Human development

Chromosome anomalies in human preimplantation embryos
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Information on the frequency of chromosome abnormalities in spontaneous abortions and in the live born population of man show that about 9% of all clinically recognised pregnancies carry a chromosome abnormality. No data is available on the frequency of chromosome anomalies in the preimplantation stage in man although it is recognised that about 40% of in vivo fertilized embryos are lost at this stage of pregnancy.

In vitro fertilization procedures have opened the way to examining the chromosomes of preimplantation embryos. From preliminary data in 10 cleaving embryos, seven have had a normal chromosome constitution. Of the three chromosomally abnormal, two i.e. 22, X, –15 and 45,XY, –15 have not been recorded in surveys of spontaneous abortions, and although viable in the early cleavage stages, are both presumably lethal beyond the preimplantation stage.

Polypeptide synthesis in human preimplantation embryos fertilised in vitro
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Human oocytes for in vitro fertilisation were donated by women undergoing laparoscopic sterilisation. Stimulation of ovulation was achieved with clomiphene (150 mg, days 2–6 or 5–9), and final oocyte maturation prompted by 5000 i.u. hCG administered 34–36 h before oocyte retrieval. Unfertilized oocytes and cleaving embryos were labelled for three hours in medium containing high specific activity ^35^S methionine, and the polypeptides synthesised were resolved by one dimensional SDS polyacrylamide gel electrophoresis. The polypeptide synthetic profiles of developing embryos, between the 1-cell and blastocyst stages, and of ageing unfertilised oocytes of the same chronological age, were compared. Although many of the polypeptides synthesised are common to developing embryos and ageing unfertilised oocytes, a number of changes occur soon after fertilisation, and several others during the ensuing cleavage divisions. Comparison of these polypeptide patterns, with those from embryos in which cleavage was retarded or arrested, has provided information which may contribute to an understanding of early human embryonic failure, and in particular the relatively low rate of successful pregnancies following therapeutic in vitro fertilisation and embryo replacement.
Knowledge is accruing on several aspects of embryonic growth, e.g. on the nature of culture media, cleavage, compaction and blastulation. Chromosomal and ultrastructural studies have been carried out on embryos. Many factors associated with follicular development influence preimplantation growth in vitro, e.g. the various forms of ovarian stimulation and the number of follicles maturing. Endometrial growth and post-implantation development in the mother could be influenced by levels of ovarian steroids in the follicular and luteal phases, and the age of the mother.

Embryonic growth in vitro can also be assessed by continued growth after replacement in the mother, e.g. the incidence and cause of abortion, the rate of identical twinning, and the effects of multireplacement on establishing pregnancy and multipregnancy. Other new developments on embryos developing in vitro will be assessed including cryopreservation, 'hatching' from the zona pellucida and embryonic metabolism.

Isolated ventricular inversion in a 4 mm human embryo

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A study was undertaken of the heart in embryos AS-1, measuring 4 mm long and aged 28 days, corresponding to O'Rahilly's stage 13, property of the Embryo Collection of the Federico Olóriz Institute, Department of Humano Anatomy, School of Medicine, University of Granada. The heart of this embryo presented ventricular inversion as a consequence of an alteration of the cardiac loop, which in turn was provoked by an L-loop type malrotation of the bulboventricular loop.

This type of anomaly, curiously, has not been previously described in the literature in terms of abnormal conformation of the heart loop in the early stages of human cardiac development, although the existence of such anomalies had been presumed in morphological studies of congenital malformations of the heart and great vessels carried out by R. Van Praagh and S. Van Praagh (1966). According to these authors, ventricular inversion without transposition of the great vessels is a rare congenital malformation which has never been correctly diagnosed in living subjects.

According to Nakamura et al., (1980) this kind of alteration in the conformation of the heart loop is simply the consequence of abnormal orientation of the linear elements which arrange themselves within Davis' cardiac jelly, and abnormal cyto-differentiation of the myocardium. However, it is possible that these abnormal developments could be induced by some teratogen which would produce its effects before the 20th day of gestation.
Placental transfer of zinc and its influence on foetal growth in man

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The zinc levels of serum and red cells of sixty mothers and their normal-delivered newborns (cord blood) and the placental tissues were determined by atomic absorption spectrophotometry in order to elucidate the mechanism of placental transfer of zinc and its influence on birth-weight in man.

Mean levels of zinc in maternal serum and red cells were found to be 1.01 ± 0.29 μg/ml and 11.76 ± 4.61 μg/g and those in foetal serum and red cells were 1.23 ± 0.24 μg/ml and 3.33 ± 0.72 μg/g, respectively. The placental content of zinc was 12.00 ± 2.24 μg/g. Spearman's rank correlations were computed between zinc level of each of the above tissues and weights of the newborns according to sex (female = 32; male = 28) and gravida. There was no significant correlation between zinc content of different tissues for the entire group. However, in the primigravida (n = 22) there was a significant positive correlation (r = 0.48; p ≤ 0.05) between zinc levels of foetal serum and foetal cells and a negative correlation (r = −0.40; p = 0.05) between placental and maternal red cellular zinc levels. High degree of correlation was observed between the total zinc content of placenta with birth-weights of the newborn of either sex and all the gravida (r = 0.49 to 0.65; p ≤ 0.5 to 0.01).

The results show that the placenta actively transfers zinc to foetus against a concentration gradient and stores an average of 5–6 mg of zinc at term. Foetal red cells contain much less zinc compared to that in mother's red cells. Probably the zinc transport mechanism in the red cells is not fully developed at birth. Furthermore, the zinc content of placenta appears to promote the foetal growth.
Human development

Chromosome abnormalities in early human embryos

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It is estimated that about 75% of all human conceptions are lost. The most common cause of early foetal loss is chromosome anomalies, which are found in about 60% of clinically recognized first-trimester spontaneous abortions. The principal mechanism leading to chromosomal in the foetus is meiotic non-disjunction, which occurs more frequently in females than in males. Advanced maternal age seems at present to be the most well-documented factor predisposing to non-disjunction. It is demonstrated how chromosome studies of early human embryos conceived in vivo as well as in vitro can provide information about the mechanisms leading to chromosome anomalies in the very early products of human conception.

Early dental development in the mandibular arch

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The early development of deciduous teeth was studied in eight embryos in O'Rahilly's stages 20–23 and seven fetuses between 16 weeks of gestation in the human.

After carrying out detailed embryological studies, the following conclusions were reached:

1. Decidual teeth germs do not all appear simultaneously, rather, the second temporary molar appears a posteriori with respect to the others, specifically in the final days of the third month of development.

2. The process of invagination and invasion of the dental plate into the underlying mesoblast is not uniform in all places. At the level of the incisors it begins horizontally and spreads toward the midline, while at the level of the canines and molars it occurs vertically.

3. Double pedicles are nearly always present at the level of the canines.

4. Involution of the dental crest commences in the second half of the third month of development, at which moment Serre's epithelial pearls appear.
The dental crest: a differentiating agent in the mandibular corpus

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The dental crest along with initiation of mandibular osteogenesis were studied in eight embryos and seven fetuses in the human. The following conclusions were reached:

1. Meckel's cartilage is the guiding element in the mandible. Nevertheless, the dental crest acts as a differentiating agent which justifies the different form and structure of the mandibular arch. The primary dental band begins to protrude in the area around the future mentonian orifice, and thus represents the first osteogenic manifestation of the mandible which suggest a differentiating role for the dental crest following the phenomenon of conjunctive ossification in that zone of the mandibular arch. This zone in time gives rise to the body of the mandible, thanks additionally to the previous appearance in the same area of Meckel's cartilage, extending from the symphisis to the otic capsule.

2. Meckel's cartilage, through ossification of its ventral-most portion, contributes not only to the organization of the future dental process, but also to Spix's spine.

3. We have confirmed that the osteogenic process in the mandible is mixed, as some parts are organized by direct ossification while others are formed by endochondral ossification.

The human mandible: a biometric study of growth

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An orthopantomographic study was carried out in a total of 100 mandibles, 18 adult subjects and 87 children ranging in age from 3 to 15 years. Curved surface laminography was used with panoramic GE-Panelipse X-ray machine.

A series of measurements were taken in each mandible which were later subjected to regression analysis with repeat observations for each value on the abscissa. After analysing male and female mandibles separately, the two responses were compared using classical statistical methods, with the following results:

1. Evolution of the indices studied is almost always linear, indicating that the increase in the indices between one year and the next is the same for all ages studied.

2. Males generally reach a specific index value at a younger age than females.

3. To sum up: No peaks or valleys appear in the development of the indices, which increase at similar rates in males and females, although male curves are always above the corresponding female curves.
Human development

Analytical morphometry of differences between infantile and adult fronto-facial profiles in *Australopithecus africanus* and modern *Homo sapiens*

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A study of allometric transformations of sagittal fronto-facial profile during post-natal growth in *Australopithecus africanus* and modern man was carried out by means of Kth order polynomial equations (least squares method) and of Fourier harmonic analysis. Bonds of such a comparison are represented by the characteristics of the chosen holotype for adult *Australopithecus africanus* (*Plesianthropus transvaalensis*, STS5, female) and for relative child (Taung skull, Taung 1,4–5 years old). The lack of the bony vault parts of the latter allowing to perform analytical evaluations only for the lower trait of the frontal bone and for the face. Equivalent segments were selected for *Plesianthropus transvaalensis* and Modern adult female and infantile subjects in a situation of normalization and standardization as requested for the use of the original SAM (Shape Analytical Morphometry) software package. Morphological distances were estimated in terms of cross punctual error and the weight of Fourier contributors in differential morphogenetic trends was also evaluated.

Influence of maternal and fetal genotypes on fetal development

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The influence of maternal and fetal blood groups on birthweight and length was studied in a group of 122 singleton births of both sexes. The blood groups were determined by using eleven commercial antisera (ABO system – A, A1, B; Rhesus system – D, C, E, c, e; MN system – M, N and Lewis system – Le}). Birthweight and length were corrected for sex difference and the corrected values were used for comparison of different genotypes. No significant influence of ABO, MN and Lewis system of mother or fetus was observed on birthweight and length. The mothers with *R1R2* genotype gave birth to significantly lighter (*P < 0.025*) babies (2775 ± 329 g) compared to those delivered by the mothers with the genotypes *R1R1* (3133 ± 431 g) and *R2R2* (3295 ± 260 g). The length of the babies was not influenced by the Rhesus blood types of the mother or fetus. The significance of the above association of the Rhesus genotypes and birthweight is not clear at present.
Human development

Study of the origin of the human thyroid gland

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In a study of the embryogenesis of the human thyroid gland it was confirmed that this gland arises from three anlagen: a single medial primordium tiroideum, and the paired lateral thyroid and ultimobranchial bodies.

The lateral thyroid anlagen are derived from the ventral margin of the pharyngeal pouch IV. In O'Rahilly's stage 16, these anlagen become distinct separate entities and begin their caudoventral migration. In O'Rahilly's stage 17 they approach the medial thyroid. In O'Rahilly's stages 18 and 19 they establish contact with the medial thyroid. In O'Rahilly's stages 20–30 the lateral thyroid anlagen are completely absorbed by the thyroid mass and acquire their definitive position in the area of the visceral aspect of the thyroid lobes.

The ultimobranchial bodies develop individually as morphological entities independent from the pharyngeal pouches IV, and are colonized during O'Rahilly's stage 14 by neuroectodermal cells. During O'Rahilly's stage 18 they are incorporated into the definitive thyroid gland.

Development of the submandibular and parotid gland capsules in the human embryos

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Twenty human embryos from 12 to 28.5 mm, have been studied in a series of microscopic slides, stained with haematoxylin-eosin. The gland capsules arise from the condensated mesenchyme in front and around the epithelial primordia. We believe that the growth of the mesenchyme and the development of the glandular epithelia are interdependent. The condensation around the epithelial primordia occurs in embryos of 12 to 13 mm – quite an early stage. The mesenchymal mass that is influenced by the glandular development is clearly different from any other that is in the neighbourhood. We have observed that mesenchymal maturation and differentiation forms a bed of glands that induces glandular growth into it. This fact is evident in 16 mm embryos with reference to the submandibular gland. In our slides we observe previous to the mesenchymal condensation, a limited clear zone with a vacuolar aspect, that surrounds the glandular primordium and which is in the site where the conjuctive capsule will be located. We believe that this is the initial phase of the capsular differentiation. We consider this differentiation is divided in two different and interconnected phases. The first one would be degeneration of mesenchymal tissue in contact with the epithelial primordium. During the second, cellular masses form from the perienvironmental mesenchyme in the sheltering of the clear zone. Then the maturated mesenchymal cells join closely with collagen fibres to initiate the process of capsular differentiation. In the embryos that we have studied, the development of the capsule of the submandibular gland is clearly more prevalent than that of the capsule of the parotid gland.
Human development

Synthesis and localization of apolipoproteins and other embryonic serum proteins in the early human embryo
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Synthesis and localization of apolipoproteins and other secretory proteins were studied in human embryos between six and ten weeks after fertilization. Newly synthesized proteins in culture supernatants of isolated embryonic tissues were labelled with $^{35}$S-methionine and immunoprecipitated respectively with antibodies directed against human apolipoprotein A1 (Apo A1), apolipoprotein B (Apo B), low density lipoprotein (LDL), transferrin and α-fetoprotein and then characterized by SDS-polyacrylamide slab gel electrophoresis and autoradiography. The results indicate that yolk sac, embryonic liver and gut are able to synthesize apolipoproteins, transferrin and α-fetoprotein. There was no evidence for the synthesis of these serum proteins by other embryonic tissues (stomach, trophoblast, adrenal, kidney and brain). In the yolk sac, three radioactively labelled species corresponding in molecular weight to 28K, 47K and over 200K Daltons were immunoprecipitated by antibodies to Apo A1 and LDL. The size of these three proteins corresponds with the known molecular weights of Apo A1, Apo AIV and Apo B, respectively. Liver was also shown to be express Apo A1 and Apo B with anti-Apo A1 and anti-LDL antibodies; but no Apo AIV was found to be co-precipitated. In addition, transferrin and α-fetoprotein were two other predominant proteins synthesized by yolk sac and liver. However, synthesis of Apo A1, transferrin and α-fetoprotein could be detected in only a few embryonic gut samples. The data suggest that the secretion of these proteins by the gut is variable during first trimester of human development. We conclude that the yolk sac and liver are primary source of apolipoproteins, transferrin and α-fetoprotein during early development of human embryo, and we also describe the localization of these serum proteins in the yolk sac.

Inductive properties of fetal human digestive mesenchyme on epithelial cytodifferentiation
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In the course of studies centered on the ontogenesis of brush border enzymes and on mechanisms underlying ontogeny of the gut, we have studied the role of epithelial-mesenchymal interactions. In a previous work we have shown that chick intestinal mesenchyme, although originating from a species which expresses sucrase activity during early development, was unable to promote the precocious appearance of sucrase in the rat endoderm (Kedinger et al. 1981, Develop. Biol. 86, 339^-347). The aim of the present study was to test the inductive properties of human mesenchymal cells on animal gut endoderm. Small intestine and stomach anlagen were taken from 8 to 10 week-old human fetuses. The presumptive small intestine was dissected out from either 54 day-old chick embryos or 14 day-old rat fetuses. Separation of mesenchyme from endoderm was achieved mechanically after a 0.03 % collagenase treatment. Due to the small quantities of human material available, primary cell cultures of intestinal and gastric mesenchymal cells were performed and used successively until 4 to 5 passages.

In a first type of experiments, human gut mesenchymal cell sheets were associated to intestinal rat endoderm; the associations were grafted for 12 days into the coelomic cavity of 3 days-chick embryos. They gave rise to well-vascularized gut segments in which the epithelium carried characteristic rat features. At the ultrastructural level, epithelial cells exhibited enhanced apical brush borders in comparison to those present in the rat endoderm reassociated with its own mesenchyme. Moreover the isolated and purified brush border membranes expressed induced-sucrase activity (16.6 ± 4.1 mU/mg prot., n = 16).

In a second set of experiments, human gastric mesenchymal cells were associated to chick intestinal endoderm and grafted in ovo. In these conditions, the mesenchymal cells induced a partial conversion of chick intestinal endoderm into gizzard-like structures (12 out of 19 cases).

In conclusion, these results indicate for the first time that human fetal digestive mesenchymal cells display inductive properties not expressed by homologous animal cell types.
First results from a human \textit{in vitro} fertilization (IVF) program in Berlin

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In 1983 an IVF-program was started as a collaboration of the laboratory for reproductive toxicology at the BGA and the facility clinic of the gynecology department. Only couples that had been intensively diagnosed for their fertility problems were accepted to the IVF-program. The diagnostic procedures also included a laparoscopy to check for motility of the uterine tubes and for the passage of sperm after artificial insemination. IVF was attempted if either the uterine tubes were blocked or if the sperm count showed a reduced motility or oligozoospermia.

The women in the IVF-program were stimulated with the hormones Clomiphene and LH to produce 2–3 mature oocytes. Follicle growth was monitored by ultrasound. The methods for obtaining the oocytes by laparoscopy, IVF, embryoculture and embryotransfer were similar to the methods described by Edwards and Steptoe (England) and by Lopata and Trownsen (Australia). The medium for the puncture of the follicles, for IVF and for the culture and transfer of the embryos to the 4-cell stage was HAM's F-10 supplemented with 10 % or 20 % human cord sera.

So far 70 IVFs have been attempted and 48 h later 30 embryo transfers could be performed, from which several 'biochemical pregnancies' and four clinical pregnancies have resulted. One of the pregnancies ended as an abortion in the second month and two tubal pregnancies had to be terminated surgically. There is one ongoing pregnancy and the expected date of birth is June 18th 1984. In contrast to other groups, hormonal stimulation in this program was very moderate so that no more than 2–3 mature oocytes were obtained during every puncture.

IVF was unsuccessful when the quality of sperm was reduced, e.g. low sperm count and low sperm motility. Chromosomal analysis of the oocytes from unsuccessful IVF attempts proved that 90 % of the oocytes had successfully maturated and reached prophase/metaphase II \textit{in vitro}. Triploidy was observed twice in zygotes from the same couple.

Establishment of primate trophoblast cell lines from marmoset embryos

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Primate trophoblast cell lines were established from marmoset preimplantation embryos (16–29 cell to blastocysts) cultured in Ham's F10+20 % F.C.S. in 35 mm plastic culture dishes in a humified atmosphere of 5 % CO$_2$ in air. Expanded blastocysts became attached to the dish 1–4 days after hatching from the zona pellucida and 1–3 days later, a trophoblast outgrowth was visible. Following attachment, there was development of visceral endoderm and inner cell mass derivatives but after 4–7 days the blastocyst collapsed and the inner cell mass derivatives degenerated. This left a monolayer composed essentially of trophoblast cells which after further culture for a variable period (1–30 days) was divided into 2–8 pieces and propagated into fresh culture dishes. The monolayer at the time of propagation was 1.5–3 mm in diameter and composed of two cell types a) a peripheral ring of large, often multinucleated cells (syncytiotrophoblast) b) a central sheet of small cells (cytotrophoblast). In some cases, the small cells had formed a vesicle up to 3 mm in diameter at the time of propagation. Propagated tissue quickly attached and formed a trophoblast outgrowth of large cells and some formed vesicles. The presence of significant numbers of cytotrophoblast cells was essential for the establishment of the cell lines as foci composed principally of syncytiotrophoblast failed to grow and degenerated after several weeks in culture. Successful cell lines were characterised by multi-vesicle formation and were propagated by cutting 2–4 mm diameter vesicles into 12–18 pieces and culturing in fresh culture dishes. They could not be propagated by trypsinization of vesicles into single cell suspensions. Cell lines of identical morphology were established from 3 of 13 embryos; one was maintained \textit{in vitro} for an excess of 16 months. The established cell lines secreted abundant quantities of chorionic gonadotrophin and tissue transferred to the marmoset uterus was capable of prolonging the luteal phase. Small pieces of tissue induced a marked decidual reaction in pseudopregnant mice.
Human development
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Ovulation and fertilization of the human oocyte
No abstract for publication

Analysis of human sperm chromosomes and factors involved in their formation in a heterospecific \textit{in vitro} environment
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In most vertebrate sperm, the DNA/protamine complex is packaged into a condensed biochemically inert form of chromatin and has ensured that direct examination of sperm chromosomes remains difficult. This problem may be tackled in a number of ways. However, the method based upon \textit{in vitro} fertilization of zona-free hamster eggs has remained the only viable one to date. After a number of modifications, our long term experience of this technique for a small group of donors has indicated a mean penetration range of 18–86 \%, while actual values have spanned the limits of 0–100 \%. The mean efficiency of analysable chromosome preparations was 15·4 \% with a range of 0–24 \%. Allowing for fixation failures this data suggests that many decondensed sperm heads/pronuclei arrest at an early stage. Attempts to increase the level of efficiency by exposing eggs to a number of polyamine putative chromosome condensing factors during serum-free Hams F10 culture will be discussed.

When fixation is optimal, chromosome morphology is well defined, though centromeres often appear stretched. Extensive banding experiments have indicated that pronuclear chromosomes do not reliably respond to any of the traditional G-banding agents or regimes employing progressive staining with dilute dyes, more suited to low cell numbers. The latter techniques merely induce some chromatid fusion and puffing with incipient bands on long arms. Selective denaturation by hydrogen peroxide in the presence of UV light seems to be the best hope of G-banding sperm chromosomes, while our routine analysis has relied on Q and R-banding. Our metaphase yield and banding success will be discussed in relation to known problems associated with analysing chromosomes from small numbers of eggs and foetal cells.

Experiments to visualize human sperm chromosomes in a factorised cell-free in vitro system will be described.
Human development

Human placental calcium-binding protein and calcium transport

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We have previously identified in the human placenta a high-molecular-weight calcium-binding protein (HCaBP) which is expressed as a function of fetal development in a manner concomitant with the accumulation of calcium by the developing embryo (Tuan, R. 1982. Placenta 3, 145–158). In this report, we have further studied the HCaBP with respect to its biochemical properties, tissue and cellular distribution, and possible involvement in placental calcium transport. Optimal calcium binding by the HCaBP occurs at pH 7–8 and in 100 mM Na\(^+\) and 3 mM Ca\(^{2+}\). The HCaBP possesses at least 10 calcium-binding sites with a \(K_d\) of \(5 \times 10^{-6}\) M [Ca\(^{2+}\)]. Highly specific rabbit-derived anti-HCaBP antibodies were used for HCaBP immunoquantitation and immunohistochemistry which revealed that the HCaBP is localized in the chorionic villi and is primarily associated with the trophoblastic cells. Placental calcium uptake was studied in vitro using preparations of cell-free, microsomal membranes isolated from term placentae. Microsomal calcium uptake was found to be an active process coupled to ATP hydrolysis. In this system, pre-incubation of microsomal membranes with anti-HCaBP antibodies significantly inhibited calcium uptake, suggesting that the HCaBP is functionally involved in placental calcium uptake. (Supported by USPHS-NIH, HD 15306).