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Review article

Methods for studying human organogenesis

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ABSTRACT

This review details methods for utilizing D & C suction abortus specimens as a source of human fetal organs to study the morphogenetic and molecular mechanisms of human fetal organ development. By this means it is possible to design experiments elucidating the molecular mechanisms of human fetal organ development and to compare and contrast human developmental mechanisms with that of laboratory animals. Finally human fetal organs can be grown in vivo as grafts to athymic mice, thus allowing ethical analysis of potential adverse effects of environmental toxicants.

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1. Introduction

The two most widely employed animal models for human development are the mouse and rat. On the whole, the overall organogenetic process is similar in animal models and in humans, and thus the tacit (but unproven) assumption is that mice and rats are valid models of human organogenesis from both a morphologic and molecular perspective. While this tacit assumption may be justified in many cases, there are notable exceptions with dramatic mouse/human differences in morphogenetic processes in certain organs. For example, studies demonstrate substantial differences in development of the mouse versus the human penile urethra. Most of the mouse penile urethra forms by direct canalization of the urethral plate within the genital tubercle (Hynes and Fraher, 2004; Seifert et al., 2008), whereas development of the human penile urethra involves “opening and closing zippers”, i.e., canalization of the urethral plate to form a widely open urethral groove and subsequent fusion of the urethral folds to form a tubular urethra (Li et al., 2014). Likewise, the male mouse has two prepuces, while the human has one (Blaschko et al., 2013; Cunha et al., in press). Prostatic lobar patterning is completely different in mice versus human (McNeal, 1976, 1981; Sugimura et al., 1986). Uterine morphology and thus morphogenesis is vastly different in humans versus mice and rats (Kurita and Nakamura, 2008; O'Rahilly, 1973). Whether these morphologic/morphogenetic differences in laboratory animal versus human development are due to differences in molecular mechanisms remains to be explored.

Resolution of whether laboratory animals provide valid models of human development can only come from a detailed morphogenetic and molecular examination of human organogenesis so that similarities/differences between mouse/rat versus human organogenesis can be compared, and the underlying molecular mechanisms revealed in laboratory animals versus humans. Moreover, based upon examination of textbooks of human embryology, it is evident that our understanding of human development is derived for the most part on classical histologic studies decades old (Koff, 1933; Felix, 1912; Jirasek et al., 1968; Lowsley, 1912; Hart, 1908; Jones, 1910; Johnson, 1920; Hunter, 1935; Andrews, 1951). It is timely to re-examine organogenesis in humans using modern morphological techniques. The goal of this paper is

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to demonstrate how research on developing human organs can be achieved.

The first hurdle to be addressed is authorization to obtain human fetal specimens for research purposes. Such authorization must be consistent with national and state laws as well as local (University, College, etc.) and national agency (NIH, MRC, etc.) guidelines. For all human fetal samples, informed consent must be obtained from the patient, and the most important issue is that the specimens must be obtained without patient identifiers to preserve confidentiality. Of course, the decision to terminate pregnancy is made solely by the patient in consultation with her physician and without any contact whatsoever with the investigators.

Facilities performing therapeutic abortions have their own protocol for handling specimens, which in most cases involves formalin fixation. For research purposes this is to be avoided so that fresh specimens can be obtained from which viable cells and tissues can be isolated.

Most abortions occur via the D&C suction procedure in which the embryo/fetus is completely disrupted. Many assume that it is difficult (if not impossible) to find human fetal organs from such specimens, which contain placental villi, endometrial tissue as well as fetal tissues. We consistently find a multitude of human fetal organs in each D&C specimen. Success requires basic anatomical knowledge and experience for what human fetal organs look like at various stages of development. Knowledge of adult human anatomy is extremely useful, since developing human fetal organs will resemble their adult counterparts at some point in development. Knowledge of the anatomy of fetal mouse/rat organ development is also useful in recognizing what an undifferentiated organ looks like. The trick is to be able to recognize and isolate developing human organs from the specimen containing clotted blood, placental villi, endometrial tissue as well as fetal parts. The goal of this paper is to demonstrate how this can be achieved.

2. Materials and methods and results

For safety, it is imperative that gloves are used by the investigator in handling abortus specimens. The D&C suction procedure is used for pregnancies in the ~6 to ~20 week age range, with most falling into the 9–14 week group. During the surgical procedure the specimen is collected in a cloth "sock", which is emptied into a 9 by 9 in. Pyrex dish containing PBS or culture medium. Forceps are used to "spread out" the specimen thus breaking up clumps of tissue. A magnification aid is useful once the specimen is fully dispersed into individual pieces. Placental villi have a characteristic morphology (Fig. 1) and can be discarded. Likewise, endometrial tissue (Fig. 1) will be recognized as intact amorphous sheets and discarded. Endometrial and placental tissue have a fleshy appearance, are pink in color and have ragged edges. In contrast, many human fetal organs (depending of the organ) will have smooth edges (see Figs. 2–5), a distinctive morphology and typically are light in color (an exception being the liver and kidney which are red). The following account describes the isolation of human fetal male and female internal and external genitalia, which are perhaps the most difficult to isolate.

The first "organ" to identify and isolate is an intact foot, from which heal–toe length will be determined and used to estimate age of the specimen (Taguchi et al., 1983; Drey et al., 2005). Information on "last menstrual period" or clinical staging of gestational age is usually unreliable. Gender is determined by reproductive tract gross appearance (see Figs. 4 and 5), and verified by PCR (Cui et al., 1994).

External genitalia are typically attached to a leg or to the pelvis and are easily identified and dissected (Fig. 2). Fig. 3 presents a series of photographs of male and female external genitalia at various gestation ages. Note that size and morphology of the external genitalia cannot be used for determination of sex as male and female external genitalia are remarkably similar until very late stages.

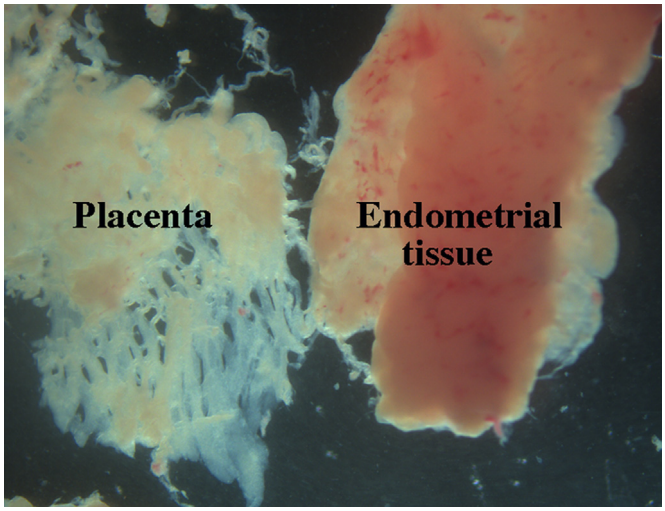

Fig. 1. Photos of human placental villi and endometrial tissue. Note their characteristic morphology.

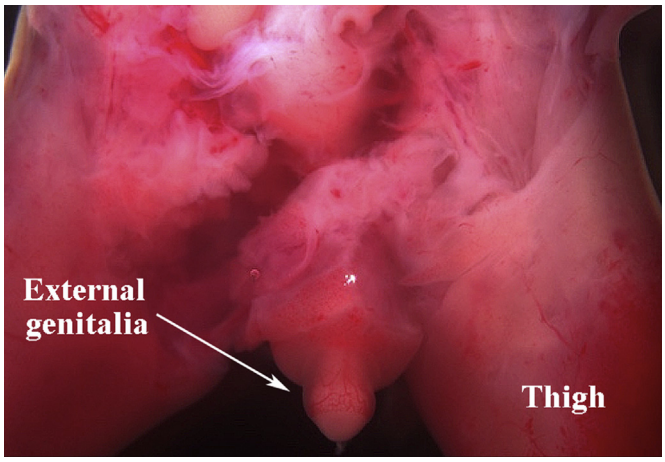

Fig. 2. A rare specimen (14 weeks of gestation) in which the legs are still attached to the pelvis. In this male specimen the external genitalia are easily identified and dissected (see prostate from same specimen in Fig. 4).

Male and female internal genitalia are more difficult to find. One method is to isolate all tissue pieces that are light in color (large and small). Place these in a Petri dish with PBS or tissue culture medium for subsequent viewing with a dissecting microscope having capability of viewing with reflected light from above as well as transmitted light from below the specimen stage. The fetal pelvis frequently remains intact during the surgical procedure. Internal genitalia may be present within the pelvis, in which case knowledge of the dorsal-ventral positioning of male and female organs is immensely helpful. In males, the bladder, prostate and urethra are ventrally situated with the rectum dorsally positioned. However, in some cases the internal genitalia may have been dislodged from their original anatomical position. Whether internal genitalia remain within the pelvis or are free floating elsewhere, for males there are two sets of associated small bilateral tubes: (a) two ureters attached to the bladder and (b) two vas deferens or Wolffian ducts attached to the posterior aspect of the bladder or urogenital sinus. All 4 small ducts may not always be present, but using anatomic principals one should try to