol** 146: 505-511.

Grant J, Keirse MJ (1989). Prelabour rupture of the membranes at term. In Chalmers I, Enkin M, Keirse MJ (eds): **Effective care in pregnancy and childbirth.** Vol 2, Oxford University Press, Oxford, pp 1112-1117.

Green-Thompson RW (1982). Antepartum haemorrhage. In : **Obstetric problems in the developing world.** Clinics in Obstetrics and Gynaecology Vol 9 (3), WB Saunders, London, p485.

Greenough A, Blott M, Nicolaides K, Campbell S (1988). Interpretation of fetal breathing movements in oligohydramnios due to membrane rupture. **Lancet** (letter) i: 182-183.

Greiss F (1967). A clinical concept of uterine blood flow during pregnancy. **Obstet Gynecol** 30: 595-604.

Grossman M, Flynn JJ, Aufrichtig D, Handler CR (1982). Pitfalls in ultrasonic determination of total intrauterine volume. **J Clin Ultrasound** 10: 17-20.

Gruenwald P (1957). Hypoplasia of the lungs. **J Mt Sinai Hosp** 24: 913-919.

Gunn GC, Mishell D, Morton DG (1970). Premature rupture of the fetal membranes. A review. **Am J Obstet Gynecol** 106: 469-483.

Hackett GA, Nicolaides KH, Campbell S (1987). Doppler ultrasound assessment of fetal and uteroplacental circulations in severe second trimester oligohydramnios. **Br J Obstet Gynaecol** 94: 1074-1077.

Halperin ME, Fong KW, Zalev AH, Goldsmith CH (1985). Reliability of amniotic fluid volume estimation from ultrasonograms: intraobserver and interobserver variation before and after the establishment of criteria. **Am J Obstet Gynecol** 153: 264-267.

Hanretty KP, Whittle MJ, Rubin PC (1988). Doppler uteroplacental waveforms in pregnancy-induced hypertension: a reappraisal. **Lancet** i: 850-852.

Hanson FW, Zorn EM, Tennant FR, Marianos S, Samuels S (1987). Amniocentesis before 15 weeks gestation: outcome, risks and technical problems. **Am J Obstet Gynecol** 156: 1524-1531.

Hanson MA, Moore PJ, Nijhuis JG, Parkes MJ (1988). Effects of pilocarpine on breathing movements in normal, chemodenervated and brain stem transected fetal sheep. **J Physiol** 400: 415-424.

Harding R (1980). State related and developmental changes in laryngeal function. **Sleep** 3: 307-322.

Harding R, Johnson P, McClelland ME (1980). Respiratory function of the larynx in developing sheep and the influence of the sleep state. **Resp Physiol** 40: 165-179.

Harding R, Sigger JN, Wickham PJD, Bocking AD (1984a). The regulation of flow of pulmonary fluid in fetal sheep. **Respir Physiol** 57: 47-59.

Harding R, Bocking AD, Sigger JN, Wickham PJD (1984b). Composition and volume of fluid swallowed by fetal sheep. **Q J Exp Physiol** 69: 487-495.

Harding R, Bocking AD, Sigger JN (1986a). Influence of upper respiratory tract on liquid flow to and from fetal lungs. **J Appl Physiol** 61: 68-74.

Harding R, Bocking AD, Sigger JN (1986b). Upper airway resistances in fetal sheep: the influence of breathing activity. **J Appl Physiol** 60: 160-165.

Harding R, Hooper SB, Dickson KA (1990). A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios. **Am J Obstet Gynecol** 163: 1904-1913.

Harrison MR, Jester JA, Ross NA (1980). Correction of congenital diaphragmatic hernia in utero. I. The model: intrathoracic balloon produces fetal pulmonary hypoplasia. **Surgery** 88: 174-182.

Harrison MR, Golbus M, RA Filly RA, Nakayama DK, Callen PW, de Lorimier AA, Hricak H (1982a). Management of the fetus with congenital hydronephrosis. **J Pediatr Surg** 17: 728-742.

Harrison MR, Nakayama DK, Noall R, de Lorimier AA (1982b) Correction of congenital hydronephrosis in utero II: Decompression reverses the effects of obstruction on the fetal lung and urinary tract. **J Pediatr Surg** 17: 965-974.

Harrison MR, Ross NA, Noall R, de Lorimier AA (1983). Correction of congenital hydronephrosis in utero. I: The model: fetal urethral obstruction produces hydronephrosis and pulmonary hypoplasia in fetal lambs. **J Pediatr Surg** 18: 247-256.

Hashimoto B, Callen PW, Filly RA, Laros RK (1984). Ultrasound evaluation of polyhydramnios and twin pregnancy. **Am J Obstet Gynecol** 154: 1069-1072.

Hellman LM, Tricomi V, Gupta O (1957). Pressures in the human amniotic fluid and intervillous space. **Am J Obstet Gynecol** 74: 1018-1021.

Hendricks CH, Quilligan EJ, Tyler CW, Tucker GJ (1959). Pressure relationships between the intervillous space and the amniotic fluid in human term pregnancy. **Am J Obstet Gynecol** 77: 1028-1037.

Hendricks CH, Gabel RA (1960). Use of intranasal oxytocin in obstetrics. I. A laboratory evaluation. **Am J Obstet Gynecol** 79: 780-788.

Hendricks CH, Eskes TKAB, Saameli K (1962). Uterine contractility at delivery and in the puerperium. **Am J Obstet Gynecol** 83: 890-905.

Hendricks SD, Smith JR, Moore DE, Brown ZA (1990). Oligohydramnios associated with prostaglandin synthetase inhibitors in preterm labour. **Br J Obstet Gynaecol** 97: 312-316.

Hill AV (1953) . The mechanics of active muscle. **Proc Roy Soc (Lond)**. 141: 104-117.

Hill LM, Platt LD, Manning FA (1979). Immediate effect of amniocentesis on fetal breathing and gross body movements. **Am J Obstet Gynecol** 135: 689-690.

Hill LM, Breckle R, Wolfgram KR, O'Brien PC (1983) Oligohydramnios: ultrasonically detected incidence and subsequent fetal outcome. **Am J Obstet Gynecol** 147: 407-410.

Hill LM, Breckle R, Thomas ML, Fries JK (1987). Polyhydramnios: ultrasonically detected prevalence and neonatal outcome. **Obstet Gynecol** 69: 21-25.

Hislop A, Fairweather DVI, Blackwell RJ, Howard S (1984). The effect of amniocentesis and drainage of amniotic fluid on lung development in *Macaca Fascicularis*. **Am J Obstet Gynecol** 91: 835-842.

Hislop A, Hey E, Reid L (1979). The lungs in congenital bilateral renal agenesis and dysplasia. **Arch Dis Child** 54: 32-38.

Hoddick WK, Callen PW, Filly RA, Creasy RK (1984). Ultrasonographic determination of qualitative amniotic fluid volume in intrauterine growth retardation: Reassessment of the 1 cm rule. **Am J Obstet Gynecol** 149: 758-762.

Hon EH (1959). The fetal heart rate patterns preceeding death in utero. **Am J Obstet Gynecol** 78: 47-56.

Hunter AGW (1987). Neonatal lung function following mid-trimester amniocentesis. **Prenat Diagn** 7: 431-441

Hutchinson DL, Gray MJ, Plentl AA, Alvarez H, Caldeyro-Barcia R, Kaplan B, Lind J (1959). The role of the fetus in the water exchange of the amniotic fluid of normal and hydramniotic patients. **J Clin Invest** 38: 971-980.

Imanaka M, Ogita S, Sugawa T (1989). Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes. **Am J Obstet Gynecol** 161: 102-6.

Itskovitz J, LaGamma EF, Rudolph AM (1983). The effect of reducing umbilical blood flow on fetal oxygenation. **Am J Obstet Gynecol** 145: 813-818.

Jacoby HE, Charles D (1966) Clinical conditions associated with hydramnios. **Am J Obstet Gynecol** 94: 910-919.

James DJK, Tindall VR, Richardson T (1983). Is the lecithin/ sphingomyelin ratio outdated? **Br J Obstet Gynaecol** 90, 995-1000.

James LS, Yeh MN, Morishima HO, Daniel SS, Caritis SN, Niemann WH, Indyk L (1976). Umbilical vein occlusion and transient acceleration of the fetal heart rate. **Am J Obstet Gynecol** 126: 276-283.

Johnson JWC, Daikoku NH, Niebyl JR, Johnson TRB, Khouzami VA, Witter RF (1981). Premature rupture of the membranes and prolonged latency. **Obstet Gynecol** 57: 547-556.

Kapuscinski J, Skoczylas B (1977). Simple and rapid fluorimetric method for DNA microassay. **Anal Biochem** 83: 252-257.

Kappy KA, Cetrulo CL, Knuppel RA, Ingardia CJ, Sbarra AJ, Scerbo JC, Mitchell GW (1979). Premature rupture of the membranes: a conservative approach. **Am J Obstet Gynecol** 134: 655-661.

Kappy KA, Cetrulo CL, Knuppel RA, Ingardia CJ, Sbarra AJ, Scerbo JC, Mitchell GW (1982). Premature rupture of the membranes at term: a comparison of induced and spontaneous labours. **J Reprod Med** 27: 29-33.

Kaye GWC, Laby TH (1973). **Tables of physical and chemical constants.** 14th edition, Longman London p29.

Kendrick FJ, Field FE (1967). Congenital anomalies induced in normal and adrenalectomised rats by amniocentesis. **Anat Rec** 159: 353-356.

Kerenyi TD, Muzsnai D (1975). Volume and sodium concentration studies in 300 saline-induced abortions. **Am J Obstet Gynecol** 121: 590-596.

Kilbride HW, Thibeault DW, Yeast J, Maulik D, Grundy HO (1988). Fetal breathing is not a predictor of pulmonary hypoplasia in pregnancies complicated by oligohydramnios. **Lancet** i: 305-306.

King JC, Mitzner W, Butterfield AB, Queenan JT (1986). Effect of induced oligohydramnios on fetal lung development. **Am J Obstet Gynecol** 154: 823-830.

Kirkinen P, Jouppila P (1978). Polyhydramnion: a clinical study. **Ann Chir Gynaecol** 67: 117-122.

Kirshon B, Moise KJ, Wasserstrum N, Ou CN, Huhta JC (1988). Influence of short-term indomethacin therapy on fetal urine output. **Obstet Gynecol** 72: 51-53.

Kirshon B (1989). Fetal urine output in hydramnios. **Obstet Gynecol** 73: 240-242.

Kirshon B, Mari G, Moise KJ (1990). Indomethacin therapy in the treatment of symptomatic polyhydramnios. **Obstet Gynecol** 75: 202-205.

Kitterman JA, Liggins GC, Fewell JE, Tooley WH (1983). Inhibition of breathing movements in fetal sheep by prostaglandins. **J Appl Physiol** 54: 687-692.

Kitzmiller JL, Cloherty JP, Younger MD, Tabatabaii A, Rothchild SB, Sosenko I, Epstein MF, Singh S, Neff RK (1978). Diabetic pregnancy and perinatal morbidity. **Am J Obstet Gynecol** 131: 560-580.

Knox WF, Barson AJ (1986). Pulmonary hypoplasia in a regional perinatal unit. **Early Hum Dev** 14: 33-42.

Kofinas AD, Espeland M, Swain M, Penry M, Nelson LH (1989). Correcting umbilical artery flow velocity waveforms for fetal heart rate is unnecessary. **Am J Obstet Gynecol** 160: 704-707.

Krause S, Ebbeson F, Lange AP (1990). Polyhydramnios with maternal lithium treatment. **Obstet Gynecol** 75: 504-506.

Kurjak A, Kirkinen P, Latin V, Ivankovic D (1981). Ultrasonic assessment of fetal kidney function in normal and complicated pregnancies. **Am J Obstet Gynecol** 141: 266-270.

Landy HJ, Isada NB, Larsen JW (1987) Genetic implications of idiopathic hydramnios. **Am J Obstet Gynecol** 157: 114-117.

Lange IR, Harman CR, Ash KM, Manning FA, Menticoglou S (1989) Twins with hydramnios: treating premature labor at source. **Am J Obstet Gynecol** 160: 552-557.

Lanier LR, Scarbrough RW, Fillingim DW, Baker RE (1965). Incidence of maternal and fetal complications associated with rupture of membranes before onset of labour. **Am J Obstet Gynecol** 93: 398-404.

Leonidas JC, Bhan I, Beatty EC (1982). Radiographic chest contour and pulmonary air leaks in oligohydramnios-related pulmonary hypoplasia. **Invest Radiol** 17: 6-10.

Liggins GC, Vilos GA, Campos GA, Kitterman JA, Lee CH (1981). The effect of spinal cord transection on lung development in fetal sheep. **J Dev Physiol** 3: 267-264.

Liggins GC (1984). Growth of the fetal lung. **J Dev Physiol** 6: 237-248.

Liley AW (1961) Liquor amnii analysis in management of pregnancy complicated by rhesus sensitisation. **Am J Obstet Gynecol** 82: 1359-1370.

Lind T, Billewicz, WZ, Cheyne GA (1971). Composition of amniotic fluid and maternal blood throughout pregnancy. **J Obstet Gynaec Brit C'wealth** 78: 505-512.

Lind T, Kendall A, Hytten FE (1972) The role of the fetus in the formation of amniotic fluid. **J Obstet Gynaec Brit C'wealth** 79: 289-298.

Lloyd JR, Clatworthy HW (1958). Hydramnios as an aid to the early diagnosis of congenital obstruction of the alimentary tract: a study of the maternal and fetal factors. **Pediatrics** 21: 903-909.

Lombardi S, Rosemund R, Ball R, Entman SS, Boehm FH (1989). Umbilical artery velocimetry as a predictor of adverse outcome in pregnancies complicated by oligohydramnios. **Obstet Gynecol** 74: 338-341.

Lumbers ER, Stevens AD (1983). Changes in fetal renal function in response to infusions of a hyperosmotic solution of mannitol to the ewe. **J Physiol** 343: 439-446.

Mahoney BS, Petty CN, Nyberg DA, Luthy DA, Hickok DE, Hirsch JH (1990). The "stuck twin" phenomenon: ultrasonographic findings, pregnancy outcome, and management with serial amniocenteses. **Am J Obstet Gynecol** 163: 1513-1522.

Major CA, Kitzmiller JL (1990). Perinatal survival with expectant management of midtrimester rupture of the membranes. **Am J Obstet Gynecol** 163: 838-844.

Mamopoulos M, Assimakopoulos E, Reece EA, Andreou A, Zheng XZ, Manalenakis S (1990). Maternal indomethacin therapy in the treatment of polyhydramnios. **Am J Obstet Gynecol** 162: 1225-1229.

Manchester DK, Pretorius DH, Avery C, Manco-Johnson ML, Wiggins J, Meier PR, Clewell WH (1988). Accuracy of ultrasound diagnoses in pregnancies complicated by suspected fetal anomalies. **Prenat Diagn** 8: 109-117.

Manning FA, Hill LM, Platt LD (1981). Qualitative amniotic fluid volume determination by ultrasound: Antepartum detection of intrauterine growth retardation. **Am J Obstet Gynecol** 139: 254-258.

Manning FA, Harrison MR, Rodeck CH, and members of the International Fetal Medicine and Surgery Society (1986). Catheter shunts for fetal hydronephrosis and hydrocephalus. **N Engl J Med** 315: 336-340.

Marsden D, Huntingford PJ (1965). An appraisal of the Coomassie blue dilution technique for measuring the volume of liquor amnii in late pregnancy. **J Obstet Gynaec Brit C'wealth** 72: 65-68.

Martell M, Belizan JM, Nieto F, Schwarcz R (1976). Blood acid-base balance at birth in neonates from labors with early and late rupture of membranes. **J Pediatr** 89: 963-967.

McLain CR (1963). Amniography studies of the gastrointestinal motility of the human fetus. **Am J Obstet Gynecol** 86: 1079-1087.

Medical Research Council Working Party on Amniocentesis (1978). An assessment of the hazards of amniocentesis. **Br J Obstet Gynaecol** 85: suppl. 2.

Mercer LJ, Brown LG, Petres RE, Messer RH (1984). A survey of pregnancies complicated by decreased amniotic fluid. **Am J Obstet Gynecol** 149: 355-361.

Mercer LJ, Brown LG (1986). Fetal outcome with oligohydramnios in the second trimester. **Obstet Gynecol** 67: 840-842.

Mescher EJ, Platzker AC, Ballard PL, Kitterman JA, Clements JA, Tooley WH (1975). Ontogeny of tracheal fluid, pulmonary surfactant, and plasma corticoids in the fetal lamb. **J Appl Physiol** 39: 1017-1021.

Millard RW, Baig H, Vatner SF (1979). Prostaglandin control of the renal circulation in response to hypoxemia in the fetal lamb in utero. **Circ Res** 45: 172-179.

Minei LJ, Suzuki K (1976). Role of fetal deglutition and micturition in the production and turnover of amniotic fluid in the monkey. **Obstet Gynecol** 48: 177-181.

Mires G, Dempster J, Patel NB, Crawford JW (1987). The effect of fetal heart rate on umbilical artery velocity waveforms. **Br J Obstet Gynaecol** 94: 665-669.

Miyazaki FS, Nevarez F (1985). Saline amnioinfusion for relief of repetitive variable decelerations: A prospective randomised study. **Am J Obstet Gynecol** 153: 301-306.

Moberg LJ, Garite TJ, Freeman RK (1984). Fetal heart rate patterns and fetal distress in patients with preterm premature rupture of membranes. **Obstet Gynecol** 64: 60-64.

Moessinger AC (1983). Fetal akinesia deformation sequence: an animal model. **Pediatrics** 72: 857-863.

Moessinger AC, Bassi GA, Ballantyne G, Collins MH, James LS, Blanc WA (1983). Experimental production of pulmonary hypoplasia following amniocentesis and oligohydramnios. **Early Hum Dev** 8: 343-350.

Moessinger AC, Fewell JE, Stark RI, Collins MH, Daniel SS, Singh M, Blanc WA, Kleinerman J, James LS (1985). Lung hypoplasia and breathing movements following oligohydramnios in fetal lambs. In: Jones CT, Nathanielsz PW, eds. **The Physiological Development of the Fetus and Newborn.** London, England: Academic Press, pp 273-278.

Moessinger AC, Collins MH, Blanc WA, Rey HR, James LS (1986). Oligohydramnios-induced lung hypoplasia: the influence of timing and duration in gestation. **Pediatr Res** 20: 951-954.

Moessinger AC, Fox HE, Higgins A, Rey HR, Al Haidieri M (1987). Fetal breathing movements are not a reliable predictor of continued lung development in pregnancies complicated by oligohydramnios. **Lancet** ii: 1297-1300.

Moessinger AC, Harding R, Adamson TM, Singh M, Kiu GT (1990). Role of lung fluid volume in growth and maturation of the fetal sheep lung. **J Clin Invest** 86: 1270-1277.

Moise KJ, Huhta JC, Sharif DS, Ou CN, Kirshon B, Wasserstrum N, Cano L (1988). Indomethacin in the treatment of premature labor. Effects on fetal ductus arteriosus. **N Engl J Med** 319: 327-331.

Moise KJ, Ou CN, Kirshon B, Cano LE, Rognerud C, Carpenter RJ (1990). Placental transfer of indomethacin in the human pregnancy. **Am J Obstet Gynecol** 162: 549-554.

Moore PJ, Parkes MJ, Nijhuis JG, Hanson MA (1989b). The incidence of breathing movements of fetal sheep in normoxia and hypoxia after peripheral chemodenervation and brain-stem transection. **J Dev Physiol** 11: 147-151.

Moore TR (1990). Superiority of the four-quadrant sum over the single-deepest-pocket technique in ultrasonic identification of abnormal amniotic fluid volumes. **Am J Obstet Gynecol** 163: 762-767.

Moore TR, Cayle JE (1990). The amniotic fluid index in normal human pregnancy. **Am J Obstet Gynecol** 162: 1168-1173.

Moore TR, Longo J, Leopold GR, Casola G, Gosink BB (1989a). The reliability and predictive value of an amniotic fluid scoring system in severe second trimester oligohydramnios. **Obstet Gynecol** 73: 739-742.

Moretti M, Sibai BM (1988). Maternal and perinatal outcome of expectant management of premature rupture of membranes in the midtrimester. **Am J Obstet Gynecol** 159: 390-396.

Moya F, Apgar V, James LS, Berrien C (1960). Hydramnios and congenital anomalies. **JAMA** 173: 1552-1556.

Murray SR (1964). Hydramnios-a study of 864 cases. **Am J Obstet Gynecol** 88: 65-67.

Naeye RL, Milic AMB, Blanc W (1970). Fetal endocrine and renal disorders: clues to the origin of hydramnios. **Am J Obstet Gynecol** 108: 1251-1256.

Nageotte MP, Freeman RK, Garite TJ, Dorchester W (1985). Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes. **Am J Obstet Gynecol** 153: 557-562.

Nakayama DK, Glick PI, Harrison MR, Villia RL, Noall R (1983). Experimental pulmonary hypoplasia due to oligohydramnios and its reversal by relieving thoracic compression. **J Pediatr Surg** 18: 347-53.

Natale R, Nasello-Paterson C, Connors G (1988). Patterns of fetal breathing activity in the human fetus at 24 to 28 weeks of gestation. **Am J Obstet Gynecol** 158: 317-321.

National Institutes of Child Health and Human Development (NICHD) National Registry for Amniocentesis Study Group (1976). Mid-trimester amniocentesis for prenatal diagnosis: safety and accuracy. **JAMA** 236: 1471-1476.

Nelson DM, Stempel LE, Zuspan FP (1986). Association of prolonged preterm premature rupture of the membranes and abruptio placentae. **J Reprod Med** 31: 423-432.

Nichols J, Schrepfer R (1966). Polyhydramnios in anencephaly. **JAMA** 197: 549-551.

Nicolaides KH, Soothill PW, Rodeck CH, Clewell W (1986). Rh disease: intravascular fetal blood transfusion by cordocentesis. **Fetal Ther** 1: 185-192.

Nicolaides KH, Peters MT, Vyas S, Rabinowitz R, Rosen DJ, Campbell S (1990). Relation of rate of urine production to oxygen tension in small-for-gestational-age fetuses. **Am J Obstet Gynecol** 162: 387-391.

Nicolini U, Nicolaidis P, Fisk NM, Vaughan J, Fusi L, Gleeson R, Rodeck CH (1990). Limited role of fetal blood sampling in prediction of outcome in intrauterine growth retardation. **Lancet** ii: 768-772.

Nicolini U, Rodeck CH, Fisk NM (1987). Shunt treatment for fetal obstructive uropathy. **Lancet** ii: 1338-1339.

Nimrod C, Varela-Gittings F, Machin G, Campbell D, Wesenberg R (1984). The effect of very prolonged membrane rupture on fetal development. **Am J Obstet Gynecol** 148: 540-543.

Normand IC, Olver RE, Reynolds EO, Strang LB (1971). Permeability of lung capillaries and alveoli to non-electrolytes in the foetal lamb. **J Physiol** 219: 303-330.

O'Brien WF, Davis SE, Grissom MP, Eng RR, Golden SM (1984). Effect of cephalic pressure on fetal cerebral blood flow. **Am J Perinatol** 1:223-226.

Owen J, Henson BV, Hauth JC (1990). A prospective randomized study of saline solution amnioinfusion. **Am J Obstet Gynecol** 162: 1146-1149.

Parmley TH, Seeds AE (1970). Fetal skin permeability to isotopic water (THO) in early pregnancy. **Am J Obstet Gynecol** 108: 128-131.

Paterson RM, Prihoda TJ, Pouliot MR (1987). Sonographic amniotic fluid measurement and fetal growth retardation: A reappraisal. **Am J Obstet Gynecol** 157: 1406-1410.

Pearce JM (1987). Uteroplacental and fetal blood flow. In **Fetal monitoring**, Balliere's Clinics in Obstetrics & Gynaecology vol 1(1), Saunders, London, pp157-184.

Pearce JM, Campbell S, Cohen-Overbeek T, Hackett G, Hernandez J, Royston JP (1988). Reference ranges and sources of variation for indices of pulsed Doppler flow velocity waveforms from the uteroplacental and fetal circulation. **Br J Obstet Gynaecol** 95: 248-256.

Pearce JM, McParland PJ (1991). A comparison of doppler flow velocity waveforms, amniotic fluid columns and the nonstress test as a a means of monitoring post-dates pregnancies. **Obstet Gynecol** 77: 204-208.

Peeters LLH, Sheldon RE, Jones MD, Makowski EI, Meschia G (1979). Blood flow to fetal organs as a function of arterial oxygen content. **Am J Obstet Gynecol** 135: 637-646.

Perlman M, Williams J, Hirsch M (1976). Neonatal pulmonary hypoplasia after prolonged leakage of amniotic fluid. **Arch Dis Child** 51: 349-353.

Perlman M, Levin M (1974). Fetal pulmonary hypoplasia, anuria and oligohydramnios: clinicopathologic observations and review of the literature. **Am J Obstet Gynecol** 118: 1119-1123.

Perry CP, Harris RE, DeLemos RA, Null DM (1976). Intrauterine growth retarded infants: correlation of gestational age with maternal factors, mode of delivery and perinatal survival. **Obstet Gynecol** 48: 182-186 1976.

Persaud TVN (1973). Meromelia and other developmental abnormalities in experimental oligohydramnios. **Anat Anz Bd** 133: 499-502.

Phelan JP, Platt LD, Yeh SY, Broussard P, Paul RH (1985). The role of ultrasound assessment of amniotic fluid volume in the management of the postdate pregnancy. **Am J Obstet Gynecol** 151: 304-308.

Phelan JP, Smith CV, Broussard P, Small M (1987). Amniotic fluid volume assessment with the four-quadrant technique at 36-42 weeks gestation. **J Reprod Med** 32: 540-542.

Phelan JP, Martin GI (1989). Polyhydramnios: fetal and neonatal implications. **Clin Perinatol** 16: 987-994.

Philipson EH, Sokol RJ, Williams T (1983). Oligohydramnios: clinical associations and predictive value for intrauterine growth retardation. **Am J Obstet Gynecol** 146: 271-278.

Pillai M, James D (1990). Hiccups and breathing in human fetuses. **Arch Dis Child** 65: 1072-1075.

Posner MD, Ballagh SA, Paul RH (1990). The effect of amnioinfusion on uterine pressure and activity: A preliminary report. **Am J Obstet Gynecol** 163: 813-818.

Potter EL (1946a). Bilateral renal agenesis. **J Pediatr** 29: 68-76.

Potter EL (1946b). Facial characteristics of infants with bilateral renal agenesis. **Am J Obstet Gynecol** 51: 885-888.

Powers WF (1973). Twin pregnancy: Complications and treatment. **Obstet Gynecol** 42: 795-808.

Pretorius DH, Manchester D, Barkin S, Parker S, Nelson TR (1988). Doppler ultrasound of twin twin transfusion syndrome. **J Ultrasound Med** 7: 117-124.

Pringle KC, Turner JW, Schofield JC, Soper RT (1984). Creation and repair of diaphragmatic hernia in the fetal lamb: lung development and morphology. **J Pediatr Surg** 19: 131-140.

Pringle KC (1986). Human fetal lung development and related animal models. **Clin Obstet Gynecol** 29: 502-513.

Pritchard JA (1966) Fetal swallowing and amniotic fluid volume. **Obstet Gynecol** 28: 606-610.

Pritchard JA, Mason R, Corley M, Pritchard S (1970). Genesis of severe placental abruption. **Am J Obstet Gynecol** 108: 22-25.

Queenan JT, Gadow EC (1970). Polyhydramnios: chronic versus acute. **Am J Obstet Gynecol** 108: 349-355.

Queenan JT, Thompson W, Whitfield CR, Shah SI (1972). Amniotic fluid volumes in normal pregnancies. **Am J Obstet Gynecol** 114: 34-38.

Rabinowitz R, Peters MT, Vyas S, Campbell S, Nicolaides KH (1989). Measurement of fetal urine production in normal pregnancy by real-time ultrasonography. **Am J Obstet Gynecol** 161: 1264-1266.

Ratten GJ, Beischer NA, Fortune DW (1973). Obstetric complications when the fetus has Potter's syndrome. 1. Clinical considerations. **Am J Obstet Gynecol** 115: 890-896.

Rayburn WF, Motley ME, Stempel LE, Gendreau RM (1982). Antepartum prediction of the postmature infant. **Obstet Gynecol** 60: 148-153.

Reale FR, Esterly JR (1973). Pulmonary hypoplasia: a morphometric study of the lungs of infants with diaphragmatic hernia, anencephaly, and renal malformations. **Pediatrics** 51: 91-96.

Reece EA, Lockwood CJ, Rizzo N, Pilu G, Bovicelli L, Hobbins JC (1987). Intrinsic intrathoracic malformations of the fetus: sonographic detection and clinical presentation. **Obstet Gynecol** 70: 627-632.

Reynolds SRM (1946). The relationship of hydrostatic conditions in the uterus to the size and shape of the conceptus during pregnancy: a concept of uterine accommodation. **Anat Rec** 95: 283-296.

Reynolds SRM (1965). Growth of the distended uterus. In : **Physiology of the uterus.** 2nd edition, Hafner, New York: pp 203-234.

Reynolds SRM, Kaminester S (1936). Distension, a stimulus for uterine growth in untreated ovariectomized rabbits. **Am J Physiol** 116: 510-515.

Richards DS, Seeds JW, Katz VL, Lingley LH, Albright SG, Cefalo RC (1988). Elevated maternal serum alpha-fetoprotein with oligohydramnios: ultrasound evaluation and outcome. **Obstet Gynecol** 72: 337-341.

Rigatto H (1984). A new window on the chronic fetal sheep model. In Nathanielsz PW (ed): **Animal models in fetal medicine.** vol III Perinatology Press, New York, pp 57-67.

Rightmire DA, Campbell S (1987). Fetal and maternal Doppler blood flow parameters in postterm pregnancies. **Obstet Gyecol** 69: 891-894.

Rivett LC (1933). Hydramnios. **J Obstet Gynaecol Br Emp** 40: 522-525.

Roberts AB, Griffin D, Mooney R, Cooper DJ, Campbell S (1980). Fetal activity in 100 normal third trimester pregnancies. **Br J Obstet Gynaecol** 87: 480-484.

Roberts AB, Goldstein I, Romero R, Hobbins JC (1991). Fetal breathing movements after preterm premature rupture of membranes. **Am J Obstet Gynecol** 164: 821-825.

Robertson EG, Neer KJ (1983). Placental injection studies in twin gestation. **Am J Obstet Gynecol** 147: 170-174

Robillard JE, Weitzam RE, Burmeister L, Smith FG (1981). Development aspects of the renal response to hypoxemia in the fetal lamb in utero. **Circ Res** 48: 128-138.

Robson MS, Turner MJ, Strong JM, O'Herlihy CO (1990). Is amniotic fluid quantitation of value in the diagnosis and conservative management of prelabour membrane rupture at term? **Br J Obstet Gynaecol** 97: 324-328.

Rodeck CH, Nicolini U (1988). Physiology of the mid-trimester fetus. In Whitelaw A, Cooke RWI (eds) **The Very Immature Infant less than 28 weeks Gestation.** British Medical Bulletin Vol 44, Churchill Livingstone, Edinburgh, pp 826-849.

Rodeck CH, Fisk NM, Fraser DI, Nicolini U (1988). Long-term in utero drainage of fetal hydrothorax. **New Engl J Med** 319: 1135-1138.

Rose MP, Elder MG, Myatt L (1987). Arachidonic acid metabolism in the human placenta. **Trophoblast Res** 2: 71-83.

Rosendahl H, Kivinen S (1989). Antenatal detection of congenital malformations by routine ultrasonography. **Obstet Gynecol** 73: 947-951.

Ross MG, Ervin MG, Oakes G, Hobel C, Fisher DA (1983). Bulk flow of amniotic fluid water in response to maternal osmotic challenge. **Am J Obstet Gynecol** 147: 697-701.

Ross MG, Sherman DJ, Schreyer P, Ervin MG, Day L, Humme J (1991). Fetal rehydration via intra-amniotic fluid: contribution of fetal swallowing. **Pediatr Res** 29: 214-217.

Ross MG, Sherman DJ, Ervin MG, Day L, Humme J (1989). Stimuli for fetal swallowing: systemic factors. **Am J Obstet Gynecol** 161: 1559-1565.

Rotschild A, Ling EW, Puterman ML, Farquharson D (1990). Neonatal outcome after prolonged preterm rupture of the membranes. **Am J Obstet Gynecol** 162: 46-52.

Royston P (1991). Constructing time-specific reference ranges. **Stat Med** 10: 675-690.

Rudolph AM, Heymann MA (1985). Methods for studying the circulation of the fetus in utero. In: Nathanielsz PW (ed) **Animal models in fetal medicine** (I), Perinatology Press, New York pp 2-58.

Russell P (1979). Inflammatory lesions of the the human placenta. I. Clinical significance of acute chorioamnioinitis. **Am J Diag Obstet Gynecol** 1: 127-137.

Sauer L, Harrison MR, Flake AW, Krummel TR (1987). Does an expanding fetal abdominal mass produce pulmonary hypoplasia? **J Pediatr Surg** 22: 508-512.

Scarpelli EM, Condorelli S, Cosmi EV (1975). Lamb fetal pulmonary fluid I. Validation and significance of method for determination of volume and volume change. **Pediatr Res** 9: 190-195.

Schneider KTM, Vetter K, Huch R, Huch A (1985). Acute polyhydramnios complicating twin pregnancies. **Acta Genet Med Gemellol** 34: 179-184.

Schroder H, Gilbert RD, Power GG (1984). Urinary and hemodynamic responses to blood volume changes in fetal sheep. **J Dev Physiol** 6: 131-141.

Schruefer JJ, Seeds AE, Behrman RE, Hellegers AE, Bruns PD (1972). Changes in amniotic fluid volume and total solute concentration in the rhesus monkey following replacement with distilled water. **Am J Obstet Gynecol** 112: 807-815.

Schulman H, Fleischer A, Farmakides G, Bracero L, Rochelson B, Grunfield L (1986). Development of uterine artery compliance in pregnancy as detected by Doppler ultrasound. **Am J Obstet Gynecol** 155: 1031-1036.

Schwarz R, Althabe O, Belitzky R, Lanchares JL, Alvarez R, Berdaguer P, Capurro H, Belizan JM, Sabatino JH, Abusleme C, Caldeyro-Barcia R (1973). Fetal heart rate patterns in labors with intact and with ruptured membranes. **J Perinat Med** 1: 153-165.

Scurry JP, Adamson TM, Cussen LJ (1989). Fetal lung growth in laryngeal atresia and tracheal agenesis. **Aust Paediatr J** 25: 47-51.

Seeds AE (1970). Osmosis across term human placental membranes. **Am J Physiol** 219: 551-554.

Seeds AE (1980). Current concepts of amniotic fluid dynamics. **Am J Obstet Gynecol** 138: 575-586.

Seeds JW, Cefalo RC, Herbert WN, Bowes WA (1984). Hydramnios and maternal renal failure: relief with fetal therapy. **Obstet Gynecol** 64: 26S-29S.

Seyberth H, Rascher W, Hackenthal R, Wille L (1983). Effect of prolonged indomethacin therapy on renal function and selected vasoactive hormones in very low birth weight infants with symptomatic patent ductus arteriosus. **J Pediatr** 103: 979-984.

Sideris & Nicolaides KH (1990). Amniotic fluid pressure during pregnancy. **Fetal Diagn Ther** 5: 104-108.

Sieck UV, Ohlsson A (1984). Fetal polyuria and hydramnios associated with Bartter's syndrome. **Obstet Gynecol** 63: 22S-24S.

Sival DA, Visser GHA, Prechtl HFR (1990). Does reduction in amniotic fluid affect fetal movements? **Early Hum Dev** 23: 233-246.

Skillman CA, Plessinger MA, Woods JR, Clark KE (1985). Effect of graded reductions in uteroplacental blood flow on the fetal lamb. **Am J Physiol** 249: H1098-H1105.

Soothill PW, Nicolaides KH, Rodeck CH, Campbell S (1986). Effect of gestational age on fetal and intervillous blood gas and acid-base values in human pregnancy. **Fetal Ther** 1: 168-175.

Spencer JADS, Giussani DA, Moore PJM, Hanson MA (1991). In vitro validation of Doppler indices using blood and water. **J Ultrasound Med** 10: 305-308.

Steel SA, Pearce JM, McParland P, Chamberlain GVP (1990). Early doppler ultrasound screening in prediction of hypertensive disorders of pregnancy. **Lancet** i: 1548-1551.

Steele MW, Breg WR (1966). Chromosome analysis of human amniotic fluid cells. **Lancet** i: 383-385.

Steer PJ, Carter MC, Gordon AJ, Beard RW (1978). The use of catheter-tip pressure transducers for the measurement of intrauterine pressure in labour. **Br J Obstet Gynaecol** 85: 561-566.

Steinberg LH, Hurley VA, Desmedt E, Beischer NA (1990). Acute polyhydramnios in twin pregnancies. **Aust NZ J Obstet Gynaecol** 30: 196-200.

Stevens AD, Lumbers ER (1985). The effect of maternal fluid intake on the volume and composition of fetal urine. **J Dev Physiol** 7: 161-166.

Strong TH, Hetzler G, Paul RH (1990b). Amniotic fluid volume increase after amnioinfusion of a fixed volume. **Am J Obstet Gynecol** 162: 746-748.

Strong TH, Hetzler G, Sarno AP, Paul RH (1990a). Prophylactic intrapartum amnioinfusion: A randomised clinical trial. **Am J Obstet Gynecol** 162: 1370-1375.

Swinyard CA (1982). Concept of multiple congenital contractures (arthrogryposis) in man and animals. **Teratology** 25: 247-258.

Symchych PS, Winchester P (1978). Animal model: amniotic fluid deficiency and fetal lung growth in the rat. **Am J Pathol** 90: 779-782.

Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B (1986). Randomised controlled trial of genetic amniocentesis in 4606 low risk women. **Lancet** i: 1287-1293.

Tabor BL, Maier JA (1987). Polyhydramnios and elevated intrauterine pressure during amnioinfusion. **Am J Obstet Gynecol** 156: 130-131.

Taylor J, Garite TJ (1984). Premature rupture of membranes before fetal viability. **Obstet Gynecol** 64: 615-620.

Thibeault DW, Beatty EC, Hall RT, Bowen SK, O'Neill DH (1985). Neonatal pulmonary hypoplasia with premature rupture of fetal membranes and oligohydramnios. **J Pediatr** 107: 273-277.

Thomas IT, Smith DW (1974). Oligohydramnios, cause of the non-renal features of Potter's syndrome, including pulmonary hypoplasia. **J Pediatr** 84: 811-814.

Tjeuw MTB, Yao FS, Van Poznak A (1986) Depressant effects of anesthetics on isolated human gravid and non-gravid uterine muscle. **Chin Med J** 99, 235-242.

Tomoda S, Brace RA, Longo LD (1985). Amniotic fluid volume and fetal swallowing rate in sheep. **Am J Physiol** 249: R133-R138.

Tomoda S, Brace RA, Longo L (1987). Amniotic fluid volume regulation: basal volumes and responses to fluid infusion or withdrawal in sheep. **Am J Physiol** 252: R380-R387.

Trimmer KJ, Leveno KJ, Peters MT, Kelly MA (1990). Observations on the cause of oligohydramnios in prolonged pregnancy. **Am J Obstet Gynecol** 163: 1900-1903.

Trudinger BJ, Knight PC (1980). Fetal age and patterns of human fetal breathing movements. **Am J Obstet Gynecol** 137: 724-728.

Turnbull AC (1957) Uterine contractions in normal and abnormal labour. **J Obstet Gynaecol Br Emp** 44: 321-323.

Turnbull AC, Anderson ABM (1965) Changes in uterine contractility following intra-amniotic injection of hypertonic saline to induce therapeutic abortion. **J Obstet Gynaec Brit C'wealth** 72: 755-762.

Uranga Imaz FA, Gascon A. (1950). Liquido amniotico; contribucion al estudio de su significacion biologica en la gestacion. **Obstet y Gynecol Latino-Am** 8: 129-140.

Urig MA, Clewell WH, Elliott JP (1990). Twin-twin transfusion syndrome. **Am J Obstet Gynecol** 163: 1522-1526.

Van den Wijngaard JAGW, Pijpers L, Reuss A, Wladimiroff JW (1987). Effect of amnioinfusion on the umbilical doppler flow velocity waveform. **Fetal Ther** 2: 27-30.

Van den Wijngaard JAGW, Wladimiroff JW, Reuss A, Stewart PA (1988). Oligohydramnios and fetal cerebral blood flow. **Br J Obstet Gynaecol** 95: 1309-1311.

Van Eyck J, Van der Mooren K, Wladimiroff JW (1990). Ductus arteriosus flow velocity modulation by fetal breathing movements as a measure of fetal lung development. **Am J Obstet Gynecol** 163: 558-566.

Van Otterlo LC, Wladimiroff JW, Wallenburg HCS (1977). Relationship between fetal urine production and amniotic fluid volume in normal pregnancy and pregnancy complicated by diabetes. **Br J Obstet Gynaecol** 84: 205-209.

Vilos GA, Liggins GC (1982). Intrathoracic pressures in fetal sheep. **J Dev Physiol** 4: 247-256.

Vintzileos AM, Campbell WA, Ingardia CJ, Nochimson DJ (1983). The fetal biophysical profile and its predictive value. **Obstet Gynecol** 62: 271-278.

Vintzileos AM, Turner GW, Campbell WA, Weinbaum PJ, Ward SM, Nochimson DJ (1985a). Polyhydramnios and obstructive renal failure: a case report and review of the literature. **Am J Obstet Gynecol** 152: 883-885.

Vintzileos AM, Campbell WA, Nochimson DJ, Weinbaum PJ (1985b) . Degree of oligohydramnios and pregnancy outcome in patients with premature rupture of the membranes. **Obstet Gynecol** 66: 162-167.

Vintzileos AM, Campbell WA, Nochimson DJ, Weinbaum PJ (1987). Preterm premature rupture of the membranes: a risk factor for the development of abruptio placentae. **Am J Obstet Gynecol** 156: 1235-1238.

Vorherr H (1975). Placental insufficiency in relation to postterm pregnancies and fetal postmaturity. **Am J Obstet Gynecol** 123: 67-103.

Vyas S, Campbell S, Bower S, Nicolaides KH (1990) Maternal abdominal pressure alters fetal cerebral blood flow. **Br J Obstet Gynaecol** 97: 740-747.

Vyas S, Nicolaides KH, Campbell S (1989). Renal flow-velocity waveforms in normal and hypoxemic fetuses. **Am J Obstet Gynecol** 161: 168-172.

Wagner G, Fuchs F (1962). The volume of amniotic fluid in the first half of normal pregnancy. **J Obstet Gynaec Brit C'wealth** 69: 131-136.

Watson WJ, Latz VL, Seeds JW (1991). Fetal urine output does not influence residual amniotic fluid volume after premature rupture of membranes. **Am J Obstet Gynecol** 164: 64-65.

Weiner CP (1987). Diagnosis and treatment of twin to twin transfusion in the mid-second trimester of pregnancy. **Fetal Ther** 2: 71-74.

Weiner CP, Heilskov J, Pelzer G, Grant S, Wenstrom K, Williamson RA (1989). Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease. **Am J Obstet Gynecol** 161: 714-717.

Weir PE, Ratten GJ, Beischer NA (1979). Acute polyhydramnios- a complication of monozygous twin pregnancy. **Br J Obstet Gynaecol** 86: 849-853

Wieloch J (1927). Uber messungen des druckes im normal graviden und hydramniotischen uterus. **Zentral Gynakol** 3: 129-136.

Wigglesworth JS, Winston RML, Bartlett K (1977). Influence of the central nervous system on fetal lung development. **Arch Dis Child** 52: 965-967.

Wigglesworth JS, Desai R (1979). Effects on lung growth of cervical cord section in the rabbit fetus. **Early Hum Dev** 3: 51-65.

Wigglesworth JS, Desai R (1981). Use of DNA estimation for growth assessment in normal and hypoplastic fetal lungs. **Arch Dis Child** 56: 601-605.

Wigglesworth JS, Desai R (1982). Is fetal respiratory function a major determinant of perinatal survival? **Lancet** i: 264-267.

Wigglesworth JS, Desai R, Guerrini P (1981). Fetal lung hypoplasia: biochemical and structural variations and their possible significance. **Arch Dis Child** 56: 606-15.

Wigglesworth JS, Desai R, Hislop AA (1987). Fetal lung growth in congenital laryngeal atresia. **Pediatr Path** 7: 515-525.

Williams EA, Stallworthy JA (1952). A simple method of internal tocography. **Lancet** i: 330-332.

Wintour EM, Barnes A, Brown EH, Hardy KJ, Horacek I, McDougall JG, Scoggins BA (1978). Regulation of amniotic fluid volume and composition in the ovine fetus. **Obstet Gynecol** 52: 689-693.

Wiqvist NE, Eriksson G (1964). Quantitative analysis of uterine motility in saline induced abortions. **Am J Obstet Gynecol** 88: 75-81.

Wladimiroff JW, Campbell S (1974). Fetal urine-production rates in normal and complicated pregnancy. **Lancet** i: 151-154.

Wladimiroff JW, Van den Wijngaard JAGW, Degani S, Noordam MJ, Van Eyck J, Tonge HM (1987). Cerebral and umbilical blood flow velocity waveforms in normal and growth retarded pregnancies. **Obstet Gynecol** 69: 705-709.

Wolf W. (1940). Aussere wehenmessung und wehenscmerz bemerkungen zu der arbeit von Dr S Lorand. **Zentralbl Gynak** 64: 311-317.

Yeh MN, Morishima HO, Niemann WH, James LS (1975). Myocardial conduction defects in association with compression of the umbilical cord. **Am J Obstet Gynecol** 121: 951-957.

Zamah NM, Gillieson MS, Walters JH, Hall PF (1982). Sonographic detection of polyhydramnios: a five-year experience. **Am J Obstet Gynecol** 143: 523-527.

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2. Fisk NM, Ronderos-Dumit, Tannirandorn Y, Nicolini U, Talbert DG, Rodeck CH (1992). Normal amniotic pressure throughout gestation. **Br J Obstet Gynaecol** 99: 18-22.

3. Fisk NM, Tannirandorn Y, Nicolini U, Talbert DG, Rodeck CH (1990). Amniotic pressure in disorders of amniotic fluid volume. **Obstet Gynecol** 76: 210-214.

4. Fisk NM, Giussani DA, Parkes MJ, Moore PJ, Hanson MA (1991). Amnioinfusion increases amniotic pressure in pregnant sheep but does not alter fetal acid base status. **Am J Obstet Gynecol** 165: 1459-1463.

5. Fisk NM, Parkes MJ, Moore PJ, Haidar A, Wigglesworth J, Hanson MA (1991). Fetal breathing during chronic lung liquid loss leading to pulmonary hypoplasia. **Early Hum Dev** 27: 53-63.

6. Nicolini U, Fisk NM, Rodeck CH, Talbert DG, Wigglesworth JS (1989). Low amniotic pressure in oligohydramnios- is this the cause of pulmonary hypoplasia? **Am J Obstet Gynecol** 161: 1098-1101.

7. Fisk NM, Talbert DG, Nicolini U, Vaughan J, Rodeck CH (1992). Fetal breathing movements in oligohydramnios are not altered by amnioinfusion. **Br J Obstet Gynaecol** in press.

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