
Male Fertility and Strategies for Fertility Preservation following Childhood Cancer Treatment

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Abstract

Infertility in the male is a potential complication of childhood cancer treatment for long-term survivors. The risk is dependent primarily on the treatment used, but also on the underlying disease. Chemotherapy (especially alkylating agents) and radiotherapy, even in low doses, may damage the seminiferous epithelium and impair spermatogenesis in both children and adults. Leydig cell function and testosterone production are generally preserved after chemotherapy and low dose radiotherapy, whilst larger doses of radiotherapy may result in hypogonadism. Patients treated with potentially gonadotoxic treatments require regular multidisciplinary follow-up including assessment of puberty and gonadal function. Currently the only option available for fertility preservation in young males treated for cancer is semen cryopreservation. For pre-pubertal patients, techniques for fertility preservation remain theoretical and as yet unproven. These include hormonal manipulation of the gonadal environment before treatment, germ cell transplantation and testis xenografting, which have all shown promise in a variety of animal studies. Refinement of these techniques requires investigations in relevant animal models. In the present chapter we include data which suggest that the common marmoset (*Callithrix jacchus*) monkey, a New World primate, exhibits important parallels with human testicular development and may help us to understand why the pre-pubertal testis is vulnerable to effects of cytotoxic therapy on future fertility.

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This chapter will describe the long-term effects of cancer treatment in childhood on male fertility. It will begin with an overview of male gonadal development with particular emphasis on the different stages in childhood, when variation in the hormonal and/or cellular environment may affect the response of the gonad to cytotoxic treatment and may also alter the effectiveness of strategies for preservation

of fertility in these patients. It will then describe the direct and indirect effects of cytotoxic therapy on the testis and the possible mechanisms involved. It will end with a review of the potential strategies for preserving fertility in survivors of childhood cancer, including established techniques as well as those that are currently experimental. Throughout this chapter studies undertaken in animals will be discussed to provide insight into gonadal development, effects of cytotoxic therapy and fertility preservation, whilst relating these findings to the situation in the human. This will also serve to highlight differences between species that may result in different effects to those seen in humans.

Male Gonadal Axis and Gonad Development

The Male Reproductive Hormonal Axis

Secretion of gonadotropins from the pituitary gland is responsible for regulating hormonal control of the gonad in the male. Although this chapter will focus mainly on the gonad itself, knowledge of this central control of the gonad is particularly important for understanding the mechanisms behind the effects of cytotoxic therapy on fertility in addition to strategies for preserving fertility in survivors of childhood cancer.

The male hypothalamo-pituitary-gonadal axis (HPG) is active from fetal life and the level of hormones produced varies at different stages throughout life. The axis regulates the onset of puberty and the establishment of spermatogenesis, in addition to the production of gonadal androgens. Gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus and stimulates the secretion of the gonadotropins in the form of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. LH acts on the testis to promote testosterone secretion from the Leydig cells and FSH acts on the Sertoli cells to initiate spermatogenesis. Two important negative feedback loops exist to regulate the secretion of gonadotropins. The testosterone negative feedback loop is established in fetal life and inhibits hypothalamic and pituitary production of GnRH and LH. Inhibin-B, produced by the Sertoli cell, exerts inhibitory effects on FSH secretion from the pituitary gland, however this negative feedback loop is only established at around puberty [1] (fig. 1).

Development of the Testis

Fetal Life and Early Infancy

During fetal life, the primordial germ cell population is thought to arise from a small group of cells in the epiblast. In humans the primordial germ cells migrate

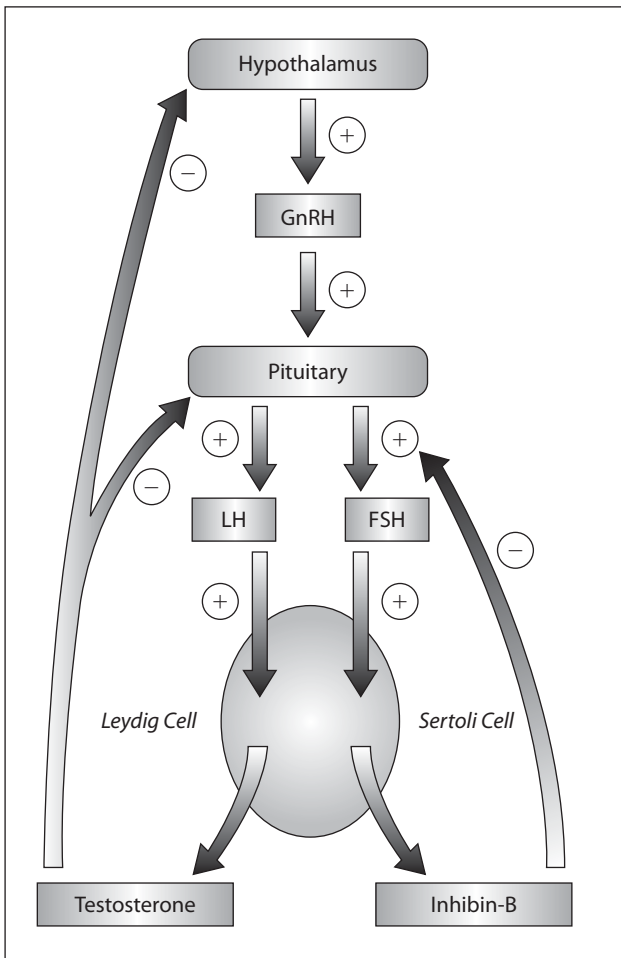


Fig. 1. The male hypothalamo-pituitary-gonadal axis. Stimulation (+) and inhibition (-) are indicated.

into the gonad during the 5th week of gestation and once they have become enclosed within seminiferous cords, they are termed gonocytes. These gonocytes begin to differentiate into spermatogonia and these in turn will ultimately give rise to spermatozoa at puberty. Also within the seminiferous epithelium are the Sertoli cells, which provide support to the developing germ cells. Leydig cells are located outside the seminiferous epithelium in the interstitial compartment and are responsible for producing androgens.

During infancy any remaining gonocytes will differentiate into spermatogonia. Differences exist between rodents and primates in terms of germ cell differentiation during this phase. In humans [2, 3] and primates such as the marmoset, gonocytes express protein markers associated with pluripotent or

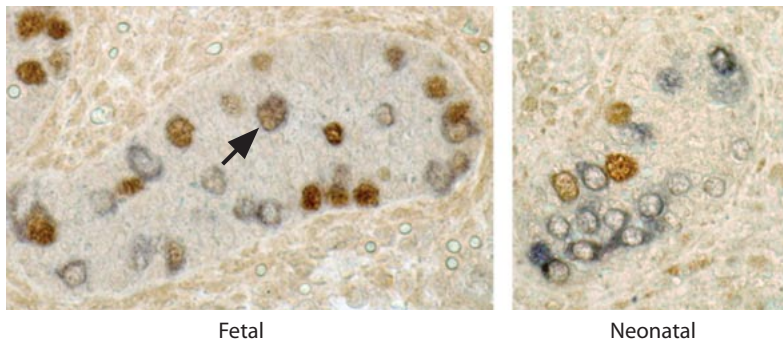


Fig. 2. Expression of OCT4 (brown) and VASA (blue) in marmoset testes from fetal (17/20 weeks gestation) and neonatal (1 day postnatal) animals. Note the progression within the cords, as the proportion of germ cells expressing OCT4 decreases and the proportion of germ cells expressing VASA increases. Cells express both markers (arrow) as they differentiate from OCT4 to VASA-positive germ cells.

undifferentiated germ cells such as OCT4 [2, 3] and AP-2 γ [4]. These cells differentiate to become spermatogonia, during which the expression of these markers is gradually reduced and germ cell-specific markers such as VASA [2, 5] and MAGE-A4 [3, 4] are expressed (fig. 2). Rodents demonstrate a homogeneous population of cells expressing markers such as OCT4 and VASA simultaneously and become negative for OCT4 in a synchronous manner without the gradual transition seen in the human and primate. A potential consequence of the mixed population of gonocytes in the primate may be differences in the effects of cytotoxic therapy, when compared to rodents. This may be particularly relevant if cancer treatment begins during infancy, when differentiation of gonocytes is still occurring.

Following birth in humans and non-human primates there is an initial rise in gonadotropins and testosterone that continues during early infancy, the so-called 'mini puberty'. In humans the rise begins at 2 weeks of life and peaks between 1 and 3 months of age, falling to low levels at 6–8 months. This pattern of secretion has also been demonstrated in many other primates, including the rhesus monkey and the marmoset [6] (fig. 3).

Childhood

In humans and non-human primates after the rise in gonadotropins and testosterone during early infancy, there follows a period of relative 'quiescence' during which levels of these hormones are relatively low [6]. This period will be referred to as the 'childhood period', which lasts from the end of infancy until the onset of puberty (fig. 3). In rodents GnRH synthesis and release is not interrupted by a post-infantile quiescent period prior to the onset of puberty, which highlights

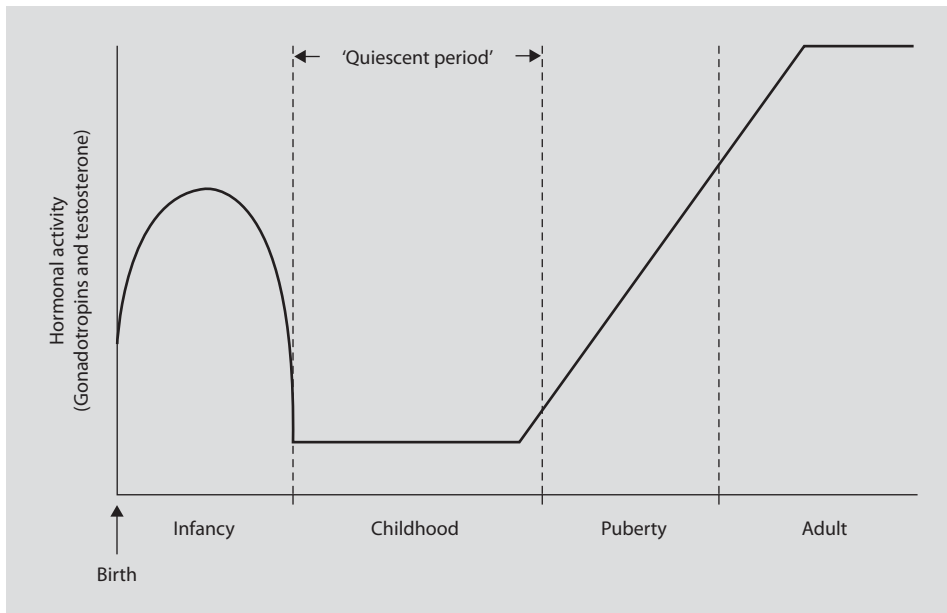


Fig. 3. The profile of gonadotropin and testosterone secretion in primates throughout life [6].

another fundamental difference between rodents and primates that may affect the response to cancer treatment.

Based on these low levels of gonadotropins and testosterone in primates, it had been assumed that the ‘childhood’ testis is a relatively quiescent organ and as a result, little germ cell proliferation occurred. As proliferating cells are considered to be the main targets of cancer therapy, in theory this should render the gonad less susceptible to the damaging effects of cytotoxic therapy. However the fact that gonadal damage occurs following cancer treatment in childhood raises doubt about whether the testis is truly quiescent during childhood. Studies have demonstrated in the human that there is pulsatile secretion of LH during sleep in mid-childhood which increases in amplitude prior to puberty [7], and that this is paralleled by incomplete spermatogenic bursts [8]. Demonstration of this activity raised the possibility that germ cell proliferation may occur in the testis during childhood and that this may render the gonad susceptible to damage by cancer treatment. To investigate this hypothesis, germ cell proliferation has been studied in the marmoset monkey during the ‘childhood’ period [9]. Immunohistochemical labelling using proliferating cell nuclear antigen showed that a proportion of germ cells are proliferating during this period [9]. A proliferation index obtained using Ki67 as the marker of mitotic activity has confirmed the presence of proliferating germ cells from birth through to adulthood in this primate species, with a lower proliferation index during the childhood period (fig. 4). This is also the

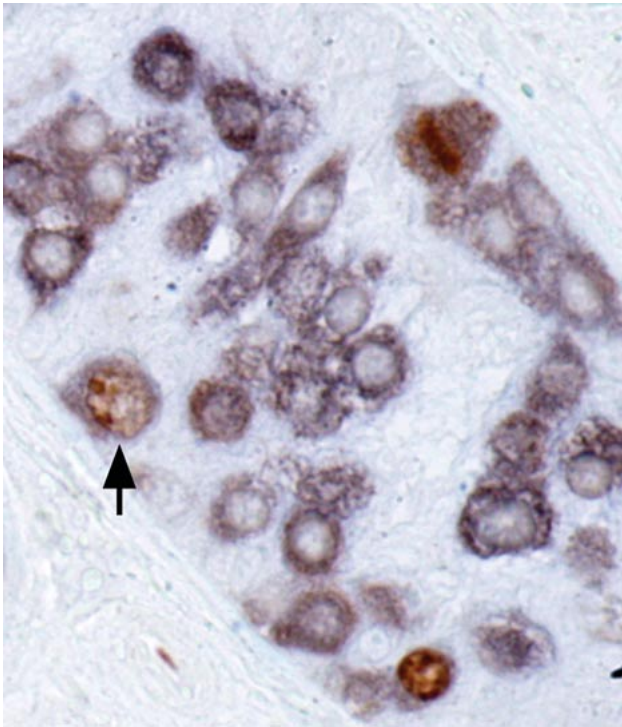


Fig. 4. Expression of VASA (blue) and Ki67 (brown) in a testis from a 35-week-old ('childhood') marmoset demonstrates the presence of proliferating germ cells (arrow) during the 'quiescent' phase of gonadotropin and testosterone secretion.

case for the human with proliferation of germ cells occurring during the childhood period. Ki67 expression has been demonstrated in 10.9% of human germ cells between the age of 1 and 6 years [10]. In the rat there is a block in G_0 of the cell cycle from late gestation until postnatal day 3–6, when proliferation resumes [11], indicating another potentially important difference between the primate and the rodent, which may have relevance for susceptibility to gonadal damage following cancer treatment.

Suppression of germ cell proliferation could in theory protect the gonad from the damaging effects of cytotoxic treatment. If germ cell proliferation is gonadotropin- or sex steroid-dependent, then the use of a GnRH antagonist might represent one strategy to achieve this. However treatment with a GnRH antagonist did not affect germ cell proliferation in the marmoset during the 'childhood' phase [9]. Indeed even treatment of marmosets with a GnRH antagonist during the neonatal period, when the levels of gonadotropins and testosterone are high, failed to have a major impact on germ cell proliferation, which remained at 70% of the control level (unpublished). This lack of complete suppression of proliferation by