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Morphological and molecular characteristics of living human fetuses between Carnegie stages 7 and 23: developmental stages in the post-implantation embryo

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TABLE OF CONTENTS

Determination of embryonic age groups or stages has been based on the Carnegie Institute collection started in 1887. Improved technology has enabled the building of a new collection of embryos of <9 weeks gestation; these were then used to compare with the original Carnegie collection. The results suggest that in providing definitive stages that are rigidly bound by developmental events, limitations are placed on categorizing the embryo. Allocation of embryos to a specific stage can assist in identifying post-ovulatory age but overlaps between stages could lead to classification into an incorrect stage.

Key words: developmental stages/embryo/mifepristone

Introduction

Much of the early work on the development of the embryo was done on non-mammalian species as the embryos were more accessible. At the turn of this century human material was collected and each embryo was thoroughly investigated; the largest and most comprehensively investigated collection of tissue was held at the Carnegie Institute, Washington, USA. The Carnegie collection of human embryos was based on the original work initiated by Franklin P.Mall in 1887. Although in 1887 His and Keibel discussed the idea of staging of the embryos (O'Rahilly and Müller, 1987), two limitations were stated by Keibel and Elze in 1908 in *Normentafeln*: (i) that 'individual embryos cannot be arranged in a perfect series, because any given specimen may be advanced in one respect while being retarded in another, and (ii) that it may prove impossible to match a new embryo exactly with any one of the illustrated norms'. In 1914, only on the basis of photographs of their external form, Mall arranged 266 fixed human embryos, 2–25 mm in length, in a series of 14 stages, lettered from H to U. In 1958, de Beer proposed that each developmental stage 'is merely an arbitrarily cut section through the time-axis of the life of an organism'. Stages are based on the apparent morphological state of development and therefore are not directly dependent on either chronological age or size; furthermore, comparison is made on a number of features of each specimen so that individual differences are less significant. In 1942, Streeter provided a definitive classification of the human embryo into 'developmental horizons', and subsequently reported that stage 23 'could be considered to mark the end of the embryonic period' and that the onset of marrow formation in the humerus was 'arbitrarily adopted as the conclusion of the embryonic and beginning of the fetal period of prenatal life'. The term 'stage' is now confined to the present day usage in all forms of embryology, such as the 46 stages of the chick (and the 46 stages of the *Ambystoma maculatum*. Stages 10–23 were published either by Streeter (1942, 1945, 1948, 1951) or with the aid of his notes (Heuser and Corner, 1957); in 1987 and again in 1993 O'Rahilly and Müller reported updates of Streeter's previous accounts. The age of embryos from stage 14 onwards proved increasingly greater than those given by Streeter which were based on comparisons with macaque embryos; such comparisons are now known not to be warranted. Stages 1–9 were published by O'Rahilly in 1973. Of the ~600 sectioned embryos in the Carnegie

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collection that are assigned to the 23 stages, the majority are listed as normal although variations and even anomalies of individual organs are known to occur. Mall and Meyer (1921) stated that 'as our knowledge of the normal becomes more complete, we find that more and more young embryos which formerly were regarded as normal are not really so... it remains impossible, even at the present time, to determine in all cases whether we are dealing with a normal or an abnormal specimen, even after it has been mounted in serial sections'. Conversely, O'Rahilly and Müller (1987) indicated 'that every minor defect would not necessarily lead to a recognizable anomaly in later life'. Drumm and O'Rahilly (1977) confirmed, ultrasonically *in vivo*, that an embryo of 30 mm is normally 8 weeks post-ovulation. Dickey and Gasser (1993) compared the crown–rump length (CRL) measurements from in-vitro fertilization (IVF) pregnancies in the first trimester with those given for each developmental stage by O'Rahilly and Müller in 1987. The findings were a 5 day difference between the earliest and latest post-ovulation ages from previously reported data (Robinson, 1973; O'Rahilly and Müller, 1987); the CRLs were greater on average than their mean measurements on any given day from their study. Also, a 2-fold difference in 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 can still develop normally. MacGregor *et al.* (1987) timed ovulation by either repeated ultrasound or by luteinizing hormone (LH) surge and subsequently measured CRL; the results from this study were compatible with those of Dickey and Gasser (1993), especially where artificial insemination was performed. In 1994, Dickey *et al.* stated that 'embryos grow at the same rate throughout the part of the embryonic period visible with ultrasound so that some embryos complete the embryonic period of development sooner than others'. This could only be explained by a variation in either the time of implantation or rate of growth and development.

A large proportion of the Carnegie Institute's material was obtained from spontaneous abortions. With regulations governing both the act of terminating a pregnancy and the subsequent disposal of the tissue differing from country to country, or even state to state, the collection of a continuous normal population of material remained difficult. Material for the Carnegie Institute was collected throughout the USA and immersed in fixative before being forwarded to the Institute. This meant that those observing the developmental features of the embryos had little access to fresh material. Consequently, the question arises of how representative a spontaneous abortion of any given stage is,

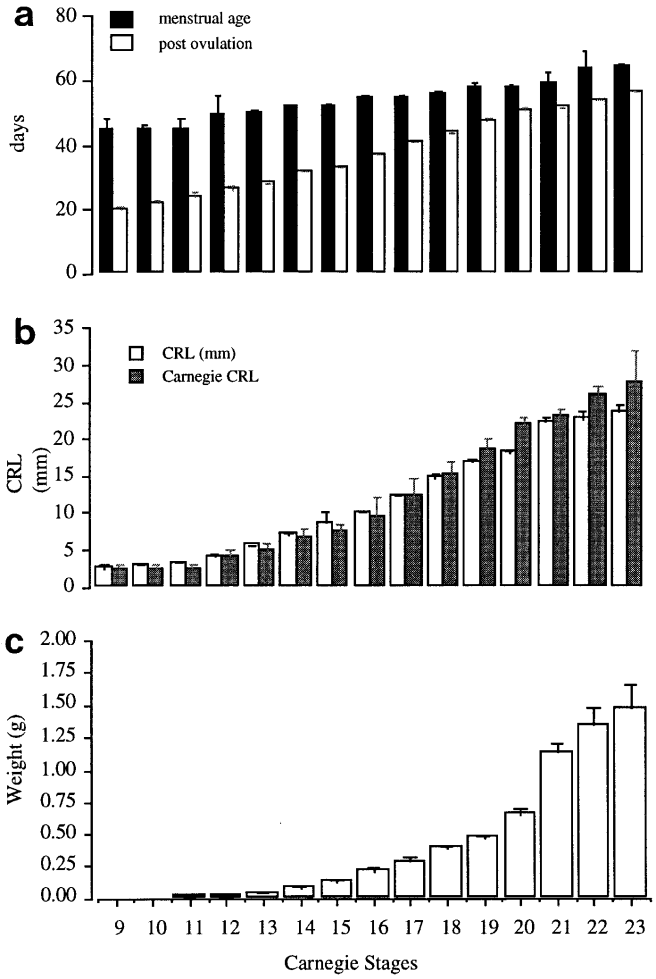

Figure 1. (a) Menstrual (calculated from last mentsrual period) and post-ovulatory (calculated from embryological development) age, mean ± SEM, at each stage of embryonic development (n = 294). **(b)** Mean ± SEM crown–rump lengths from the current study and from O'Rahilly and Müller (1987) at each of the developmental stages (Carnegie, n = 353; current study, n = 310). **(c)** Mean ± SEM weight of the embryos at each stage of development (n = 282).

and, therefore, how accurate previous observations were. A new study was therefore initiated with first trimester human embryos, from a normal population of women requesting a medical termination of pregnancy for social reasons and compared with the established Carnegie Institute series (Figure 1).

Materials and methods

Collection of specimens

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 the measurement

of serum human chorionic gonadotrophin (HCG; Biostat Ltd., Stockport, Cheshire, UK) and, in some cases, by transabdominal ultrasound scanning. Exclusion criteria included those with evidence (ultrasound scanning) of multiple pregnancies, those with a history of serious medical disorders and those aged <17 years. All patients were administered varying doses of mifepristone (RU486) on their first day of admission onto the programme and varying doses of prostaglandins 48 h later as part of a series of studies arrived at developing a safe, effective method of inducing abortion medically (Cameron *et al.*, 1986; Rodger and Baird, 1987; for review Baird, 1993). On the day of administration of prostaglandins, the patients were admitted to hospital, examined 4 h after the administration, and any products of conception found were carefully removed from the cervical os, vagina or collected in bedpans. The tissue was transferred to the laboratory in a sterile container and a dissection and thorough examination, using a light dissecting microscope (Wild) with the addition of an external fibre optic light source was carried out. A careful record of the condition the specimens arrived in, weights (on a four decimal place balance) and measures (using 1 mm graph paper under a dissecting microscope) of the embryo (where applicable) and photographic records were produced. Varying parameters were established to identify the embryonic specimens at different stages of development, these stages having previously been established by the careful examination of many specimens at the Carnegie Institute in Washington, US. Based on their external developmental anatomy, each embryo was allocated to a group which was defined on the basis of previous work published by the Carnegie Institute, and the results compared with those previously published.

This work was approved by the sub-ethics committee for Paediatrics and Reproductive Medicine of the Lothian Research Ethics Committee, UK.

Data collection

In order to identify the anatomical differences in a normal population of embryos the analysis has had to rest on a visual description, drawings and photographs, the major difficulty occurring when the more advanced embryos in one stage and the least advanced in the next stage seemed to overlap in most of the points of reference. Varying parameters were established to contribute in identifying different stages and these were routinely noted along with other relevant features.

The features used were the appearance of the primitive streak and the progressive fusion of the neural folds, leading to the development of the caudal and rostral neuropores and later their closure; the number of visible paired somites, used only in the early stages of development, the emergence of the forebrain vesicle and the IVth ventricle, the development of the optic vesicle from the appearance of the optic sulcus through the appearance and closure of the lens disc in the optic evagination; the appearance of retinal pigment, the development of the eyelids to the point where they completely cover the eye; and the appearance of the pharyngeal arches through their subsequent development and then separation into the different parts of the external ear and mouth; the appearance and development of both of the limb buds, at differing times, from the ectodermal ridges through the differentiation of the limb bud into the hand/foot, arm/leg, and forearm/thigh to the appearance of the digits, the regression of the interdigital notches and, occasionally, the formation of the outline of the joints and nails; also included, on older embryos, was the development of the umbilicus with the consequent regression of the secondary yolk sac and the development and regression of the tail bud. For each given stage, the embryos had to agree on a majority of points before having their classification accepted.

All developmental measurements were the responsibility of one person thus avoiding inter-operator variation. The results section gives the parameters by which the Carnegie Institute reported their results. This is followed by a summary of the findings from this study.

Results

The descriptions of all stages were based on the data collected by the Carnegie Institute, Washington, USA (O'Rahilly and Müller, 1987). A total of 310 embryos in good or excellent condition were collected and the range of embryonic lengths, and the age allocated by the Carnegie Institute for each of the embryonic stages are listed in Table I. Figure 1 demonstrates the relationship between the post-ovulatory (established from embryological determination of age) and menstrual ages, the CRLs set by the Carnegie Institute and those measured in the current study, and the weight of the embryos, against each of the given Carnegie stages. In the current study the number of good or excellent embryos collected, the embryonic length (measured on fresh tissue), and the menstrual age were all reported for comparison. All individual descriptions of the embryos used in the current study are found in the Appendix.

Table I. Comparison of data from Carnegie study with current data

Carnegie data				Present study[a]		
Stage	No. embryos	CRL (mm)	Post-ovulatory age (days)	No. embryos	CRL (mm) direct	Menstrual age (days)
7	7	0.4	16	2	45.5 ± 8.5	
8	21	1–1.5	17–19	3	2.5 ± 0.5	39.5 ± 3.7
9	2	1.5–2.5	19–21	2	2.5 ± 0.5	45 ± 0
10	11	2–3.5	21–23	13	2.9 ± 0.1	45.2 ± 1.6
11	20	2.5–4.5	23–25	14	3.2 ± 0.2	44 ± 0.9
12	23	3–5	25–27	28	4.2 ± 0.2	49.5 ± 1.2
13	22	4–6	28	34	5.6± 0.2	50.2 ± 0.8
14	37	5–7	32	27	7.2 ± 0.2	51.9 ± 0.8
15	24	7–9	33	25	8.7 ± 1.2	52.2 ± 0.8
16	36	8–11	37	28	10.0 ± 0.2	54.3 ± 1.2
17	27	11–14	41	30	12.2 ± 0.3	54.8 ± 1.0
18	33	13–17	44	21	14.8 ± 0.5	55.9 ± 0.9
19	22	16–18	47–48	24	16.9 ± 0.3	58.3 ± 0.7
20	15	18–22	50–51	24	18.1 ± 0.3	57.9 ± 0.6
21	17	22–24	52	18	22.3 ± 0.6	59 ± 1.9
22	15	23–28	54	8	22.8 ± 1.0	64 ± 5.0
23	21	27–31	56–57	9	23.7 ± 0.8	63.9 ± 1.1

[a]Data included were only from patients with known last menstrual period dates.

Stages 7 and 8

Stage 7 is characterized by the appearance of the notochord process rostral to the primitive node and streak, 16 days post-ovulation.

Stage 8 is characterized by the appearance of the primitive pit, the notocordal canal, and the neurenteric canal and is found 18 ± 1 days post-ovulation.

Prior to stage 9, four specimens were collected and are briefly tabulated as follows.

Summary

As there are few parameters set for these stages, and due to the undeveloped state of the embryos, it was found that the differences between our findings and those of the Carnegie Institute were negligible. At stage 7 only two embryos were identified; neither had a primitive groove and although tissue in the central area gave the appearance of a compacted ball of cells, there were no gross features identifiable. Three embryos were found to have the features described by O'Rahilly and Müller at stage 8 (Figure 2). All gave the appearance of a primitive groove, two showed a slight decrease in the area of attachment to the secondary yolk sac, and one showed the beginnings of the development of the pericardial cavity. The menstrual age was 45.5 ± 8.5 days for stage 7, and 39.5 ± 3.7 days for stage 8. The embryonic length for stage 8 was 2.5 ± 0.5 mm, with a maximum length of 3 and a minimum length of 2 mm; no attempt was made to measure the embryonic length in group 7.

Stage 9

Stage 9 is reached at ~20 ± 1 days post-ovulation, and is defined by the number of paired somites present (between one and three); the neural folds should also be developing. The size of this embryo can vary from ~1.5–3 mm in length depending on the turning and curvature of the specimen. From the dorsal plane the embryo is frequently described as shaped like the sole of a shoe. Many embryos display dorsal concavity or lordosis, and although abrupt bends and kinks are artefacts 'anything from a gentle convexity to a moderate dorsal concavity must be considered normal' (Heuser and Corner, 1957). Two specimens were recovered from intact termination material.

Summary

The differences between the two embryos at this stage ranged from having no differentiation of the cardium, a visible optic disc and three somites, to cardiac differentiation, no visible optic disc and three somites. The embryonic length was 2.5 ± 0.5 mm, and the menstrual age 45 days. Little difference was found between the two embryos studied and those previously reported by the Carnegie Institute.

Stage 10

In stage 10, at a post-ovulatory age of ~22 ± 1 days, the characteristic features include 4–12 paired somites; the possible appearance of the optic sulcus in the forebrain and an indication of the invagination of the optic disc; the

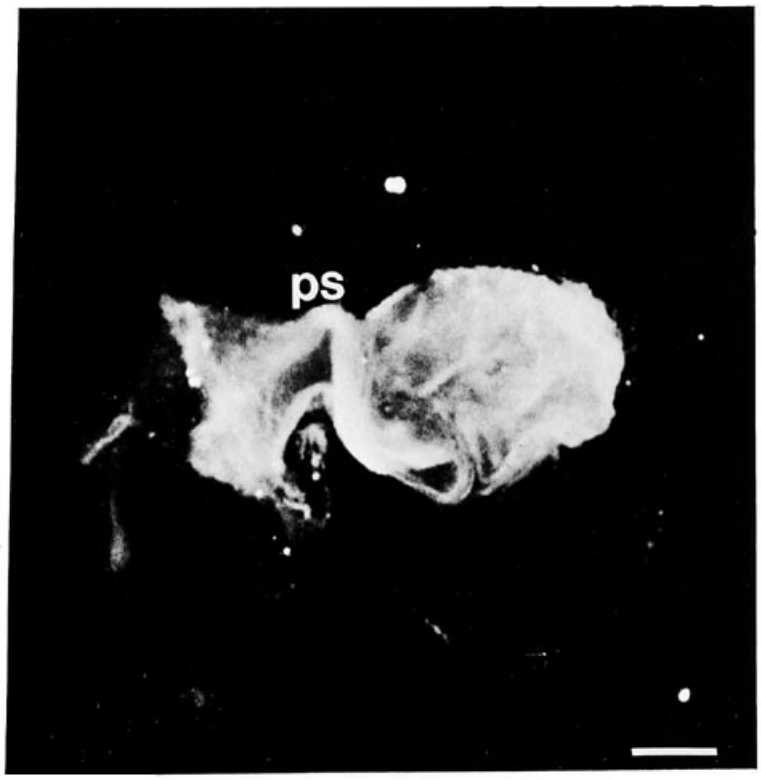

Figure 2. Photograph illustrating a medial view of a stage 8 (Ru 377) embryo showing the secondary yolk sac and the primitive streak (ps) and the connecting stalk. Stage 8 is characterized by the appearance of the primitive pit, the notocordal canal and the neurenteric canal. Bar = 1 mm. The photograph was taken prior to fixation.

visible development of the first pharyngeal arch, with the possible formation of the hyoid arch and the maxial processes and the fusion of the neural folds (either imminent or in progress), thus forming the rostral and caudal neuropores. This is the start of the formation of the neural tube; at this point its stage of development can vary from completely open to closed from the rhombencephalon to below the level of the last visible somite. By the end of this stage, the cardiac tube and pericardial cavity are becoming dominant features of the developing embryo, the umbilical vesicle is developing although the secondary yolk sac is still dominant. There is also an increase in the embryonic length and, at this stage, any degree of lordosis (concavity) is considered normal.

A total of 13 embryos were collected from this stage after termination of pregnancy, of which 11 of the gestational sacs were received in an intact condition (Figure 3).

Summary

The overall findings among this group of embryos were that the neural groove did not start to close until between the formation of the sixth and seventh somites, a pericardial cavity could be easily identified in 11/13 samples (two were not noted), the range of paired somites was 5–12, whereas the optic sulcus was noted in only 3/13, could not be seen in 5/13 specimens and no note was taken of its status in five specimens. The menstrual age was 45.2 ± 1.6 days and the embryonic length 2.9 ± 0.12 mm.

Stage 11

Approximately 24 ± 1 days post-ovulatory; prominent features include 13–20 paired somites; the otic invagination is occasionally apparent as a slight depression—this is due to the refraction of its thick margins; both pharyngeal arches (mandibular and hyoid) are in evidence and the rostral neuropore is closing (usually seen with ≥19 somites in the more advanced embryos of the group) or is near to closing (in the least advanced members of the group) during this stage. The neural tube is the key determinant for the identification of this stage in which, in the least advanced members of the group, the fusion of the neural folds has extended rostrally to the region of the midbrain. A total of 14 specimens were collected from this stage (Figure 4).

Summary

At this stage, the embryos were mainly classified according to the number of somites and the condition of the

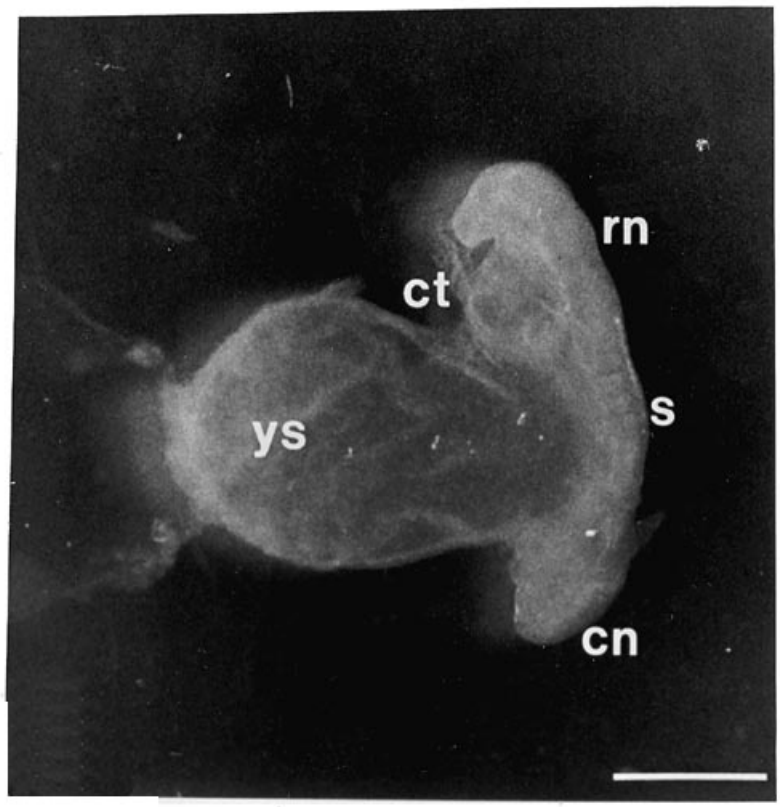

Figure 3. Photograph illustrating a left lateral view of a stage 10 embryo (Ru 124). Stage 10 is characterized by the appearance of 4–12 paired somites, the optic sulcus, the otic invagination, the development of the first pharyngeal arch and the fusion of the neural folds. This embryo demonstrates the neural closure extending from the level of the last somite (s) to the cardiac tube leaving the caudal (cn) and rostral (rn) neuropores open. The cardiac tube (ct) in the pericardial cavity is highly visible, as is the secondary yolk sac (ys). Bar = 1 mm. The photograph was taken prior to fixation. Further information on these and other stage 8 and 10 embryos can be found in the Appendix.

closing neural tube. The specimens in this stage were found to have 13–20 somites; in only 3/14 specimens was the rostral neuropore found to have closed, in 9/14 it was still open, its status was not noted in two, and there was no difference in the number of somites as to whether the rostral neuropore was closed or open. In all specimens a pericardial cavity was noted and in 10/14 samples some type of cardiac convolutions were apparent. The number of pharyngeal arches noted ranged from none (two specimens, at either end of the range of paired somites), and the mandibular and hyoid arches present (10 specimens, found throughout the sample range). All specimens but one were found to have a visible otic invagination. The menstrual age was 44.0 ± 0.9 days and the embryonic length 3.2 ± 0.22 mm. Key findings of this stage were that the number of somites had no influence on the development of the pharyngeal arches or the closure of the neural tube.

Stage 12

The characteristics by which the 12th stage is recognized are as follows; 26 ± 1 days post-ovulation; a 3–5 mm CRL which is conditioned by the amount of flexion or curvature; three pharyngeal arches are found by the end of this stage, although on occasion four may be noted; the caudal neuropore is closed by the end of the stage; the otic vesicle is almost closed and the fore or upper limb buds may make an appearance. The number of somites recognized in this group are from 21 to 29, but one is often not visible, unless sections have been cut, as it is contributing to the hypoglossal canal. With this in mind the number of somites accepted into this stage for this study has ranged from 21–28. A total of 28 embryos were found to match the criteria for this stage (Figure 5).

Summary

The 28 specimens allocated to this stage were found to have 20–28 somites. In 15/28 the rostral neuropore was found to be closed, 4/28 were found with it still open, and the rest were not noted; for the caudal neuropore 15/28 were found to be closed, 3/28 open, and the rest not noted; in 4/28 specimens the caudal neuropore was found to be closed before the rostral neuropore. In only 3/28 samples was the upper limb bud present, with the cardiac tube

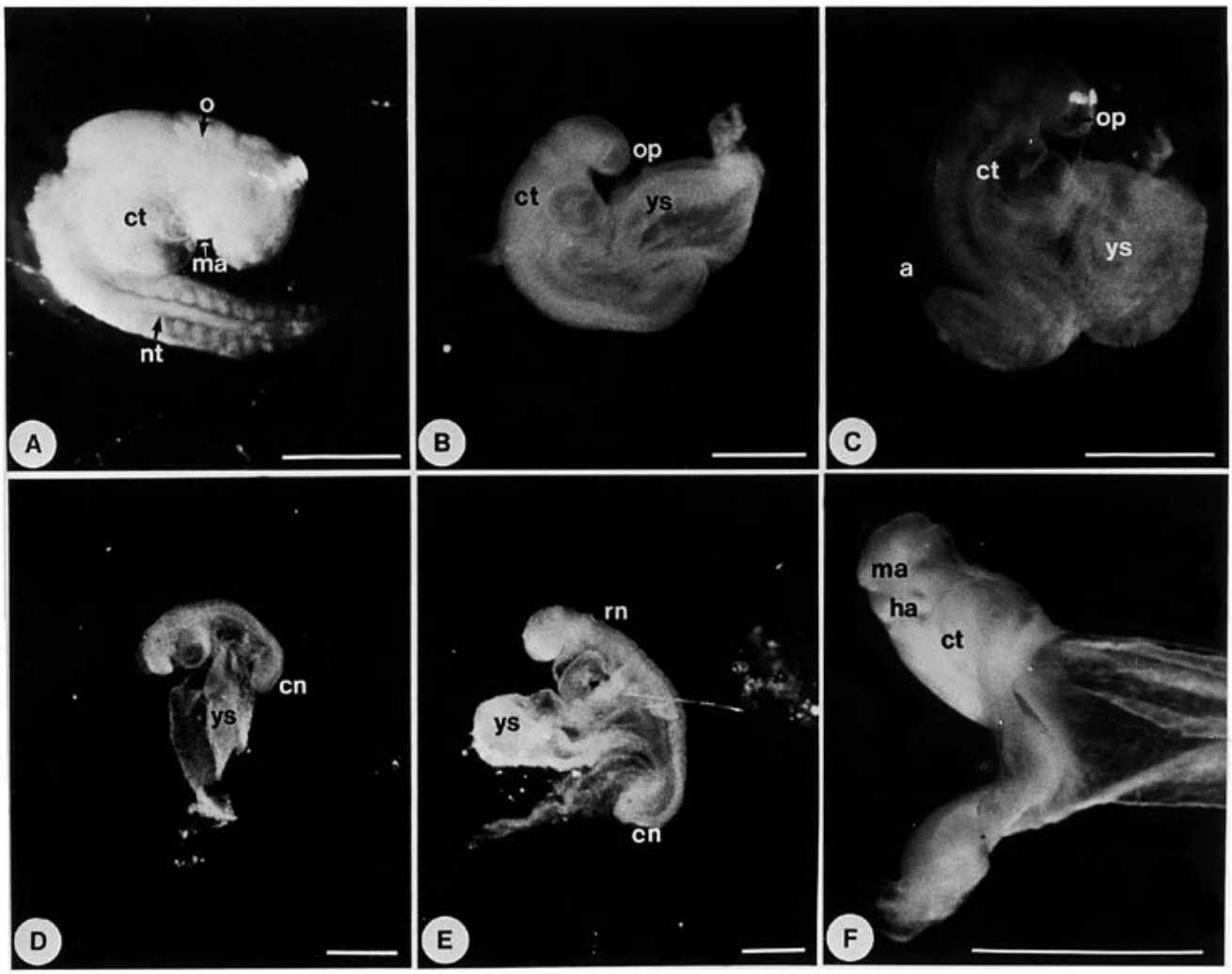

Figure 4. Photographs illustrating the variations found in the external form encountered in stage 11 embryos. Stage 11 is characterized by the appearance of 13–20 somites, two pharyngeal arches and the closure of the rostral neuropore. Bar = 1 mm, and the photographs were taken prior to fixation. (**A**) Ru 52; the right lateral view showing the cardiac tube (ct), the otic invagination (o), the mandibular arch (ma) and the neural tube (nt). The somites are distinguishable, slight damage can be seen toward the top of the spinal cord and the yolk sac attachment is missing. (**B**) Ru 87; the right lateral view showing the optic evagination (op), the cardiac tube (ct) and the secondary yolk sac (ys). The somites and the spinal cord are distinguishable. (**C**) Ru 87; the right lateral view showing the embryo in the intact amnion (a), the cardiac tube (ct), the optic evagination (op) and the secondary yolk sac (ys). (**D**) Ru 136; the left lateral view illustrating the yolk sac (ys) and the unclosed caudal neuropore (cn). The cardiac tube, somites and rostral neuropore are all distinguishable. (**E**) Ru 181; the left lateral view showing the closure of the neural tube from below the last somite to opposite the otic invagination, leaving the rostral (rn) and caudal (cn) neuropores open; also shown is the yolk sac (ys). The somites and the cardiac tubes are distinguishable; note that pericardial covering is absent. (**F**) Ru 568; the right lateral and ventral view showing the mandibular (ma) and hyoid (ha) arches, and the cardiac tube. Also distinguishable are the yolk sac, the somites and the umbilical connection, found below the yolk sac stalk.

showing convolutions in 13/28, still seen as a tube in 10/28 of specimens and not noted in the rest. The majority of specimens were found to have three pharyngeal arches, 19/28 with an equal distribution having one, two, four or not being noted as to their status. The weight range for this stage was 0.0011–0.0444 g, with a mean of 0.0146 ± 0.0021 g. The menstrual age was 49.5 ± 1.2 days and the embryonic length or CRL 4.2 ± 0.21 mm. The rostral neuropore was found to be open in 46.4% of the specimens allocated to this group. Based on previous work it was assumed that the fusion of the neural folds had extended rostrally into the region of the midbrain.

Stage 13

Stage 13, at 28 days post-ovulation, is characterized by a 4–6 mm CRL (post-fixation); >30 somites, which become increasingly difficult to determine and exceeding 30 in number they are not regarded as an accurate way of determining the gestational age. The otic vesicle is closed;

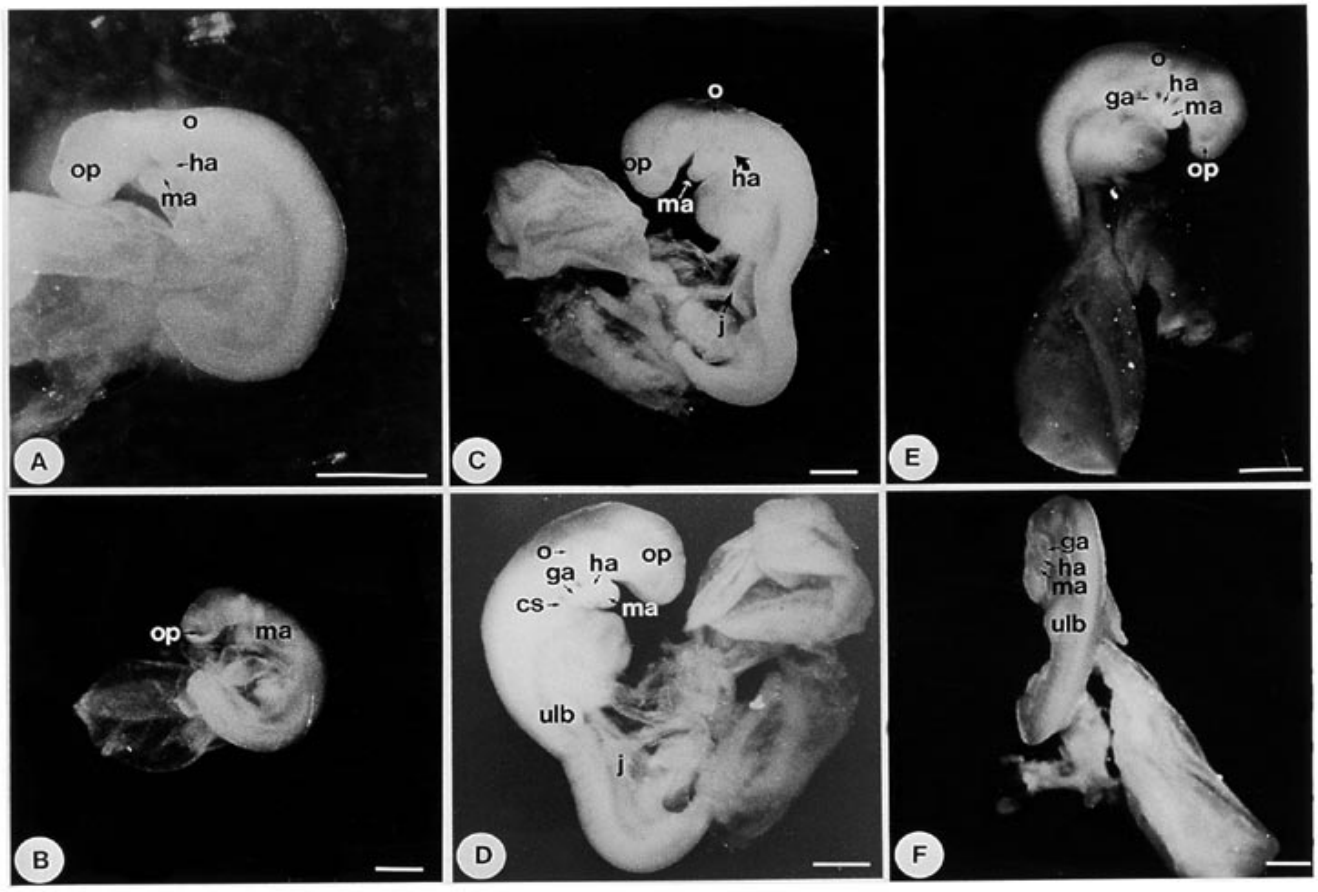

Figure 5. Photographs illustrating stage 12 embryos. Stage 12 is characterized by 21–30 paired somites, three to four pharyngeal arches, the closure of the caudal neuropore and the appearance of the upper limb bud. Bars = 1 mm, and the photographic plates were taken prior to fixation. (**A**) Ru 123; the left lateral view showing the otic invagination (o), the optic evagination (op), and the mandibular (ma) and hyoid (ha) arches. Also distinguishable are the somites and the secondary yolk sac stalk. (**B**) Ru 144; the left lateral view showing the optic evagination (op) and the mandibular arch (ma). The cardiac region and the secondary yolk sac are translucent. (**C**, **D**) Ru 576; the left (**C**) and right (**D**) lateral views showing the otic invagination (o), the optic evagination (op), the mandibular (ma), hyoid (ha) and glossopharyngeal (ga) arches, the cervical sinus (cs), the appearance of the upper limb bud (ulb) and the junction of the umbilical vesicle and the intestine (j). Also distinguishable are the amnion, the secondary yolk sac, the somites, the cardiac tube, the hepatic trabeculae and the IVth ventricle. (**E**, **F**) Ru 582; the right lateral and dorsal views showing the otic invagination (o), the optic evagination (op), the mandibular (ma), hyoid (ha) and glossopharyngeal (ga) arches, and the upper limb bud (ulb). (**E**) shows the cardiac tube as translucent with the hepatic trabeculae slightly below and behind it.

the lens disc in the optic evagination is not usually indented but appears internally; the external contour of the neural tube is the same as that of the embryo; the hyoid arch starts to lie across the glossopharyngeal arch so that it moves caudal, posterior to a depressed triangle where the surface ectoderm sinks to come into contact with the pharyngeal ectoderm, thus creating the cervical sinus. The upper limb buds form definite ridges, and the beginning of the lower limb buds becomes apparent; in tissue after fixation with formalin the outline of the brain and optic vesicle can sometimes be detected.

Summary

In this stage (Figure 6), 34 specimens were found to fulfil the correct criteria. The CRL range was 4.2–8 mm with a mean of 5.57 ± 0.21 mm. For the Carnegie Institute's data, the CRL range fell between 4 and 6 mm, and 80% of their embryos were found within this range. In the current study, it was found that 21/34 of the CRL were within this range, 11/34 were larger and 2/34 smaller; this could be due to the fact that the Carnegie Institute measurements were made on fixed specimens whilst the measurements from the current study were made on fresh tissue. The menstrual age was 50.2 ± 0.8 days. In 32/34 embryos the otic vesicle was closed, while in all 100% the optic evagination was noted. In 19/34 specimens the hyoid arch was found to overlap the glossopharyngeal arch, while in 12/34 specimens three pharyngeal arches were noted although no overlapping was seen to be occurring; also in 14/34 samples the cervical sinus was found to be present. Upper limb buds were found

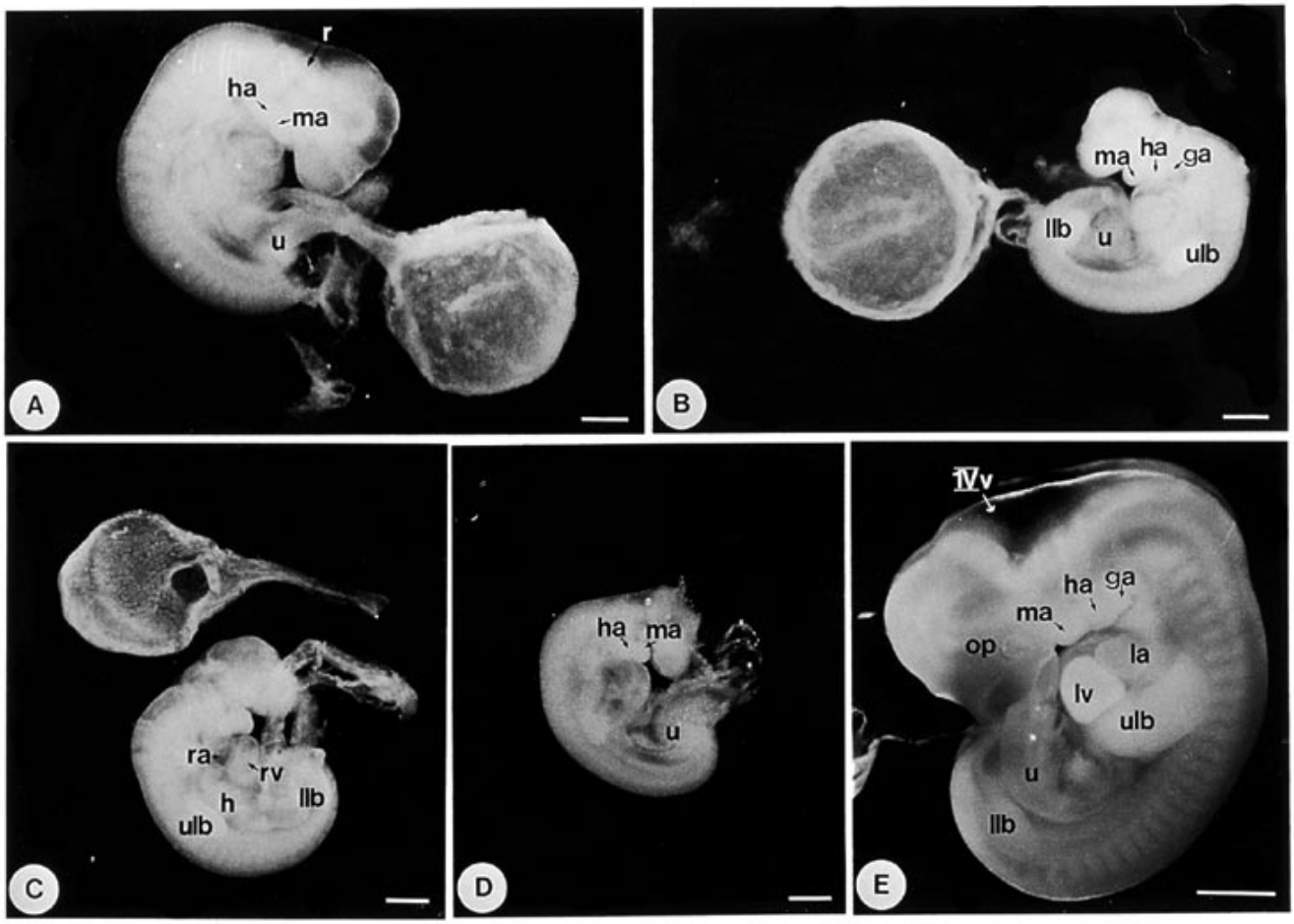

Figure 6. Photographs illustrating the variations in the external form encountered in stage 13 embryos. Stage 13 is characterized by second pharyngeal arch overlapping the third arch, the appearance of the upper limb buds as definite ridges and the appearance of the lower limb bud. Bar = 1 mm. All photographs were taken prior to fixation. (**A**) Ru 101; the right lateral view showing the mandibular (ma) and hyoid (ha) arches, the umbilicus (u) and rhombomeres (r) in the IVth ventricle. The brain is showing as a translucent area in the head, to the front of the IVth ventricle. (**B**) Ru 157; the left lateral view illustrating the mandibular (ma), hyoid (ha) and glossopharyngeal (ga) arches, the upper (ulb) and lower (llb) limb buds, and the umbilicus (u). The left atrium and left ventricle are distinguishable, as is the hepatic trabeculae which lies below the cardium. (**C**) Ru 137; the right lateral view illustrating the right atrium (ra), the right ventricle (rv), the hepatic trabeculae (h) and the upper (ulb) and lower (llb) limb buds. Also distinguishable are the mandibular and hyoid arches, rhombomeres in the IVth ventricle, the umbilicus and, detached from the embryo, the secondary yolk sac. (**D**) Ru 538/1; the right lateral view illustrating the mandibular (ma) and hyoid (ha) arches, and the umbilicus (u). The upper limb bud is recognisable, although faint, as are the right atrium and ventricle. (**E**) Ru 567; the left lateral view showing the IVth ventricle (IVv), the optic vesicle (op), the mandibular (ma), hyoid (ha) and glossopharyngeal (ga) arches, the left atrium (la), the left ventricle (lv), the upper (ulb) and lower (llb) limb buds and the umbilicus (u). The otic invagination and the forebrain vesicle are also distinguishable.

in 26/34 specimens, while lower limb buds were seen in 11/34.

Stage 14

At stage 14, 32 days have elapsed since ovulation and the fixed, average, CRL is 5–7 mm with a maximal range of 5–8 mm. By now the upper limb buds are rounded, curving ventrally, and starting to taper towards the tip; the lower limb buds are present, but usually only as raised ectodermal ridges; the mandibular and hyoid arches are large and conspicuous, the glossopharyngeal arch is relatively small and often concealed with the depression of the cervical sinus, and the ectoderm of the nasal plate is thickening and its opaque rim may stand out.

Summary

In this stage (Figure 7), 27 embryos were found to match the majority of the Carnegie Institute criteria. The CRL was 7.15 ± 0.22 mm, with a minimum length of 5 mm, and a maximum length of 9 mm. Overall, 12/27 specimens had CRL that ranged between the mean lengths of 6–7 mm

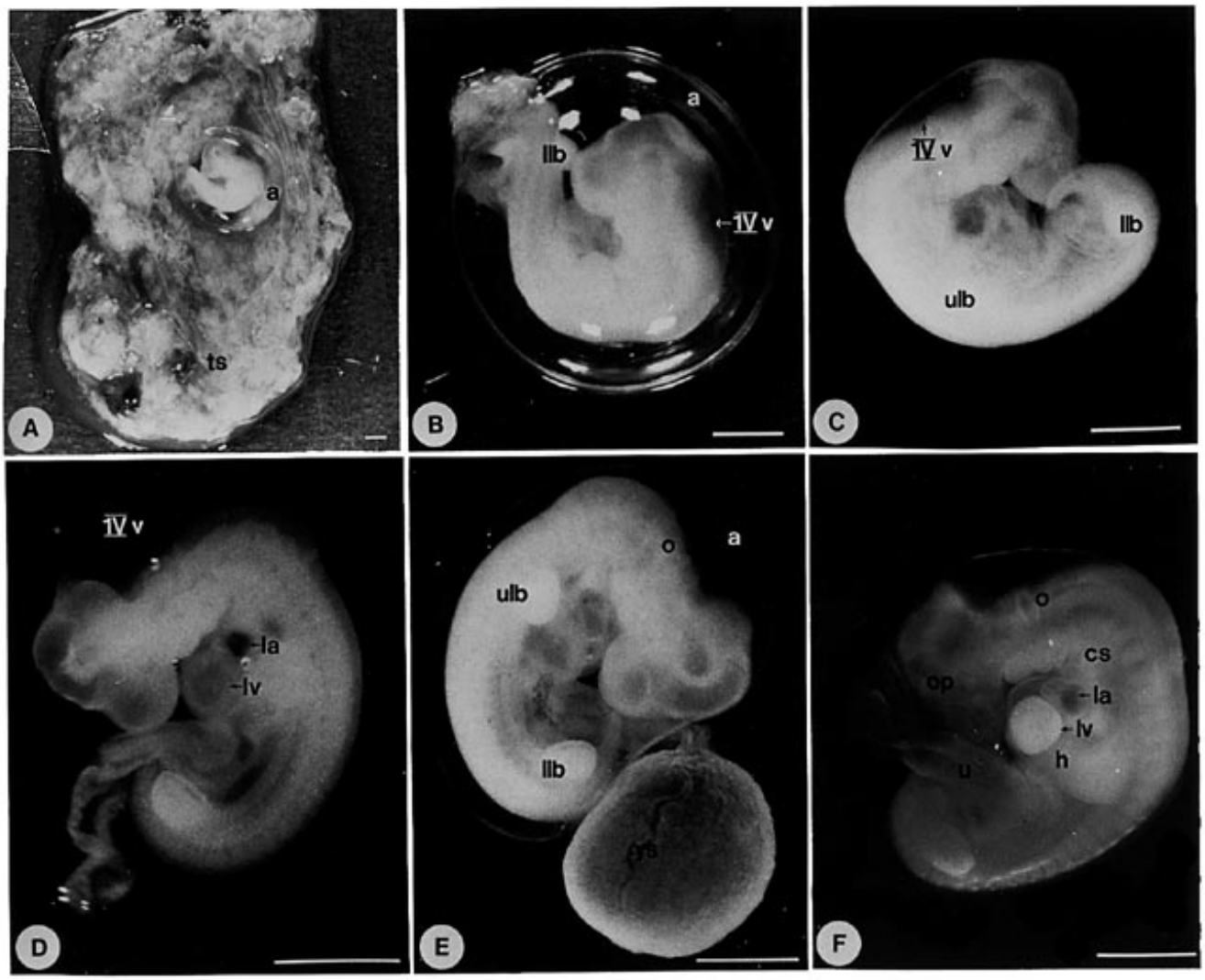

Figure 7. Photographs illustrating five embryos encountered at stage 14. Stage 14 is characterized by thickening of the nasal plate, the elongation of the upper limb buds and the conspicuousness of the first and second pharyngeal arches. Bar = 2 mm; all photographs were taken before fixation. (**A**, **B**) Ru 58; (**A**) the left lateral view showing the attachment of the embryo to the trophoblast sac (ts), with the embryo still in the intact amnion (a). (**B**) illustrates the embryo in the intact amnion (a) with the IVth ventricle (IVv) and the lower limb buds (llb) noted. (**C**) Ru 72; the right lateral view illustrating the IVth ventricle(IVv), and the upper (ulb) and lower (llb) limb buds. The amnion is distinguishable between the tail bud and the cephalic region, and the right atrium and ventricle are also visible. (**D**) Ru 83; the left lateral view showing the IVth ventricle (IVv), and the left atrium (la) and left ventricle (lv). The cephalic region, above the IVth ventricle, is translucent and the formation of the fore brain vesicle can be seen. (**E**) Ru 141; the right lateral view illustrating the intact amnion (a), the otic vesicle (o), the upper (ulb) and lower (llb) limb buds, and the yolk sac (ys). The cardiac area is distinguishable showing the right atrium and ventricle and the hepatic trabeculae, which lies slightly beneath it. (**F**) Ru 580; the left lateral view illustrating the otic vesicle (o), the optic vesicle (op), the cervical sinus (cs), the left atrium (la), the left ventricle (lv), the hepatic trabeculae (h), and the umbilicus (u). The upper and lower limb buds are also distinguishable.

given by the Carnegie Institute. For comparison, they found that ~70% of their embryos were within the range, with a minimum length of 5.5 mm and a maximum length of 8.2 mm. The menstrual age was 51.9 ± 0.8 days. The weight of the embryos was 0.0772 g ± 0.01 g, with a minimum of 0.021 g, and a maximum of 0.2524 g. Developmentally, 18/27 embryos were found to have the upper limb bud elongating, while 9/27 of the upper limb buds were still paddle shaped; all embryos from this stage had the appearance of a lower limb bud; 7/27 had a visibly thickening nasal plate; 21/27 had both the mandibular and hyoid arches large and conspicuous, whereas 5/27 had the first three pharyngeal arches equal in size.

Stage 15

At this stage the average CRL of a fixed embryonic specimen is 7–9 mm, although a range of 6.5–8.5 has been

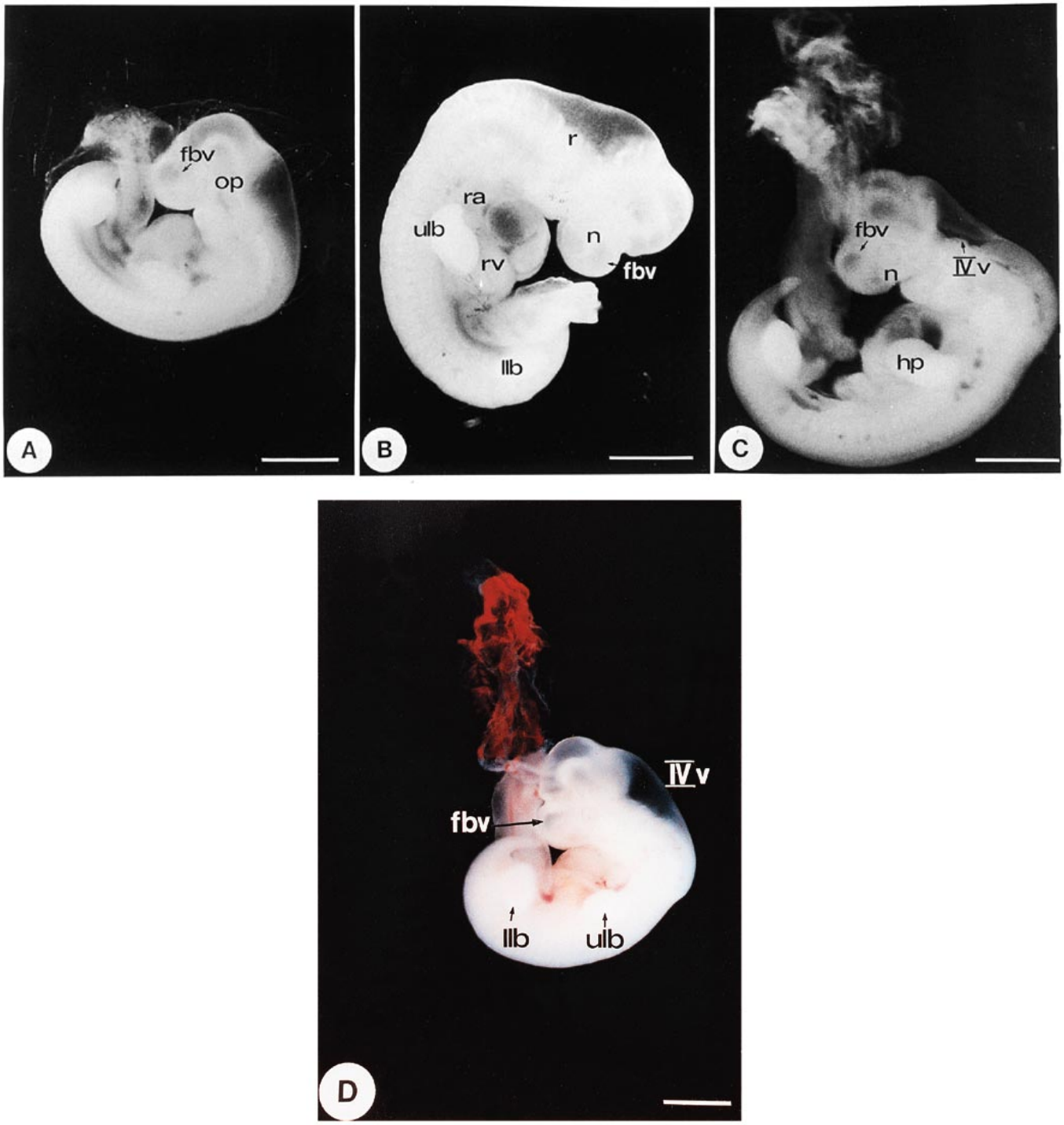

Figure 8. Four embryos illustrating the variations in the external form found at stage 15. Stage 15 is characterized by the closure of the lens vesicle, the formation of the nasal pits, the segmentation of the hyoid arch, the differentiation of the upper limb bud, and the elevation of the somites and spinal ganglia. All photographs were taken prior to fixation; measurement bar = 2 mm. (**A**) Ru 129; the left lateral view showing the forebrain vesicle (fbv) and the optic vesicle (op). The amnion is visible around the umbilical attachment, also distinguishable are the upper and lower limb buds, the left atrium and ventricle and the hepatic trabeculae. (**B**) Ru 176; the right lateral view illustrating the rhombomeres (r) in the IVth ventricle, the raised nasal discs (n), the forebrain vesicle (fbv), the right atrium (ra) and ventricle (rv), and the upper (ulb) and lower (llb) limb buds. The somites and umbilicus are distinguishable. (**C**) Ru 185; the left lateral view showing the IVth ventricle (IVv), the forebrain vesicle (fbv), the raised nasal discs (n), and the hand plate (hp) on the upper limb bud. The lower limb bud, umbilicus and tail bud are also distinguishable. (**D**) Ru 159; the left lateral view showing the IVth ventricle (IVv), the forebrain vesicle (fbv), and the upper (ulb) and lower (llb) limb buds. The amnion, still attached to the umbilicus, is visible, the crown of the head is translucent, the optic vesicle is distinguishable, and the cardium and hepatic trabeculae are visible.

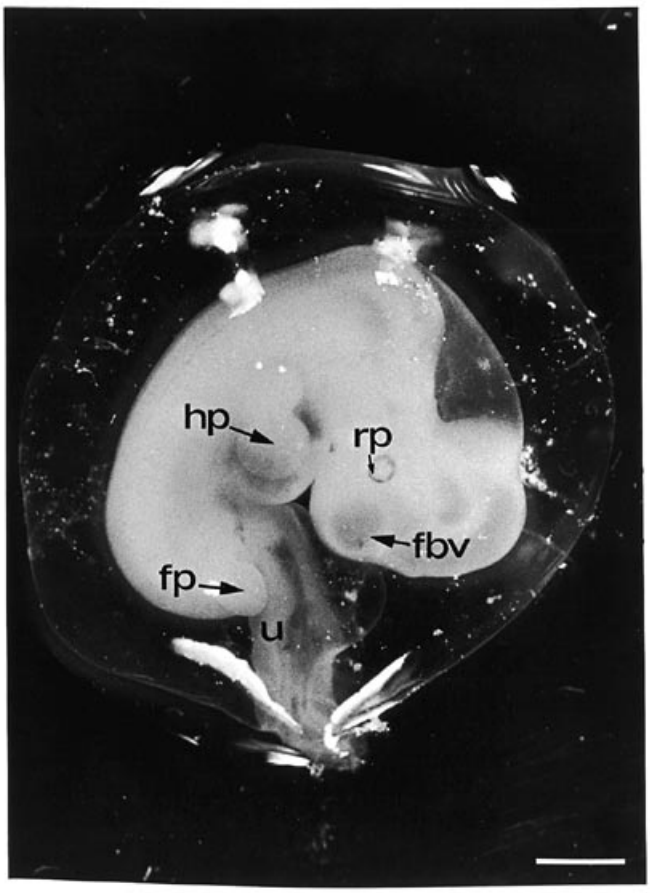

Figure 9. One photograph illustrating the right lateral view of a stage 16 embryo (Ru 78). Stage 16 is characterized by the turning of the nasal pits to face ventrally, the appearance of retinal pigment in the optic vesicle, the formation of the auricular hillocks and the differentiation of the lower limb bud. Points of development noted are the forebrain vesicle (fbv), the hand (hp) and foot (fp) plates, the umbilicus (u) and the retinal pigment (rp) in the optic vesicle. Measurement bar = 2 mm, and the photograph was taken prior to fixation.

recorded, and the post-ovulatory age of the embryos in this group is ~33 days. The relative width of the embryo has become greater due to growth of spinal ganglia, muscular plates, and mesenchymal tissues associated with them. Five characteristics are associated with this stage: (i) the lens vesicles have closed and the surface pores through which they communicate have disappeared; (ii) the nasal discs begin to recede from the surface acquiring the form of large oval depressions or nasal pits; (iii) the ventral segment of the hyoid arch subsegments into the primordium of the antiragus; (iv) the upper limb bud becomes regionally subdivided into a distal hand plate, a proximal forearm, arm and shoulder region and the lower limb bud elongates; (v) the fifth characteristic is that the somites, spinal ganglia and muscular plates all produce characteristic elevations.

Summary

In this stage (Figure 8), 25 embryos were found to approximate to the Carnegie Institute criteria. The CRL was 8.71 ± 1.19 mm, with a minimum and a maximum recorded length of 7–11 mm. In 18/25 of the specimens, the embryos were within the mean limits of the 6.5–8.5 mm CRL given by the Carnegie Institute; in fact for comparison, they had found 80% to lie within these limits with a minimum of 6 mm and a maximum of 11 mm. The menstrual age was 52.2 ± 0.8 days. The embryonic weight for this stage was 0.1295 ± 0.009 g, with a minimum recorded weight of 0.0612 g and a maximum of 0.2166 g. Of the five characteristics associated with this stage, 23/25 were found to have the lens vesicle closed; 17/25 had the nasal discs receding from the surface and the appearance of nasal pits; the primordium of the antiragus was only noted in 3/25 of the embryos; 24/25 were showing primary differentiation of the upper limb bud into the hand, arm and forearm, among the 25 specimens the lower limb buds were found to be elongating in 24 (one was still paddle shaped), and in 20/25 of the embryos the forebrain vesicle was seen to be prominent.

Stage 16

The characteristics associated with this stage include: an average CRL of 11–14 mm (total range 7–14 mm); 37 days post-ovulation; the nasal pits, turning to face ventrally from their slightly raised lateral position disappear from the profile view and only the prominent lips forming the lateral boundary can be seen with its marginal fold overhanging the floor of the nasal pits. The retinal pigment becomes visible towards the end of the stage; the hyoid arch becomes much more conspicuous, forming the auricular hillocks, and conversely, the glossopharyngeal arch recedes and is no longer visible by the end of stage 16. The upper limb bud starts to show the formation of a hand plate in which, occasionally, the marginal vein can be seen; the lower limb bud begins to differentiate into the thigh, leg and foot.

Summary

In this stage (Figure 9), 28 embryos were found within the majority of the Carnegie Institute criteria. The mean CRL was 10.03 ± 0.22 mm, with a minimum length of 8 mm and a maximum of 12 mm. In 5/28 of the specimens studied, the embryos were within the Carnegie Institute's mean range for the CRL of 11–14 mm. The menstrual age was 54.3 ± 1.2 days. The weight of the embryos in this stage was 0.2124 ± 0.026 g, with a minimum of 0.099 g and a maximum of 0.775 g. The developmental characteristics associated with this stage show that 26/28 embryos had

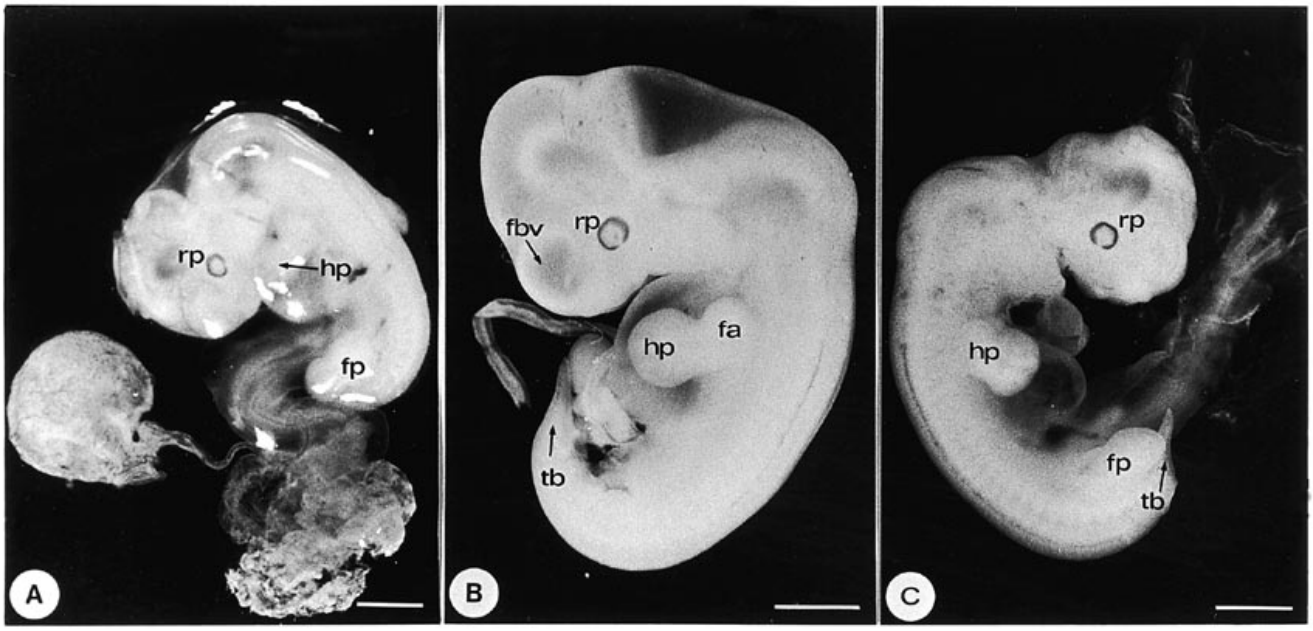

Figure 10. Three photographs to illustrate the differences encountered in the external form at stage 17. Stage 17 is characterized by an increase in the head size, the straightening of the main axis and the appearance of digital rays in the hand plate. All photographs were taken prior to fixation, and the measurement bar = 2 mm. (**A**) Ru 69; the left lateral view showing the development of retinal pigment (rp) in the optic vesicle, and the differentiation of the upper and lower limb buds into the hand (hp) and foot (fp) plates. The amniotic sac, still attached to the umbilicus, is distinguishable and the yolk sac is visible. (**B**) Ru 154; the left lateral view illustrating the development of the retinal pigment (rp) in the optic vesicle, the forebrain vesicle (fbv), the differentiation of the upper limb bud into the forearm (fa) and the hand plate (hp), and the regression of the tail bud (tb). The yolk sac stalk is distinguishable although the yolk sac and umbilicus are absent. Some damage can be seen to the lower abdominal area where the umbilical attachment and left lower limb bud should be. (**C**) Ru 183; the right lateral view illustrating the development of the retinal pigment (rp) in the optic vesicle, the differentiation of the upper and lower limb buds into the arm/leg and hand (hp) and foot (fp) plates, and the regression of the tail bud (tb). The cardiac region shows distinctly the right atrium and right ventricle with the hepatic trabeculae lying beneath them.

retinal pigment in the optic vesicle; in 11/28, the nasal pits had turned to face ventrally, while in 13/28 they were still facing laterally; in 12/28 the hyoid arch was conspicuous, while in 14/28 it was still overlapping the glossopharyngeal arch, which was receding in 10/28; the auricular hillocks were only seen in 1/28 of the specimens; the lower limb bud was found to show primary differentiation into the foot, leg and thigh in 12/28 embryos while it was still elongating in 12/28 of specimens; no hand plates were seen.

Stage 17

The characteristics that identify this stage include: a mean CRL of 11–14 mm (total range of 10–14.5 mm), and 41 days post-ovulation. The physical characteristics of the embryonic growth at this stage are; an increase in the size of the head due, to precocious growth; the main axis of the trunk is straighter with, perhaps, a slight indication of the lumbar curvature; the nasal pits are further medial and directed ventrally so that the nostril is not visible at all in profile; a full complement of auricular hillocks are present on the mandibular and hyoid arches; the hand plate on the upper limb bud shows digital rays, and a rounded digital plate starts to appear on the lower limb bud.

Summary

In this stage (Figure 10), 30 embryos were found to be within the Carnegie Institute criteria. The mean CRL was 12.24 ± 0.28 mm, with a minimum and maximum length range of 10–15 mm. In 18/30 of the specimens, the CRL of the embryos was found to be within the mean range of 11–14 mm established by the Institute. From the findings of the Carnegie Institute, 68% of the embryos fell within a range of 11–13.6 mm, with a minimum recorded length of 10 mm and a maximum recorded length of 14.5 mm. The menstrual age was 54.8 ± 1.0 days. The weight for this stage was 0.2874 ± 0.023 g, with a minimum and maximum weight of 0.183 and 0.8322 g. Developmentally, the characteristics of this stage showed that in 25/30 the main axis of the trunk was straightening, with 17/30 having a slight lumbar curvature; in 9/30 the nasal pits had moved

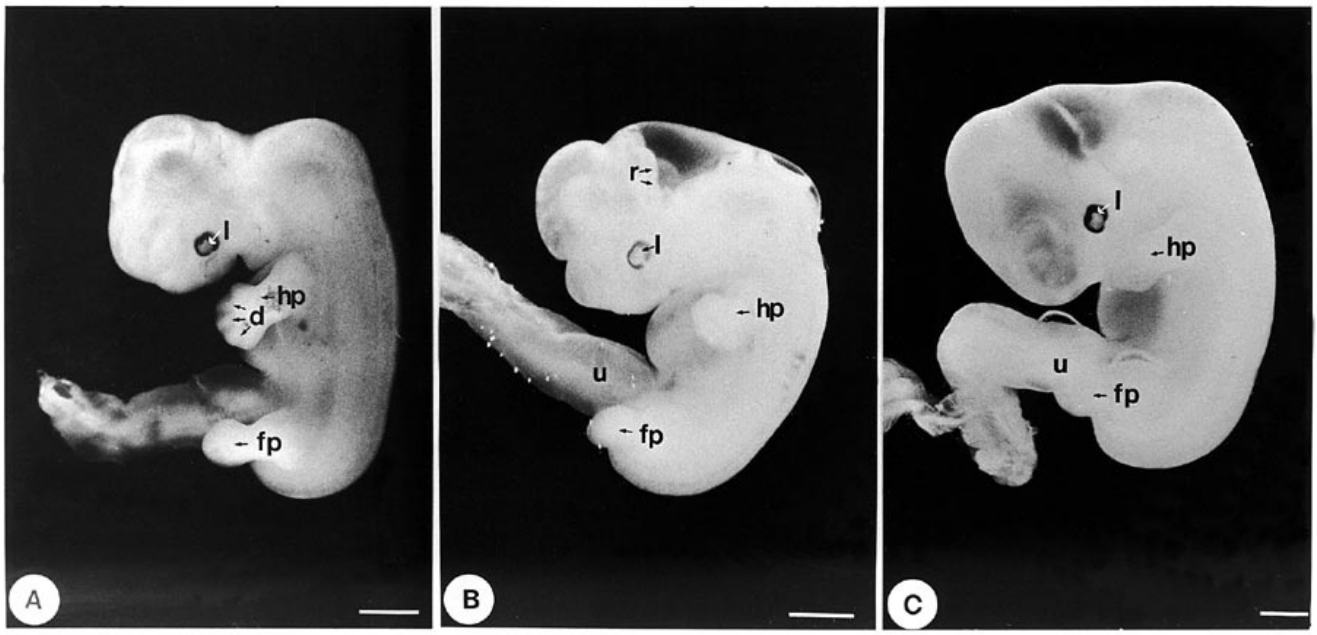

Figure 11. Three photographs illustrating the variations in external development seen in stage 18 embryos. Stage 18 is characterized by the increase in length of the limb buds, the appearance of interdigital notches on the hand plate, the elbow region becomes discernible, the visualization of digits on the foot plate and the eyelids take on a more advanced form. Measurement bar = 2 mm, and all photographs were taken prior to fixation. (**A**) Ru 171; the left lateral view showing the lens (l) in the optic vesicle, the digits (d) appearing in the hand plate (hp), and the foot plate (fp). Other distinguishable features include the retinal pigment in the optic vesicle, the forebrain vesicle, the receding tail bud and the umbilicus. (**B**) Ru 204; the left lateral view showing the lens (l) in the optic vesicle, the rhombomeres (r) in the IVth ventricle, the hand plate (hp), the foot plate (fp), and the umbilicus (u). The retinal pigment in the optic vesicle is distinguishable, although it does not, as yet, completely surround the lens. (**C**) Ru 564; the left lateral view showing the lens (l) in the optic vesicle, the hand (hp) and foot (fp) plates, and the umbilicus (u). The forebrain vesicle, IVth ventricle and the hepatic trabeculae are distinguishable.

so far ventrally that they could no longer be seen and in 20/30 the pits were still turning to face ventrally; in 22/30 a full complement of auricular hillocks were found, but in 6/30 these were still seen as pharyngeal arches; all embryos were found to have digital rays in the hand plates and 22/30 were showing foot plates in the lower limb buds.

Stage 18

This stage is characterized by a CRL of 13–17 mm and a post-ovulatory age of ~44 days. The shape of the embryo is more uniformly cuboidal, and both the cervical and lumbar flexures are indicated; both limbs are longer and the digital plate of the hand is notched; the elbow region is usually discernible; the lower limb bud shows that toe rays forming in the digital plate are identifiable in some specimens; the eyelid folds have taken a more advanced form and a distinct tip to the end of the nose is found; the auricular hillocks have transformed into specific parts of the ear.

Summary

In this stage (Figure 11), 21 embryos were found to have the criteria set by the Carnegie Institute. The mean CRL was 14.79 ± 0.47 mm, with a minimum and maximum length of 11–20 mm. In 16/21, the specimens had a CRL of between 13 and 17 mm which are the minimum and maximum lengths previously recorded; from the findings of the Institute two thirds of the embryos had a CRL of 14–16 mm. The menstrual age was 55.9 ± 0.9 days. The weight of the specimens was 0.3885 ± 0.023 g, with a minimum and maximum weight of 0.196–0.619 g. Developmentally, 18/21 showed a lumbar flexure, and 19/21 showed a cervical flexure; in 19/21 the limb buds had increased in length and, in 13/21, interdigital notches were appearing in the hand plates; in 17/21 digital rays were seen in the foot plates; elbow regions were discernible in 6/21 individuals, while 8/21 had a distinct tip to the nose; no evidence was found to support a more uniform shape nor was any eyelid development noted.

Stage 19

The characteristics of stage 19 include: a 17–20 mm CRL; 47–48 days post-ovulation; in the embryo proper the trunk is elongating and straightening, and the head is no longer at right angles to the trunk; the limbs are found to extend directly forward and the toe rays are more prominent, although no interdigital notches have been seen.

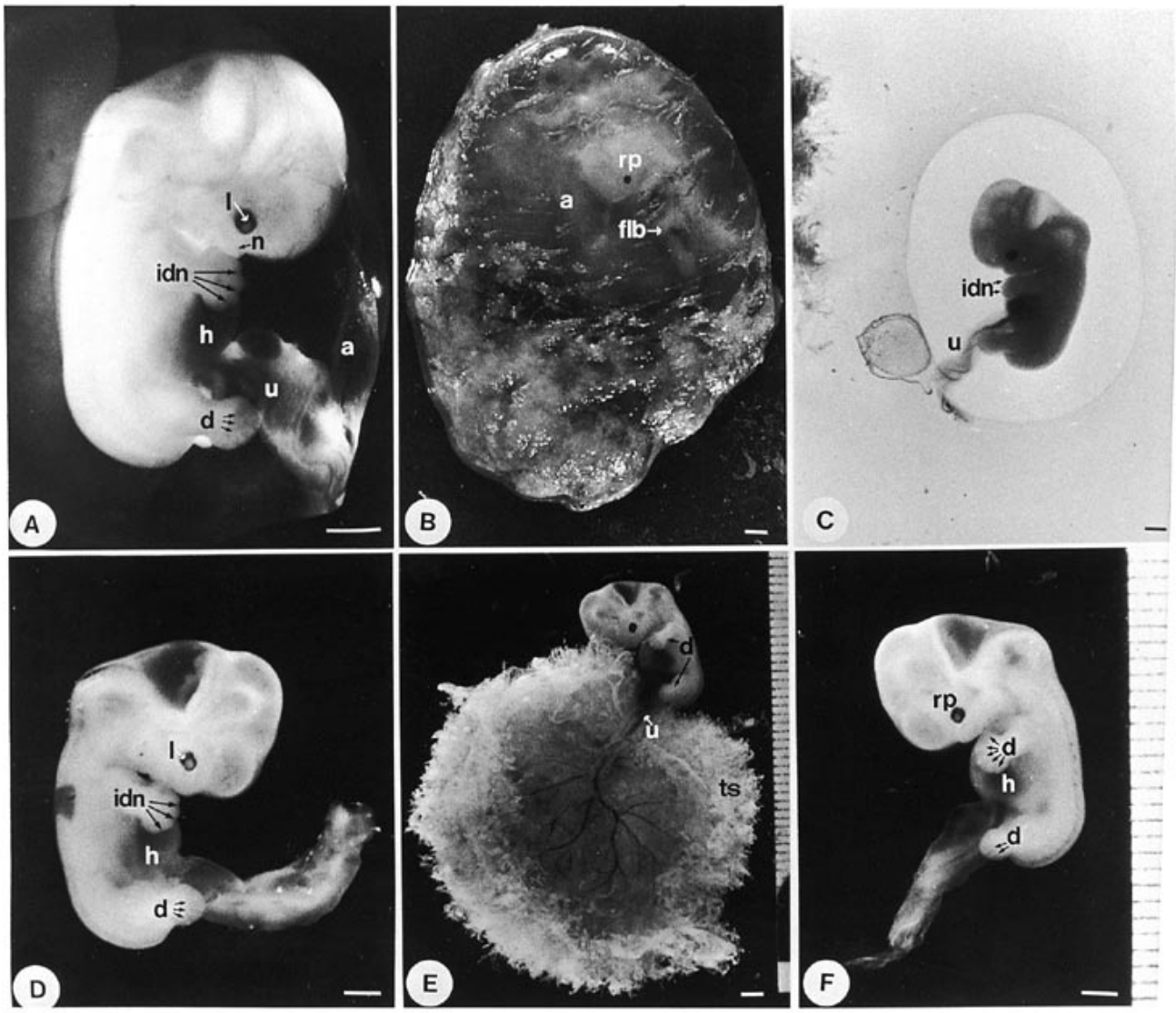

Figure 12. Photographs of four embryos belonging to stage 19. Stage 19 is characterized by the elongation and straightening of the trunk and the more prominent appearance of the toe rays. Measurement bar = 2 mm, and all photographs were taken prior to fixation. (**A**) Ru 45; the right lateral view showing the embryo in the intact amniotic sac (a), the lens (l) of the optic vesicle illustrating that the optic vesicle is moving towards a more ventral position, the nose (n) tip taking a prominent shape, interdigital notches (idn) are seen to be separating the digits on the hand plate, the digits (d) on the foot plates are distinguishable, as are the hepatic trabeculae (h) in the lower abdomen. The IVth ventricle is identifiable and the start of the physiological hernia can be seen in the umbilicus (u). (**B**, **C**) Ru 46; the left lateral view of the intact gestational sac (**B**) through which the retinal pigment (rp), amniotic sac (a) and the forelimb buds (flb) can be distinguished. (**C**) illustrates the left lateral view of the same embryo, removed from the gestational sac but in the intact amniotic sac, and with the secondary yolk sac attached. The interdigital notches (idn) are distinguishable on the hand plate, and the umbilical veins and artery can be seen in the umbilicus (u). (**D**) Ru 168; the right lateral view showing the IVth ventricle, the interdigital notches (idn) on the hand plate, the digits (d) on the foot plate, the optic vesicle, which has moved to a more central placement, shows the lens (l), and the hepatic trabeculae (h) in the lower abdomen. (**E**, **F**) Ru 170; both photographs illustrate the left lateral view of this embryo, with plate (**E**) showing the umbilical attachment (u) between the embryo and the trophoblast (t). The digits (d) on both the hand and foot plates are distinguishable. (**F**) shows in more detail the digits (d) on the hand and foot plates, the hepatic trabeculae (h) in the lower abdomen, and the retinal pigment (rp) in the optic vesicle. The IVth ventricle and the forebrain vesicle are both distinguishable.

Summary

At this stage (Figure 12), 24 specimens were found to have the majority of the criteria established by the Carnegie Institute. The mean CRL was 16.85 ± 0.31 mm, with a minimum and maximum length of 14–20 mm. In 10/24 specimens, the embryos collected were within the mean range of 17–20 mm with 14/24 measuring less than this range. The menstrual age was 58.3 ± 0.7 days. The weight of these embryos was 0.4655 ± 0.022 g, with a minimum weight of 0.285 g and a maximum weight of 0.672 g.

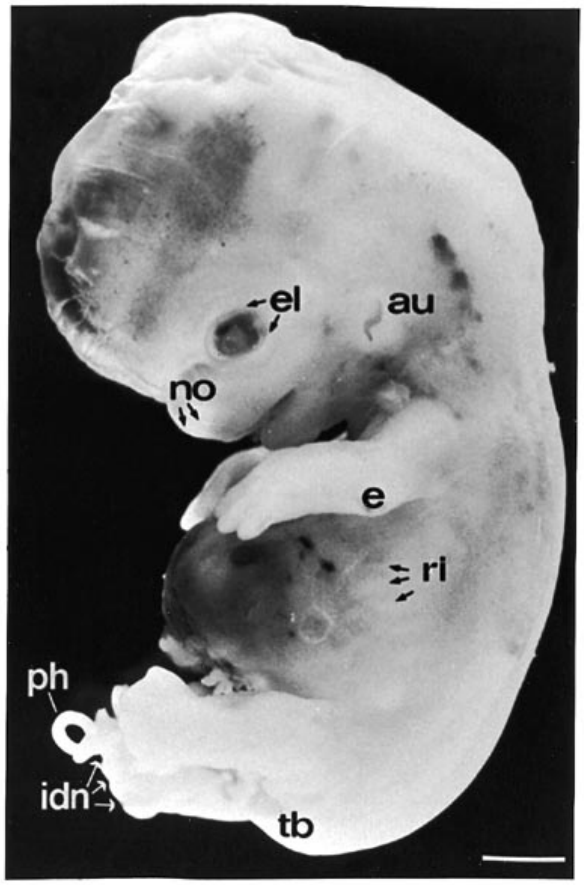

Figure 13. One photograph illustrating the left lateral view of a stage 20 embryo (Ru 180). Stage 20 is characterized by the lengthening of the upper limbs and the beginnings of flexion in the region of the elbow, the hands curve towards the cardiac region and interdigital notches are appearing on the foot plates. The photograph was taken after fixation, the measurement bar = 2 mm. The features identified on this embryo include the eyelids (el), the external form of the ear (au), the nostrils (no), the developing elbow (e), the faint outline of the ribs (ri) in the abdominal cavity, the physiological hernia (ph), the interdigital notches (idn) on the foot plates, and the regressing tail bud (tb).

Developmentally, the head was moving away from a right angle position to the trunk in 16/24 of the specimens, while the main axis of the body was straightening in all 100%; toe rays were becoming prominent in all of the specimens, and the upper limb buds were extending forward in all specimens. In all of the specimens the auricular hillocks were starting to form more specific parts of the ear.

Stage 20

Stage 20 is characterized by a CRL of 21–23 mm and a post-ovulation age of 50–51 days. The embryo proper, lengthens in the upper limb bud and is slightly bent at the elbows, the hand plate curves towards the cardiac region, and a growth centre is found above the temporal frontal region. The beginnings of interdigital notches are seen in the foot plate.

Summary

In this stage (Figure 13), 24 embryos were found to have the same developmental features as those allocated by the Carnegie Institute. The mean CRL was 18.12 ± 0.31 mm, with a minimum length and maximum length of 15.5 to 21 mm. Only 1/24 specimen was found to be 21–23 mm which were mean lengths previously recorded by the Institute; all of the other measurements were <21 mm. The menstrual age was 57.9 ± 0.6 days. The weight was 0.6533 ± 0.036 g with a minimum weight of 0.2945 g and a maximum weight of 0.9433 g. Developmentally, the optic cups were centring in 23/24 of the specimens; the elbow region was bent in all of the specimens; the hand plates were curving towards the cardiac region in 1/24 of the specimens, while the digits on the hand plates were separated in 22/24; interdigital notches between the toes were found in 12/24 of the specimens; in all specimens a faint outline of the developing scapula was seen and in 7/24 the outline of ribs noted; in a couple of specimens the faint outline of joints was seen in the digits of the hand plate; in all specimens the tail bud was receding.

Stage 21

This stage is characterized by a CRL of 22–24 mm and a post-ovulation age of 52 days; the embryo proper shows the distal phalangeal portions to be slightly swollen and showing the beginning of tactile pads; the hands become flexed at the wrists and nearly meet together above the cardiac area.

Summary

In this group (Figure 14), 18 embryos were found to have a majority of criteria established for this stage by the Carnegie Institute. The mean CRL was 22.3 ± 0.62 mm, with a range of 17–27 mm. In 6/18 of the specimens, the CRL was found to be 22–24 mm, the size given by the Institute that the majority of previous specimens were between. Of the rest of the specimens allocated to this stage, 7/18 had a CRL <22 mm and 5/18 had a CRL >24 mm. The menstrual age was 59.0 ± 1.9 days. The weight recorded for this stage was 1.1314 ± 0.071 g, with a minimum weight of 0.635 g and a maximum weight of 1.728 g. Developmentally, all specimens showed flexion of the hands at the wrists; in all specimens the interdigital notches on the foot plates were more pronounced; in 17/18 the upper limb buds were curved ventrally, and in 16/18 of specimens the outlines of ribs were seen in the abdominal cavity. Other developmental characteristics not previously

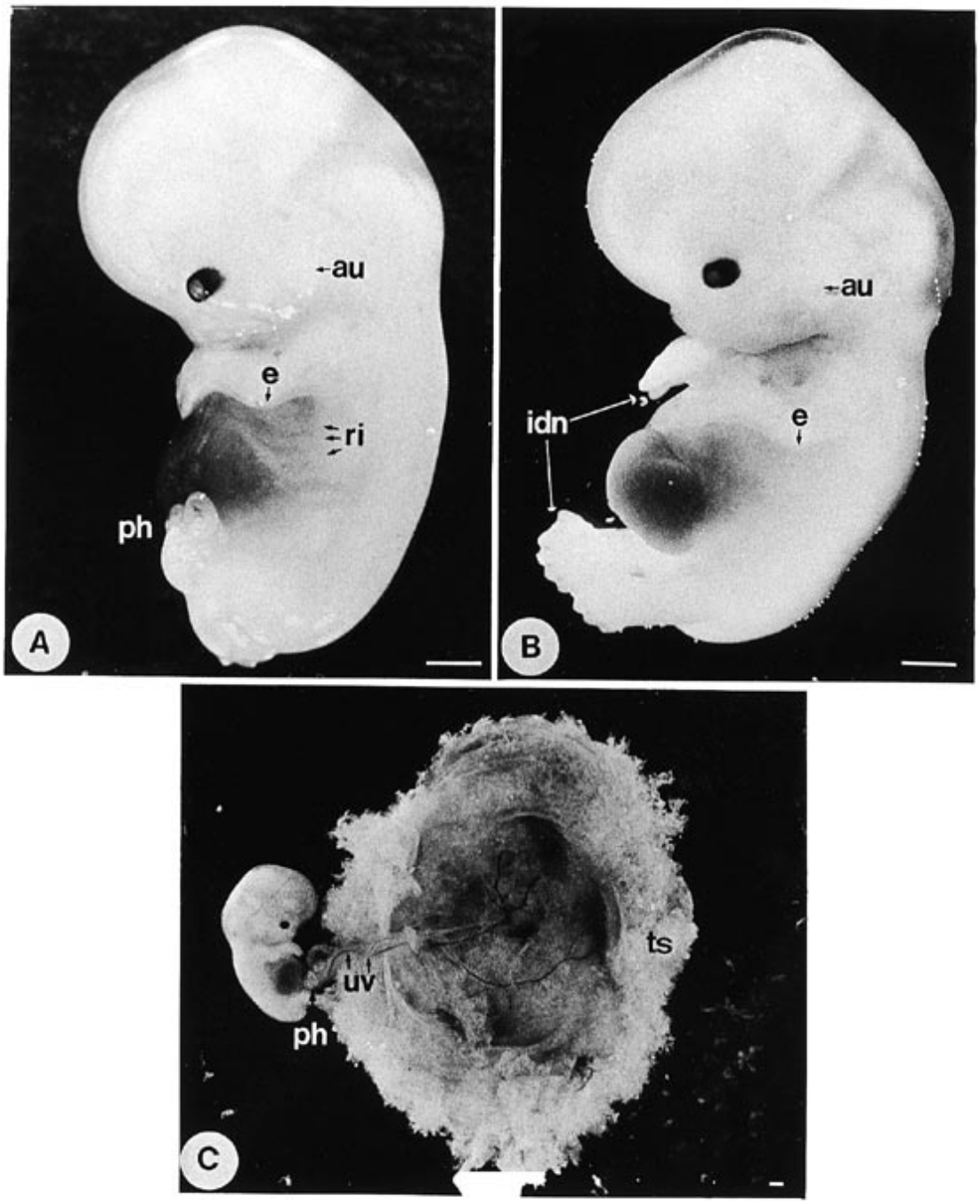

Figure 14. Photographs of three embryos belonging to stage 21. Stage 21 is characterized by the appearance of tactile pads on the hand and foot plates and the flexion of the wrists bringing the two hand plates almost to meet across the cardiac area. All photographs were taken prior to fixation, the measurement bar = 2 mm. (**A**) Ru 59; the left lateral view clearly showing the external development of the ear (au), and the elbow (e) region, the ribs (ri) are visible in the abdominal cavity, and the physiological hernia (ph) can be distinguished where the umbilical attachment would normally be found. The upper limbs are now meeting across the cardiac region and the lower limbs can be seen to be folded across each other. (**B**) Ru 200; the left lateral view showing the developing external ear (au), the elbow region (e) and the interdigital notches (idn) on both the hand and foot plates. The attachment via the umbilicus is absent showing the liver protruding through the damaged tissue. Both the upper and lower limb buds are stretched forward with the upper showing slight inclinations towards each other across the cardiac area. (**C**) Ru 310; the right lateral view showing the attachment between the embryo and the trophoblast (ts). The umbilical vein (uv) and the physiological hernia (ph) can clearly be seen in the umbilicus. The upper and lower limb buds are both stretched forward and the tail bud has almost receded.

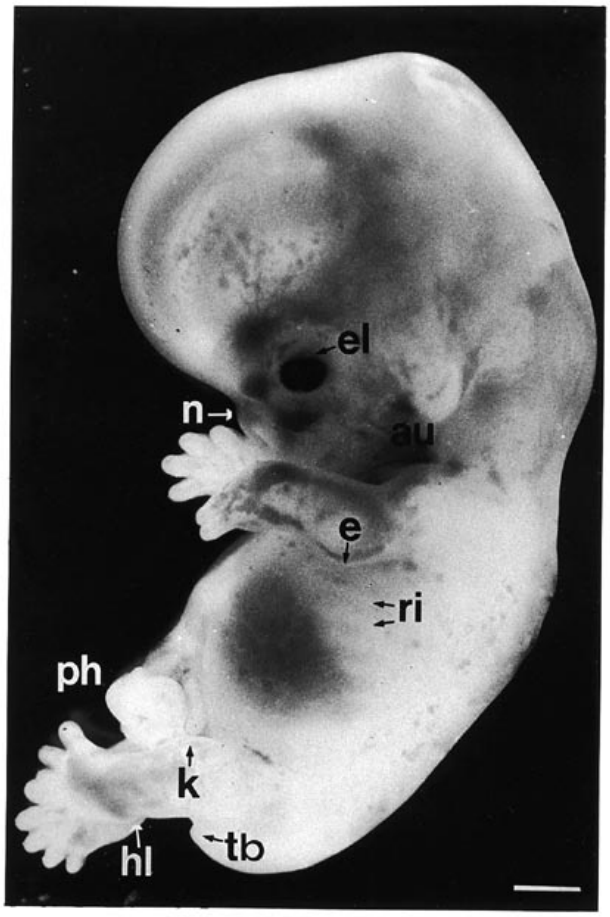

Figure 15. One photograph illustrating the left lateral view of a stage 22 embryo (Ru 135). Stage 22 is characterized by the encroachment of the eyelids across the eyes and the extension of the hands away from the body. The photograph was taken before fixation, and the measurement bar = 2 mm. The features identified on this embryo include the eyelids (el), the external form of the nose (n) with the nostrils beneath it, the external form of the ear (au), the developing elbow (e), the appearance of the ribs (ri) in the abdominal cavity, the extrusion of the physiological hernia (ph) into the umbilical attachment (which is missing), the development of the knees (k) and heels (hl) on the lower limb bud, and the regression of the tail bud (tb).

mentioned by the Institute for this stage were found: 15/18 showed a straighter axis of the trunk; 1/18 showed the development of the knees; in 1/18, eyelids were seen to be encroaching across the eyes which were very opaque; in 4/18, the heels could be identified; and in 2/18, the faint outline of joints and nails could be seen in the digits of the hand plates.

Stage 22

This stage is characterized by a CRL of 25–27 mm and a post-ovulation age of 54 days; the development of the embryo has progressed so that the eyelids are rapidly encroaching onto the eyes; the tragus and antitragus of the auricle have a more definite form; the hands extend further from the body and the fingers may, on occasion, overlap each other.

Summary

At this stage (Figure 15), eight embryos were found to have the correct criteria established by the Carnegie Institute. The mean CRL was 22.75 ± 0.98 mm, with a range of 18–26 mm. In 2/8 of the specimens, the CRL was found to be 25–27 mm, the sizes given by the Institute that the majority of previous specimens were between. For the rest of the specimens allocated to this stage, 6/8 had a CRL of <25 mm. The menstrual age was 64.0 ± 5.0 days. The weight recorded for this stage was 1.3462 ± 0.13 g with a minimum weight of 0.8048 g, and maximum weight of 1.9224 g. Developmentally, 7/8 had progressed so far that the eyelids rapidly encroached onto the eyes; in 4/8, the tragus and antitragus had a more definite form; in all of the embryos the hands were extending forward, with 4/8, having overlapping fingers; in 4/8 specimens, heels had developed at the edge of the foot plates; and in 4/8 the digits on the foot plates were completely separate; in 2/8, the external genitalia were well formed; in 2/8, the chin took on a more defined form; in 1/8, the nose was more defined than had been previously seen; and in 3/8 there was an increase in the length of the limbs.

Stage 23

This stage is characterized by a CRL (full range) of 23–32 mm, although the majority of specimens fall between 28–32 mm; and a post-ovulation age of 56–57 days. By the end of this stage, the head has made rapid progress in its path towards an erect position; it is distinctly rounded out, and the cervical region and trunk are of a more mature shape; the eyes are largely open, but in some specimens the eyelids may show some fusion; the limbs have increased markedly in length and show an overall more advanced state in the differentiation of their subdivision; with, also, the forearm ascending to or above the level of the shoulder. The superficial external genitalia are well developed but do not suffice for the determination of sex.

Summary

In this stage, nine embryos were found to have the correct criteria as defined by the Carnegie Institute. The mean CRL was 23.67 ± 0.8 mm, with a range of 10–26 mm. None of the specimens were found to have a CRL between 25–27 mm, the size given by the Institute that the majority of previous specimens were between; all of the specimens allocated to this stage had a CRL of <25 mm. The menstrual age was 63.9 ± 1.1 days. The weight for this

stage was 1.477 ± 0.18 g with a minimum weight of 0.5292 g, and maximum weight of 2.278 g. Developmentally, 5/9 had progressed so that the eyelids were rapidly encroaching onto the eyes and in 4/9 the eyelids had fused; in all of the embryos there was an increase in the length of the limbs; in 2/9, the foot plates met and in 4/9, the hands were overlapping each other; in 2/9, the hands had moved away from the front of the body; in 1/9 the arms were wrapped around the embryo; in 6/9 the edges of the foot plates were developing into heels; in 6/9 the head was more erect and in 5/9 the axis of the trunk was more mature. In a few samples, the faint outline of joints on the digits of the upper limb buds were noted, the neck area was developing, and the chin and faint outline of nails were found.

Discussion

The embryonic period is identified as the third to eighth week of pregnancy, or, developmentally, from stage 9 to stage 23. This is the period during which the three germ layers give rise to their own tissues and organ systems, and therefore organ formation becomes a major feature. The embryo develops from a flattened disc, through the formation of the neural tube and somites, the closure of the neural tube through the rostral and caudal neuropores, the appearance of the pharyngeal arches, and otic and optic vesicles, the development of the umbilical vesicle, the appearance of the limb buds and their subsequent differentiation, occurring in conjunction with the overall lengthening and maturation of the body form.

In this comparative study it has been shown that although the majority of parameters established by Streeter (1942, 1945, 1948, 1951), Heuser and Corner (1957) and subsequently O'Rahilly and Müller (1987, 1993), were the same, they can only be used as a guide to developmental embryology. In the present study, other features were noted which may help to identify the stages for different embryos. At stage 10, it was noted that the neural groove did not start to close until between the formation of the sixth and seventh somites. In stage 11, the rostral neuropore is reputed to be closing or near closing depending on the number of somites present. Although O'Rahilly and Müller (1987) stated that at ≥19 somites the neuropore had usually closed, in this study it was found closed in 21.4% of specimens and the number of somites present were not a determining factor as to how far the closure had progressed. In support of this, only 53.6% of specimens were found to have a closed rostral neuropore at stage 12, when, according to previous reports (O'Rahilly and Müller, 1987), the rostral neuropore is closed. The caudal neuropore should, according to previously reported data (O'Rahilly and Müller, 1987), also be closed by the end of the stage; however findings from the current study indicated that only 53.6% of the specimens had a closed caudal neuropore by the end of stage 12.

The CRLs recorded in the present study at stage 12 differed from the results reported by O'Rahilly and Müller (1987). Those reported by O'Rahilly and Müller ranged from 3–5 mm for fixed tissue; the measurements from this study ranged from 2.5–7 mm. Some of the differences noted in the range could be due to the degree of lordosis in the embryos or to shrinkage of the tissue post-fixation. Again, at stage 13, there was a difference between the previously recorded CRLs (range 4–6 mm) and those found in the current study (4.2–8 mm). In the present study 69.7% of the CRLs fell within previous estimates for this age group and 30% had a greater CRL than the estimates reported by O'Rahilly and Müller (1987). As noted in previous studies (O'Rahilly and Müller, 1987), not all of the specimens from stage 13 showed upper limb bud development, and in only 33.3% were lower limb buds seen. Up to this stage, the groups were mainly identified by the number of paired somites.

For the primary identification of the remaining stages, more than one parameter was used, but classification was generally given according to the development of the limb buds. At stage 14, again the CRLs differed, with a general trend towards a greater length (prefixation) in comparison with previously published data (O'Rahilly and Müller, 1987; Dickey and Gasser, 1993; Dickey *et al.*, 1994) post-fixation. All of the embryos at stage 14 had a definite upper limb bud with 69.2% of them showing elongation and the other 30.8% still paddle shaped. The mandibular and hyoid arches were not always conspicuous.

The majority of the criteria described by O'Rahilly and Müller (1987) were met by the embryos that had been categorized for stage 15. At stage 16 the CRL range was found to be shorter than previously reported by O'Rahilly and Müller; but whether this was due to the degree of lordosis or an actual difference is difficult to determine. In 39.3% of the specimens the nasal pits were turned to face ventrally, whereas the rest still showed the pits laterally. The retinal pigment, usually visible at the end of this stage, (Arey, 1942; Hamilton, *et al.*, 1966; Sadler, 1990; O'Rahilly and Müller, 1987) was evident in all but two specimens.

At stage 17 there was only one difference noted between previously reported characteristics and the findings reported in the current study, which was that the movement of the nasal pits seemed to be slightly slower than expected (O'Rahilly and Müller, 1987). At stage 18 the CRL measurements in the current study were greater on both

sides than the previously given CRL, and no evidence was found to support the idea of a more uniform shape, nor was any eyelid development noted, although both of these had been reported previously (O'Rahilly and Müller, 1987). The CRLs of those specimens from the current study allocated to stage 19 were in general smaller than those previously reported. The degree of lordosis is unlikely to be the reason for the apparent decrease in size, so it can be assumed that the embryos were indeed smaller than those previously reported. Again at stage 20 a general trend towards smaller CRLs was found in the present study. In addition to the criteria already established as parameters of development at stage 20, the movement of the eyes from a lateral position towards a more ventral one was found to be well established in 95.8% of specimens, and in 91.3% the digits on the upper limb bud were found to have completely separated.

At stage 21 the range for the CRLs noted in the current study was greater than those previously communicated (O'Rahilly and Müller, 1987), but the CRLs seemed to be evenly distributed throughout the range. Also at this stage, the majority (88.9%) of the embryos showed the outline of developing ribs in the abdominal cavity; several additional characteristics were noted: the development of the heels on the outer edge of the foot plate; the slight bend of the legs at the knees; the faint outline of joints and nails on the digits of the upper limb buds; and the eyelids starting to encroach across the eyes.

Stage 22 showed a decrease in the range of CRLs with only 25% lying within the range previously reported (O'Rahilly and Müller, 1987); the remainder of the CRLs measured in the present study had a smaller length. At stage 23 the CRLs in the current study were, again, smaller than those previously reported. More developmental features were noted at this stage, but since only nine specimens were found and the developmental range of parameters across the group was broad it was difficult to describe the characteristics of this stage.

The conclusions from this study are that there are different degrees of embryonic development not only between groups, but within each group. When taking all parameters into account, it cannot be said that there is a definite division between one stage and another; instead, there seems to be an area of development at each stage that could be classified into two groups. Menstrual age proved to be too variable to identify the developmental age with any accuracy and without large discrepancies occurring at all stages. The CRL in latter stages of development proved to be too erratic to be regarded as anything more than a guide for categorizing the developmental stages. O'Rahilly and Müller (1987, 1993) and Nishimura *et al.* (1968, 1974) have previously shown that early human embryos of markedly different sizes could be at the same developmental stage, but no indication was given as to whether the size differences were due to dissimilar growth rates or because the age of the embryo was unknown. Dickey and Gasser (1993) demonstrated that identically aged embryos could differ in embryonic length by up to two fold. They also demonstrated that embryos of an identical age could differ by as much as 5 days in time according to the onset of cardiac activity. Dickey and Gasser's (1993) results came from studies involving ultrasound scanning of patients undergoing IVF or GIFT. The current study has provided evidence that each individual embryo has a different time scale for the development of different features. In providing definitive stages that are rigidly bound by developmental events, limitations are placed on categorizing the embryo. Although the allocation of embryos to a specific stage can assist in identifying the post-ovulatory age, the overlaps between stages could lead to classification of the embryo into a wrong age group or stage.

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