Morphological and molecular characteristics of living human fetuses between Carnegie stages 7 and 23: ultrasound scanning and direct measurements

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The developmental age of an embryo in the first trimester of pregnancy is generally determined by ultrasound scanning and/or by calculation from menstrual age. In the original studies, validation of the estimate of gestational age by ultrasound was not possible as the exact date of conception was unknown. Variation in growth rates of identically aged fetuses has previously been reported after assisted conception and with the use of ultrasound scanning. As these pregnancies were ongoing the accuracy of the scanning results could not be determined. Comparison of scanning and direct measurements after termination of pregnancy and menstrual age were carried out to determine the accuracy in fetal dating. The results suggest that the use of ultrasound scanning to determine gestational age is of less use than previously thought, and that the use of menstrual age is severely limited.

Key words: crown–rump length/embryo/gestational age/menstrual age/ultrasound

Introduction

An embryo can be defined as the product of the union of male and female germ cells and the consequent rearrangement of DNA to produce an individual. In mammals the embryonic period is defined as that time between implantation of the blastocyst and the formation of marrow in the developing cartilage. The aim of this series of papers was to study the development of the human embryo from post-implantation to the end of the embryonic period. During this time the major organ systems develop and consequently this is the time that major malformations can occur. It is therefore important that detailed studies are carried out on normal populations to assess the developmental ranges and to set parameters for normal development. This series of papers aims to update previously reported data with a series of new human embryos, collected after medical termination of pregnancy, by the study of four aspects related to the development of the human embryo. The aspects studied were: (i) the compilation and comparison of measurements made during routine ultrasound scanning, to confirm a pregnancy of <9 weeks gestation, and measurements made directly on the same tissue after termination of the pregnancy; (ii) the assessment of developmental parameters previously recorded, and comparison of their validity with a new series of embryos through morphological studies after termination of pregnancy; (iii) the detection of the patterns of protein expression of one of the possible growth factors during the embryonic period of development by immunolocalization techniques; and (iv) the identification of one of the possible growth factors affecting embryonic development through its detection by in-situ hybridization techniques throughout the period of embryonic development.

Ultrasound scanning and direct measurements during the first trimester

Until 1973 the method of assessing gestational age in the first trimester of pregnancy depended on a subjective
impression of the size of the uterus and the appearance of the size of the gestational sac, with little attention paid to the size of the fetus. In 1973, Robinson reported a method by which the crown–rump length (CRL) of a fetus could be measured in utero by ultrasound and the results compared with direct post-abortion measurements of fetuses from missed abortions. Sonographic cephalometry performed later in gestation, i.e. after the 14th week of pregnancy, was found to be of limited value because of the wide range of normality; the normal range increases from ±1 week in the second trimester (Campbell and Newman, 1971) to at least ±2 weeks towards term (Willocks et al., 1964; Campbell, 1969). In 1973, Robinson reported that the biological range of fetal criteria was smaller in the first trimester and thus, that the accuracy in determining maturity should be correspondingly greater. Robinson provided a normal growth curve for fetal CRL which was derived from 214 examinations on 80 patients. Consequently, in a further ‘blind’ series using the growth curve as a basis, Robinson found it possible to predict the maturity of pregnancy to within 3 days between weeks 6–14. Kohorn and Kaufman (1974) used sonar during the first trimester to determine confidence limits for the uterine and gestational sac sizes, and discussed the consequences for threatened abortions, deciding that single readings to determine the likelihood of abortion were not sufficient. In 1975, Robinson continued the sonar series with a publication on gestational sac volumes as determined by sonar, but decided that as a method of assessing the maturity of a pregnancy the technique was of less value than sonar measurements of CRL. However it was found useful in identifying early blighted ova or anembryonic pregnancies. Again in 1975, Robinson and Fleming assessed CRL measurements to determine the reproducibility and accuracy of sonar techniques in the measurement of in-vivo CRLs. Potential sources of random error were identified to be operator judgement, movement of the embryo or mother, machine sensitivity settings, and measurement of the photograph. Sources of systematic error were those of oscilloscope scale factor, velocity calibration inaccuracies and the effect of beam width. In 1975, the original data were re-analysed to determine, on a statistical basis, the accuracy of the technique as a method of assessing maturity and it was shown that an estimate could be made to within 4.7 days, with a 95% probability on the basis of a single measurement, and to within 2.7 days if three independent measurements were made. Drumm et al. (1976) measured the fetal CRL by pulsed ultrasound from 47–101 days gestation in a cross-sectional study of 253 patients. The findings from this study were that the CRL range was to be within 3 days of the menstrual age with a maximum variation of 3 days for any given occasion. Drumm et al. (1976) also reported that the ‘acceleration in the rate of change in the CRL was a constant, and that intrapatient variability, where measured, was low’ (95.8% of patients had gestational ages of ±3 days of the predicted values).

In these original studies it was not possible to validate the estimate of gestational age by ultrasound because the exact date of conception was unknown. In 1988, Rossavik et al. reported ultrasound measurements of gestational sac and CRL in early pregnancies in which conception had occurred following in-vitro fertilization (IVF). The findings showed that the 95% confidence limits were 7.7 days before and 7.5 days after the mean for the gestational sac diameter; and 5.2 days before and 5.6 days after the mean for the CRL. Rossavik et al. (1988) also noted that embryos grow at the same rate throughout the part of the embryonic period visible with ultrasound so that some embryos completed the embryonic period of development sooner than others. These differences were confirmed by Dickey et al. (1994), who postulated that the difference in embryo size and the date of the appearance of a heart beat could be explained either by variation in the time of implantation or rate of growth and development prior to the time that cardiac activity could be detected by ultrasound. Dickey and Gasser (1993) reported that variability was found in the size and development of normal human embryos after assisted reproductive technology and before the 10th week post-insemination. They noted that marked differences, of 5 days between the earliest and latest post-ovulation ages, occurred in the rate of early human development prior to 27 days post-insemination, and that these differences were minimized after 68 days post-insemination. A 2-fold difference in the size between embryos of identical post-ovulation age following IVF and gamete intra-Fallopian transfer (GIFT) proved that human embryos differ in their early growth and yet still develop normally (Dickey and Gasser, 1993). Since the routine use of ultrasound scanning in pregnancy and the introduction of assisted reproductive techniques it has been possible to determine accurately a growth curve from post-ovulation dates. However whether the growth of fetuses in pregnancies occurring as a result of IVF is similar to those occurring spontaneously is not known. The development of medical methods of inducing abortion in early pregnancy has provided a source of intact normal fetuses. In this study we report the accuracy of growth curves by comparison of measurements taken during routine scanning before medical termination of pregnancy, and measurements performed directly after termination of pregnancy on fresh tissue.
**Developmental embryology**

Each of the stages has established a different set of developmental features that assist in placing the embryo in its stage (Table 1). Almost all stages include the following criteria as part of the staging process. The Carnegie collection has been used as the ‘gold standard’ which has been used to assess the stage of development of individual embryos. It should be remembered, however, that the material on which this collection is based was obtained from fetuses following spontaneous abortion and hence it is not known how well they represent normal development.

**Embryonic length**

Most previous data have been published on tissue that was immersed in fixative before examination. Streeter (1942) thought it more practical that measurement after fixation was used as standard. In the present study it was decided to measure the tissue before fixation for direct comparison with measurements made during ultrasound scans on the same tissue, before termination of pregnancy. The measurement of CRL appears to have been introduced into embryology by Arnold in 1887 (Keibel and Mall, 1910). In the human embryo it becomes practicable, from stage 12 onwards, to use the CRL as a measurement of age; preceding this the measurement is less than satisfactory due to the degree of flexion. Prior to stage 12 the greatest length (GL) should be measured without any attempt to straighten the natural curvature of the specimen.

**Table I. Developmental stages in human embryos (from O’Rahilly and Müller, 1987)**

<table>
<thead>
<tr>
<th>Carnegie stage</th>
<th>Pairs of somites</th>
<th>Size (mm)</th>
<th>Age (days)</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.1–0.15</td>
<td>1</td>
<td>Fertilization</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.1–0.2</td>
<td>1.5–3</td>
<td>From 2 to about 16 cells</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.1–0.2</td>
<td>4</td>
<td>Free blastocyst</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.1–0.2</td>
<td>5–6</td>
<td>Attaching blastocyst</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.1–0.2</td>
<td>7–12</td>
<td>Implanting although previllous</td>
</tr>
<tr>
<td>5a</td>
<td></td>
<td>0.1</td>
<td>7–8</td>
<td>Solid trophoblast</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>0.1</td>
<td>9</td>
<td>Trophoblastic lacunae</td>
</tr>
<tr>
<td>5c</td>
<td></td>
<td>0.15–0.2</td>
<td>11–12</td>
<td>Lacunar vascular circle</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.2</td>
<td>13</td>
<td>Chorionic villi; primitive streak may appear</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td></td>
<td></td>
<td>Chorionic villi</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td></td>
<td></td>
<td>Primitive streak</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.4</td>
<td>16</td>
<td>Notocord process</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>1.0–1.5</td>
<td>18</td>
<td>Primitive pit; notocord and neurteric canals; neural folds may appear</td>
</tr>
<tr>
<td>9</td>
<td>1–3</td>
<td>1.5–2.5</td>
<td>20</td>
<td>Somites first appear</td>
</tr>
<tr>
<td>10</td>
<td>4–12</td>
<td>2–3.5</td>
<td>22</td>
<td>Neural folds begin to fuse; 2 pharyngeal bars; optic sulcus</td>
</tr>
<tr>
<td>11</td>
<td>13–20</td>
<td>2.5–4.5</td>
<td>24</td>
<td>Rostral neuroepic closes; optic vesicle</td>
</tr>
<tr>
<td>12</td>
<td>21–29</td>
<td>3–5</td>
<td>26</td>
<td>Caudal neuroepic closes; 3–4 pharyngeal bars; upper limb buds appearing</td>
</tr>
<tr>
<td>13</td>
<td>30–7</td>
<td>4–6</td>
<td>28</td>
<td>Four limb buds; lens disc; otic vesicle</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>5–7</td>
<td>32</td>
<td>Lens pit and optic cup; endolymphatic appendage distinct</td>
</tr>
<tr>
<td>15</td>
<td>7–9</td>
<td>33</td>
<td></td>
<td>Lens vesicle; nasal pit; antiragus beginning; hand plate; trunk relatively wider; future cerebral hemispheres distinct</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>8–11</td>
<td>37</td>
<td>Nasal pit faces ventrally; retinal pigment visible in intact embryo; auricular hillocks beginning; foot plate</td>
</tr>
<tr>
<td>17</td>
<td>11–14</td>
<td>41</td>
<td></td>
<td>Head relatively larger; trunk straighter; nasofrontal groove distinct; auricular hillocks distinct; finger rays</td>
</tr>
<tr>
<td>18</td>
<td>13–17</td>
<td>44</td>
<td></td>
<td>Body more cuboidal; elbow region and toe rays appearing; eyelid folds may begin; tip of nose distinct; nipples appear; ossification may begin</td>
</tr>
<tr>
<td>19</td>
<td>16–18</td>
<td>47.5</td>
<td></td>
<td>Trunk elongating and straightening</td>
</tr>
<tr>
<td>20</td>
<td>18–22</td>
<td>50.5</td>
<td></td>
<td>Upper limbs longer and bent at elbows</td>
</tr>
<tr>
<td>21</td>
<td>22–24</td>
<td>52</td>
<td></td>
<td>Fingers longer; hands approach each other; feet likewise</td>
</tr>
<tr>
<td>22</td>
<td>23–28</td>
<td>54</td>
<td></td>
<td>Eyelids and external ear more developed</td>
</tr>
<tr>
<td>23</td>
<td>27–31</td>
<td>56.5</td>
<td></td>
<td>Head more rounded; limbs longer and more developed</td>
</tr>
</tbody>
</table>
Embryonic age

The embryonic age, calculated from the menstrual history, is seldom of use (O’Rahilly and Müller, 1987). Allowance should be made for considerable variability in both the follicular and luteal phases of the menstrual cycle, with the date of ovulation only estimated at midcycle. The age of very early human embryos (those of the first 3–4 weeks, post-ovulation) has been estimated chiefly by comparison of their development with that of the monkey conceptuses of known post-ovulative ages (Rock and Hertig, 1944). Coital history has not always been noted but where the information is available the post-ovulatory age is easier to identify.

Other parameters

Other parameters used to assist in establishing the embryos in the different stage categories include: the appearance of the primitive streak; the progressive fusion of the neural folds, leading to the creation and closure of the caudal and rostral neuropores; the number of paired somites; the appearance and subsequent division of the cardiac tube; the development of the optic and otic vesicles; the appearance of the pharyngeal arches to their separation of the different parts into the ear and the development of the jaws and neck; the development and differentiation of the limb buds; and the position and maturation of the developing body.

The normality of the collected embryos is difficult to establish as every minor defect will not necessarily lead to a recognizable anomaly later in life. In this study embryos found to be grossly abnormal have not been included as this is, primarily, a study to define the relationship of age to the developmental processes of a normal population of human embryos in the first trimester of pregnancy.

The use of mifepristone (RU486) for the termination of pregnancy in the first trimester

The first antiprogestin was discovered in the 1980s by Roussel scientists searching for an antiglucocorticoid and was given the generic name of mifepristone; the company code name was RU486. Along with other antiprogestins, since synthesized, it has a wide range of actions including disruption of folliculogenesis, inhibition of ovulation, induction of menses, management of labour, treatment of certain types of tumours, treatment in endometriosis, cervical ripening, treatment of fibroids, meningioma and breast cancer; and through the result of antiglucocorticoid activity, the treatment of Cushing’s syndrome. Ample progesterone must be produced by the corpus luteum to maintain early pregnancy; low progesterone secretion in the luteal phase has been implicated in habitual abortion (Giudice et al., 1989) although this is a controversial opinion. It was therefore logical to postulate that antiprogestins given during early pregnancy might act as abortifacients, thus providing an alternative to current techniques of surgical abortion. Mifepristone was initially tested as a means of an efficient termination of pregnancy in successful animal experiments (Baulieu, 1993; Herrmann et al. (1982), successfully performed the first studies in humans, documenting the ability of mifepristone to interrupt early pregnancy. These results were confirmed by a dose-finding study conducted under the auspices of the World Health Organization (Bygdeman, 1993). Two conclusions were drawn from the results; (i) that the frequency of successful complete abortion using mifepristone decreased with advancing age of the fetus, with ~60–70% incidence of complete abortion up to 8 weeks and (ii) there appeared to be no relationship between the success rate and the treatment regime employed for women at the same stage of gestation (Donaldson et al., 1993). In an attempt to interrupt early gestation more effectively different prostaglandin preparations were used in combination with mifepristone. The prostaglandins (PG) tried have included sulprostone (an injectable PGE2 analogue); gemeprost (vaginal pessary PGE1 analogue) and misoprostol (an oral preparation). In 1991, a protocol was approved for the medical interruption of pregnancy in the UK, using a combination of mifepristone and gemeprost for up to 63 days amenorrhoea. Efficiency rates of 96% complete expulsions, 1% continuing pregnancies, 2.1% incomplete expulsions, and 0.9% requiring dilation and curettage were reported in the UK Multicentre Trial (1990).

The collection of the tissue after first trimester termination of pregnancy was a by-product of the studies carried out in the Royal Infirmary of Edinburgh since 1986 (Cameron et al., 1986; Rodger and Baird, 1987; Baird et al., 1988; Baird 1993), to develop effective methods of inducing abortion in early pregnancy. With the permission of the local medical ethics committee, from 1986 until 1993, tissue from patients taking part primarily on studies, was routinely collected. The tissue was examined, fixed and then stored for future studies to be carried out.

Materials and methods

The subjects used in this study were all referred by local family planning services and general practitioners with <63 days amenorrhoea and requesting termination of pregnancy. Pregnancy was confirmed by measurement of serum human chorionic gonadotrophin (HCG, Serono
Ultrasound scanning and direct measurements

Figure 1. Comparison between direct and scanning gestational sac measurements (mm) during the first trimester. Slope of regression line: 

\[ y = 2.2925 + 0.52675x; \]

\[ y = \text{scanning diameter}; \]

\[ x = \text{direct diameter}. \]

Figure 2. Comparison between gestational sac mean size with menstrual age from scanning data, during the first trimester.

Diagnostics kit, Stockport, Cheshire, UK). Exclusion criteria included those with evidence of multiple pregnancies, those with a history of serious medical disorders, and those aged <17 years. All candidates were allocated varying doses of mifepristone (RU486) and varying doses of prostaglandins. Pelvic ultrasound scans were carried out to help determine the stage of gestation, and to confirm the presence of the embryo and gestational sac. The first ultrasound scan, to confirm an ongoing pregnancy, was performed on the day the women received the tablets of mifepristone, or on the day the women were accepted onto the study. A follow-up scan was performed the day the women came in for prostaglandin treatment, 48 h after administration of mifepristone. Measurements during the follow-up ultrasound scanning included the gestational sac size, the CRL of the embryo, the presence and/or absence of a fetal heart (to confirm that the embryo was not a missed abortion) and secondary yolk sac. At 4 h after PG administration the women were examined and any products of conception removed. These were then assessed and measurements taken to compare with those measurements collected from the ultrasound scans. Only a small percentage of those women treated aborted intact gestational sacs; the majority of tissue that was collected came in a variety of conditions, from totally macerated to a burst sac, but with excellent fetal material still attached to the placenta.

**Gestational sac data**

All measurements were made by one investigator using an abdominal ultrasound scanner (Diasonics; DRF 250 series, BMS Scotland, Bothwell, Strathclyde, UK) with a 3.5 mHz probe. Two or three measurements of the gestational sac diameter (D) were taken during the ultrasound scanning on the day of PG administration from these the geometric diameter was calculated \((D1 \times D2 \times D3)/3 = \text{geometric diameter}\). In the same gestational sac, two diameters (as most of the gestational sacs were burst three measurements were not considered relevant) were taken post-abortum, and again the geometric diameter \((D1 \times D2)/2\) calculated. The geometric diameters were subsequently plotted against each other, and a regression line calculated and drawn through the points. The geometric diameters of both the scanning measurements and the direct measurements of the gestational sac were plotted against the menstrual age (calculated from the last menstrual period), and a regression line calculated through the points. On analysis some of the subjects were found to have menstrual ages >63 days gestation, but, as this was only 4.77% of the data for the direct measurements and 1.82% of the data for the scanning measurements, the data were included in all statistical tests.

**Embryonic data**

The CRL measured, by ultrasound, on the day of PG administration was compared with measurements taken after products of conception were passed. The results were plotted as a scatter graph and a simple regression line calculated to determine if any relationship could be found between the different groups of data. The CRLs, from both the scanning and direct measurements, were plotted, as a scatter graph, against the menstrual age (calculated from the last menstrual period) and a simple regression line calculated to show if any correlation occurred. Some of the points from the menstrual age were found to be >63 days amenorrhea but as they only represented a small proportion of the data (1.79% for the scanning data and
Intact data

The number of intact gestational sacs received were totalled, and an overall percentage of intact sacs against all tissue calculated. To demonstrate if any differences were found between intact gestational sac data and overall gestational sac data post-abortum, the mean ± SEM of the intact data and the mean ± SEM of all direct measurements were plotted against the scanning data, and a regression line calculated and drawn through the points. The weight of the intact sac was also plotted against the size and a polynomial growth curve drawn through the points.

Results

Gestational sac data

The results from the gestational sac geometric diameters for both the scanning and the direct measurements were plotted as a scatter graph and a simple regression line calculated to ascertain any relationship between the different groups of data (Figure 1). Although there was a good correlation between the two measurements the direct measurements were almost always larger than the diameters estimated by scanning. From the regression line the mean deviation from linearity was calculated, where x equalled the direct measurements and y equalled the scanning measurements. The mean ± SD was 3.3 ± 2.71, with a maximum difference of 14.23 mm and a minimum difference of 0.23 mm. The correlation of the geometric diameter measurements taken during ultrasound scanning with those measurements taken directly from the tissue post-abortum, was very high (n = 90; r = 0.84; P <0.001).

The geometric diameter of the scanning measurements and the menstrual age were plotted (Figure 2) as a scatter graph and a regression line passed through the points. There was a wide spread of points around the regression line (y = –8.4105 + 0.58872x). There was a significant correlation between the size of gestational sac as measured directly or by ultrasound and the menstrual age (r = 0.48, P <0.001, n = 112). The positive correlation is not surprising since gestational sac size would be expected to increase with menstrual age. However, the degree of scatter and relatively poor correlation coefficient reinforce the view
that menstrual age is a poor measure of gestational age. The geometric diameter of the direct measurements and the menstrual age were plotted as a scatter graph and a regression line drawn through the points (Figure 3). As before the points were widely scattered about the regression line ($y = -4.7627 + 0.788514x$). Nevertheless, the correlation coefficient was statistically significant ($r = 0.47; P < 0.01; n = 482$).

**Embryonic data**

The CRL estimated data, from the ultrasound scans and the direct measurements, were plotted against each other on a scatter graph and a simple regression line ($y = 2.2709 + 0.71256x$) was calculated to determine if any relationship was likely between the different groups of data (Figure 4). The CRL measurements taken during ultrasound scanning had a high correlation with those measurements taken directly from the tissue post-abortum ($n = 70; r = 0.81; P <0.001$). The CRL, from the scanning measurements, was plotted as a scatter graph, against the menstrual age, calculated from the LMP, and a simple regression line ($y = -14.7312 + 0.4635$) calculated (Figure 5). Although the points were distributed over a wide area, a statistically significant positive correlation was found ($n = 99; r = 0.57; P <0.001$).

The CRL from the direct measurements was plotted against the menstrual age as a scatter graph and a line of regression ($y = -13.025 + 0.45373x$) passed through the points (Figure 6). Although the data points were widely scattered, a statistically significant correlation was found ($n = 354; r = 0.57; P <0.01$) showing that the direct measurement of CRL increased with menstrual age. However, the relatively low correlation coefficient implies that menstrual age is too inaccurate as a measure of gestational age.

Both direct measurements of the CRL and gestational sac were larger than the scanning measurements, although this difference was only significant for the sac measurements. An explanation for this could be that, due to the usual rupture of the sac during its passage through the cervical os, or due to the effect of gravity on the aborted sac and consequent flattening of the tissue, the measured diameters were an overestimate of the in-vivo diameter.

**Intact data**

This part of the study was designed to investigate whether the correlation between direct measurements of sac diameter and ultrasound was due to inaccuracies in the direct measurement of burst sacs. Out of 583 terminations performed over a 7 year period, 107 (18.4%) intact gestational sacs were received.

A comparison was carried out between the diameters of the intact gestational sacs and the direct gestational sac against the diameter of the scanning gestational sac, and a scatter graph was then drawn (Figure 7). The regression line was drawn through the points of the gestational sac direct measurements and a very high degree of correlation was found ($n = 89; r = 0.84; P <0.001$). When a regression line was passed through the sub-population of intact gestational sac data, an almost exact degree of correlation was found ($n = 15; r = 0.94; P <0.001$). This correlation was not unexpected as direct gestational sac measurements would increase with increased scanning measurements.

The intact gestational sac diameters were plotted, as a scatter graph, against their corresponding weights and an exponential growth curve fitted through the points (Figure 8). A high degree of correlation between the two was found ($n = 106; r = 0.9; P <0.001$).

**Discussion**

In this study it has been shown that there are highly significant differences in gestational sac diameter between the measurements collected directly and those compiled during ultrasound scanning. As the two groups of data are known to be from the same population the postulated hypothesis would be that one of the groups had a greater degree of inaccuracy. Overall the measurements of the gestational sac that were taken directly from the tissue are significantly greater than those from the ultrasound scans. It is possible that the direct measurements of sac diameter are an underestimate of the true size due to the fact that the majority of sacs were burst prior to measurement. However
there was no significant difference in sac diameter in the subset of sacs which were examined intact and it is likely, therefore, that the ultrasound measurements consistently underestimate the true size.

The same result occurred with the regression line of both the scanning and direct measurements against menstrual age. As the $r$ value for both groups of data was similar it was thought that the differences could be more a reflection of variability in menstrual cycles—taking differences in ovulation, length of cycle, and the amount of time needed for implantation into account, as well as vagaries in the dates of last menstrual period, rather than differences and anomalies in the measurements. These findings correlate well with the overall results from Robinson (1975) but his results did not start until 5 weeks (menstrual age) into the pregnancy. Rossavik et al. (1988) found (from calculations by linear equation) an indication that the ultrasound findings from the gestational sac increased by 1 mm/day starting 16 days post-conception, with a 10 mm gestational sac diameter age at 40 days (menstrual age). Our earliest scans were performed at 36 days (menstrual age); calculations (from linear regression) for 40 days menstrual age predicted a larger figure of 15.14 mm gestational sac size.

The relationship between the two measurements of the CRL was similar to that found between the scanning and direct measurements of the gestational sac size. With an $r$ value equal to 0.78 ($n = 58$), a good linear correlation was found, thus confirming that clinically measured CRL by ultrasound scanning provides a reasonably accurate estimate of gestational age.

Previous studies have either correlated ultrasonography with tissue from missed or spontaneous abortions, or ultrasonographic measurements with accurate post-implantation dates from assisted conception cycles. This could lead to inaccuracies in the data being collected for the following reasons: (i) different growth rates in identically aged fetuses have been observed (Dickey et al., 1992; Dickey and Gasser, 1993), therefore, small differences in CRL may or may not indicate different post-ovulatory age; (ii) direct measurements of tissue, in cases studied from assisted conception cycles, were not possible for comparison with ultrasonic measurements as the pregnancies were ongoing and (iii) tissue collected from either spontaneous or missed abortions has limitations: the degree of growth and development could be affected and therefore date inaccurately the embryo.

In conclusion, the comparison of the scanning and direct measurements of gestational sac size and the CRL against menstrual age show an increase in growth as age increases. Ultrasound measurement of gestational sac diameter is a less accurate estimate of gestational age, but is a useful way of diagnosing anembryonic pregnancies and blighted ova. Various reasons for the degrees in error seen in the scanning data could include operator error, movement of the subject during scanning procedures, lack of calibration of the scanning machine, and lack of reproducibility of
measurements. The use of menstrual age to predict the time of gestation has severe limitations.

References


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