

COMMENTARY

The triphasic nature of Leydig cell development in humans, and comments on nomenclature

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Abstract

Leydig cell development in humans, although for years described as being biphasic, with fetal and adult phases of maturation, is better considered as a triphasic developmental phenomenon. The morphological literature is summarized in this commentary. Although the majority of studies are of a qualitative nature and many questions remain as to the relative and absolute numbers of cells involved in these developmental phases, this literature is more consistent with a triphasic developmental pattern.

This view of Leydig cell development is in accord with the well-known triphasic history of testosterone production, i.e. peaks at 14–18 weeks of fetal life, 2–3 months after birth, and from puberty throughout adult life. It is also significant that the neonatal phase of testosterone production is dependent upon reactivation of the hypothalamic–pituitary–testicular axis (HPT). The current interest in the functional implications of the neonatal period will be better served by considering human Leydig cell development as triphasic.

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For decades, the developmental history of Leydig cells in humans has been widely described as being biphasic, i.e. with a fetal stage followed by the adult generation beginning at puberty. This view of cellular development, however, is not consistent with the biochemical studies which clearly indicate a triphasic pattern of testosterone production during human development, the initial testosterone peak occurring at the end of the first trimester of fetal life (Reyes *et al.* 1974), the second peak occurring at 2–3 months of postnatal life (Forest *et al.* 1973, Faimen *et al.* 1974, Winter *et al.* 1976), and the third peak being the pubertal rise that lasts throughout adulthood. Excellent early ultrastructural studies of fetal (Pelliniemi & Niemi 1969, Holstein *et al.* 1971) and adult Leydig cells (e.g. Fawcett & Burgos 1960, Christensen and Gillim 1969, Christensen 1975, Schulze 1984) documented the basic characteristics of these steroid-producing cells. The vast literature on Leydig cell development in the rat and in numerous other mammals reinforced the concept of a biphasic developmental pattern. The pig was long considered the exception to the rule in that there were three waves of development. It would appear that this extensive literature on mammals, coupled with the fact that human Leydig cells had only been studied from the fetal and adult time periods, led to a ‘blind spot’ in the study of the cell biology of human Leydig cells.

I first suggested that human Leydig cell development is best considered triphasic in 1985 in an abstract in the

Anatomical Record, and elaborated on this in a later paper (Prince 1985, 1990). This conclusion is based on the existing morphological literature. Although the majority of these studies are qualitative in nature and exhaustive morphometric studies, such as those performed using rats, are not available for humans, the evidence is more consistent with human Leydig cell development being triphasic rather than biphasic. The strengths of the qualitative studies have been the range of ages examined (which encompass the full extent of fetal life, the neonatal period, childhood, and the pubertal period into adulthood) and the morphological evidence of cellular regression and degeneration.

The fetal period in humans has been studied by Mietkiewski *et al.* (1966), Pelliniemi & Niemi (1969), Holstein *et al.* (1971), Gondos & Golbus (1976), Codesal *et al.* (1990) and Waters & Trainer (1996). It can be concluded, from a review of this literature, that there is some continuity between the cellularity of the fetal Leydig cell development peak at 14–18 weeks and the neonatal population present at 2–4 months. Although Pelliniemi & Niemi (1969) and Holstein *et al.* (1971) favor cell degeneration in the late fetal period, evidence of cell regression is also present in their studies. The ultrastructural study of Gondos & Golbus (1976) and the histological study of Mietkiewski *et al.* (1966) both favor cell regression during the late fetal period. All studies report a major decline in Leydig cell numbers after the peak fetal development at

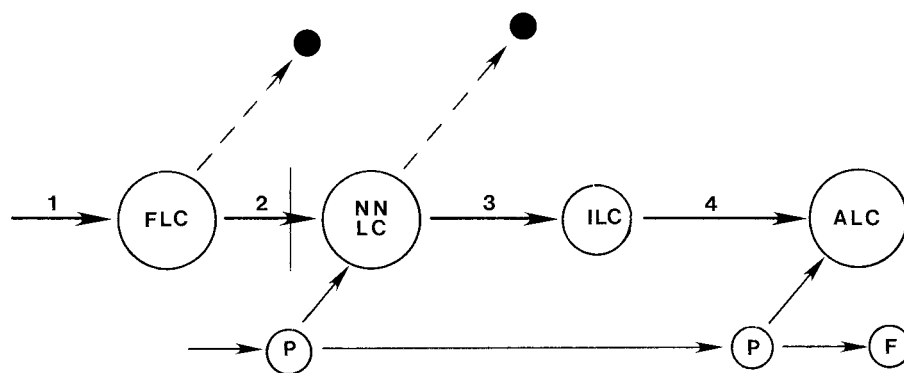


Figure 1 Flow chart of the three phases of Leydig cell development in humans. Although much remains unclear regarding the source of precursor cells and the relative and absolute numbers of cells involved, this scheme should serve as a foundation from which to interpret future studies of Leydig cell development in humans. The main horizontal axis indicates: (1) Development of the fetal Leydig cell population (FLC) from undifferentiated precursors (mesenchymal cells) (Pelliniemi & Niemi 1969, Holstein *et al.* 1971). (2) Regression of fetal Leydig cells, with subsequent maturation into mature neonatal Leydig cells (NN LC): the vertical line represents birth; a broken line leading to a black circle represents the component of cells which degenerates following the fetal phase of development (Mietkiewski *et al.* 1966, Pelliniemi & Niemi 1969, Holstein *et al.* 1971, Gondos & Golbus 1976, Codesal *et al.* 1990, Prince 1990). (3) Regression of the neonatal Leydig cell population: a component regresses to immature Leydig cells (ILC), which are present throughout childhood; a broken line leading to a black circle represents the component of the neonatal Leydig cells which degenerates (Prince 1984, 1985, 1990, Codesal *et al.* 1990). (4) Maturation of the immature Leydig cells at puberty into a segment of the adult Leydig cell population (ALC): this pubertal developmental phase also involves recruitment of precursor cells (P) (precursor cells include primitive fibroblasts of interstitium and peritubular fibroblasts); F, fibroblastic cells of the adult interstitium (F Prince, unpublished observations, Chemes 1996).

14–18 weeks, mature cells being relatively inconspicuous at term.

Leydig cells are again prominent a few months after birth, with a peak development at 2–3 months (Prince 1985, 1990, Codesal *et al.* 1990). The diverse cell population present at 4 months after birth (slightly past the peak of neonatal development), a mix of mature well-developed Leydig cells and smaller cells which are consistent with regressing cells, supports a continuity, in part, of the neonatal Leydig cell population with the small immature Leydig cells present during childhood (Prince 1984). Evidence of cell degeneration is also present within this time-frame. The finding that immature Leydig cells are present throughout childhood (Prince 1984) suggests that these cells are conserved and develop at puberty into a segment of the mature, adult Leydig cell population. It is probable that the relatively undifferentiated cells, previously referred to as primitive fibroblastic cells (Prince 1984), which are numerous in the interstitium during childhood, also contribute substantially to the cell population in the adult.

Thus, the morphological studies support both cell regression and cell degeneration as the fetal Leydig cell population recedes and, likewise, as the neonatal Leydig cell population recedes after the second developmental peak at 2–3 months after birth. The most likely scenario is that a combination of regressed Leydig cells from the fetal

period and recruited precursors form the neonatal Leydig cell population, and, similarly, a combination of regressed neonatal Leydig cells, via immature Leydig cells, and recruited precursors form the adult Leydig cell population. This developmental scheme is summarized in Fig. 1.

The complete cellular dynamic of these waves of Leydig cell maturation remains unclear. Although the evidence indicates both cell regression and degeneration after the fetal and neonatal periods, the relative percentages and absolute numbers of the cells involved are currently not established. The increase in mature Leydig cell numbers at 2–3 months (Codesal *et al.* 1990) does indicate recruitment of precursors within this time-frame. Also, the extensive development at puberty indicates recruitment of precursor cells during development of the adult Leydig cell population. Interestingly, whilst a biphasic developmental pattern in the rat is well accepted, the evidence indicates that the fetal Leydig cell population does not degenerate, but contributes to the adult Leydig cell population (Kerr & Knell 1988, Russell *et al.* 1995, Ariyaratne *et al.* 2000). The bulk of the adult cell population is derived from precursor recruitment postnatally, however.

Unnecessary confusion exists in the current literature regarding human Leydig cell development. Although some recent reviews and research papers have adopted the concept of triphasic development of human Leydig cells (e.g. Kerr 1992, Saez 1994, Berensztein *et al.* 1995,

Rivarola *et al.* 1995), others continue to view the development of these cells as a biphasic phenomenon. A recent review by Chemes (1996), devoted exclusively to human Leydig cell development, generally discusses this topic from the biphasic viewpoint, but in the same review does state that the triphasic concept may be more accurate. Typically, reviews of Leydig cells which support the biphasic pattern tend to discuss this topic in a general fashion, including human and rat studies in the same review, but relying heavily on data from rat studies (e.g. Huhtaniemi & Pelliniemi 1992, Ge *et al.* 1996, Pelliniemi *et al.* 1996). Numerous differences exist between rats and humans with respect to the cell biology of Leydig cells. Notably, the initial development of the fetal Leydig cell population in the rat is independent of gonadotropic hormone control (Huhtaniemi 1996). In contrast, during human development, chorionic gonadotropin is present at the outset of fetal Leydig cell differentiation, and luteinizing hormone (LH) secretion occurs soon afterwards, with the activation of the HPT axis. The consensus of the extensive morphological literature on the rat is that Leydig cell development is biphasic, having a fetal phase and an adult phase.

A contributing factor in this confusion, I believe, is the use of the terminology 'fetal-type Leydig cell' to describe the Leydig cells present during the neonatal period; this term appears in numerous papers (e.g. Nistal *et al.* 1986, Codesal *et al.* 1990, Huhtaniemi & Pelliniemi 1992, Chemes 1996, Ge *et al.* 1996). The main premise of the biphasic description of Leydig cell development is that there are two generations of Leydig cells, fetal and adult, and that any that are present neonatally are part of the fetal generation. From a nomenclatural standpoint, the use of the term 'fetal' to describe cells present 3 months after birth is awkward. More importantly, there is no evidence, in humans, to support two separate and distinct cell generations. A study of postnatal human Leydig cell development by Nistal *et al.* (1986) unfortunately labels mature, postnatal Leydig cells as 'fetal', and labels cells with a modest development of smooth endoplasmic reticulum (SER) as 'infantile', regardless of whether the cells are found in a 1-year-old child or a 6-year-old child. This quantitative study did not report a neonatal rise in Leydig cell numbers. Their choice of age groups may very well have been the reason why the neonatal peak at 2–3 months was missed. Interestingly, a later study from the same laboratory, using shorter time intervals (1 month) between age groups, did provide quantitative support for the neonatal peak at 2–3 months of age (Codesal *et al.* 1990), though it also labeled postnatal cells as 'fetal'.

A triphasic pattern of Leydig cell development and testosterone production may, in fact, be more common than is currently appreciated. Numerous non-human primates have been shown to exhibit fetal, neonatal and pubertal periods of testosterone secretion (marmoset monkey – Dixson 1986, Lunn *et al.* 1994; rhesus monkey

– Robinson & Bridson 1978; crab-eating monkey – Steiner & Bremner 1981, Fouquet *et al.* 1984; chimpanzee – Winter *et al.* 1975) that are dependent upon activation of the HPT axis. Morphological studies of primate Leydig cell development are uncommon, however, especially during the fetal period. The series of studies by Fouquet *et al.* (1978, 1983, 1984) documents the cellular maturation and involution that occurs in the crab-eating monkey from fetal life to adulthood, and correlates these changes with the fluctuations in testosterone production. These studies also support both cellular regression and degeneration following the fetal and neonatal periods, which is similar to that found in humans. The diverse cellularity found following the neonatal maturational peak (Fouquet *et al.* 1984) is strikingly similar to that which is found in the human (Prince 1990). Marmoset Leydig cells are also found to exhibit a neonatal maturational peak with subsequent regression (F Prince, unpublished observations). Leydig cell development in the pig has long been known to be triphasic, having fetal, perinatal and pubertal periods (e.g. Moon & Hardy 1973, van Vorstenbosch *et al.* 1984).

Further support for the view that the neonatal phase of Leydig cell development in humans is distinct from the fetal phase comes from the fact that this phase of Leydig cell development and testosterone secretion is dependent upon a reactivation of the hypothalamic–pituitary axis (Mann & Fraser 1996). The elevated neonatal blood testosterone level in humans has been shown to be associated with increased levels of gonadotrophic hormones (Faiman *et al.* 1974). Furthermore, a non-human primate model has shown that blockade of the neonatal activation of the hypothalamic–pituitary axis via gonadotropin-releasing hormone antagonist (GnRH Ant) prevents the maturation of Leydig cells (Prince *et al.* 1998) and ablates the neonatal rise in testosterone secretion (Lunn *et al.* 1994).

In sum, I suggest that the Leydig cells present during the fetal period (peak, 14–18 weeks) be referred to as the 'fetal Leydig cell population', that the Leydig cells found during the neonatal phase (peak, 2–3 months) be referred to as the 'neonatal Leydig cell population', and that the Leydig cells found from puberty onwards be referred to as the 'adult Leydig cell population'. The population of small cells with a minimal to modest development of SER, which are present throughout childhood are perhaps best termed 'immature Leydig cells', rather than 'infantile', as used by Nistal *et al.* (1986). The term 'infantile', applied to cells in children 5 or 10 years of age, seems inappropriate. The view of human Leydig cell development in this triphasic pattern is in accord with long-standing biochemical data, and with the literature on human Leydig cell morphology. This concept of Leydig cell development will also serve as a better foundation from which to interpret future studies relating to the significance of the neonatal phase of activation of the HPT axis.

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