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Effect of suppression of pituitary–testicular function during fetal life with a GnRH agonist on reproductive development in ram lambs during the first 28 weeks of life

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Introduction

Over the past 50 years, total numbers of spermatozoa in man has declined by approximately 50% (Carlsen *et al.*, 1992). One explanation for this decrease is that factors that affect the development of the fetal reproductive system may limit reproductive function in adults (Sharpe, 1993). We have demonstrated that suppression of fetal gonadotrophin secretion during the last half of gestation with a GnRH agonist results in a 40–45% reduction in the number of Sertoli cells and testicular size at birth (Thomas *et al.*, 1994). Thus we have a model whereby we can manipulate the reproductive system during fetal life and study the consequences of such an intervention in later life. The aim of the present study, therefore, was to investigate whether impairment of gonadotrophic drive during fetal life influenced testicular size and function in neonatal sheep.

Materials and Methods

Mature pregnant Scottish Blackface ewes with known single insemination dates were used for the study. On day 75 of gestation (term = 145 days), a biodegradable implant containing 3.0 mg of the GnRH agonist, buserelin [D-Ser(tBu)^6, Pro^9-NHEt]-GnRH$_{1-9}$ (Hoechst AG, Frankfurt) was placed s.c. into the tail of 12 male fetuses (Brooks and McNeilly, 1992). Sham operations were performed in an additional 12 male fetuses (controls).

On the day after birth, the lambs received a bolus i.v. injection of 500 ng GnRH. Blood samples were collected by venepuncture at −15, 0, 5, 20, 40, 60 and 90 min relative to injection for LH and testosterone assay. The implant was then removed by docking the tail and blood samples collected twice weekly for 28 weeks for LH, FSH and inhibin assay. The GnRH treatment was repeated at 2, 4, 6, 8, 10, 12, 16, 20 and 28 weeks of age, when the lambs were also weighed and the testicular diameter measured using callipers. The concentrations of LH, FSH, testosterone (Baird *et al.*, 1981) and immunoreactive inhibin (McNeilly *et al.*, 1989) were measured by previously described radioimmunoassays.

The influence of buserelin was analysed by a two-way analysis of variance using a repeated measures design. When a significant ($P < 0.05$) treatment effect was found, a Duncan's multiple-range test was used to test for significant differences between individual means.

Results

Treatment with buserelin from day 75 of gestation until birth resulted in a significant ($P < 0.05$) reduction in the diameter of the testes at birth and in the first 8 weeks of life compared with control ram

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lambs (Fig. 1a). By 10–12 weeks of age, however, mean testis diameter was similar in both groups. Mean plasma concentrations of FSH in GnRH agonist pretreated lambs were significantly ($P < 0.05$) higher between 3 and 8 weeks of age compared with controls (Fig. 1b). Treatment with buserelin had no effect on plasma concentrations of immunoreactive inhibin (Fig. 1c) or LH (data not shown). Lamb birth masses (control, 4.3 ± 0.2 kg versus buserelin, 4.6 ± 0.3 kg) and growth rates (controls, 1.23 ± 0.03 kg week^{-1} versus buserelin, 1.22 ± 0.07 kg week^{-1}) were similar between groups.

At birth, injection of 500 ng GnRH elicited an immediate increase in LH secretion in all control lambs (Fig. 2). Plasma testosterone concentrations subsequently increased 40–90 min later (Fig. 2). These responses were completely abolished in newborn lambs treated with buserelin during the last half of gestation. After removal of the buserelin implant, the magnitude of the LH response remained significantly ($P < 0.01$) lower in GnRH agonist pretreated lambs between 2 and 12 weeks of age compared with controls. However, in both groups, there was a progressive increase between 2 and 8 weeks of age in the magnitude of the LH response to GnRH injection (Fig. 2). Between 16 and 28 weeks of age, there was no significant difference between the two groups in the amplitude of the LH response to GnRH administration; the size of the response decreased with age in both groups.

Despite the reduced LH response between 2 and 12 weeks of age in lambs pretreated with GnRH agonist, both groups exhibited similar testosterone responses to the GnRH-induced LH release (Fig. 2). In both groups, the plasma testosterone concentrations progressively increased between 2 and 20 weeks of age. At 28 weeks of age, following puberty, there was a two- to threefold increase ($P < 0.01$) in plasma testosterone concentrations, with control lambs having significantly ($P < 0.01$) higher concentrations of testosterone compared with buserelin pretreated lambs (Fig. 2).

Discussion

The present data show that administration of a GnRH agonist during the last half of gestation results in a transient decrease in testicular size during the first 8 weeks of life. Furthermore, the compensatory growth of the testis in newborn lambs after GnRH agonist removal was associated with increased plasma concentrations of FSH. Since the regulation of numbers of Sertoli cells is a major determinant of testicular size (Berndtson *et al.*, 1987) and replication of Sertoli cells during fetal and neonatal life is predominantly under FSH control (Mann *et al.*, 1989), it is likely that the increased rate of testicular growth observed following GnRH agonist pretreatment reflects an increase in Sertoli cell proliferation in response to increased concentrations of FSH. This is analogous to the situation following unilateral castration in prepubertal lambs, whereby compensatory hypertrophy of the remaining testis is accompanied by high concentrations of FSH and blockade of this increase prevents compensatory testicular growth (Jenkins and Waites, 1983).

The increase in FSH concentrations between 3 and 8 weeks of life in GnRH agonist pretreated lambs is likely to result from a reduction in the negative feedback by testicular factors. Although immunoreactive inhibin concentrations were similar in GnRH agonist pretreated and control lambs, a reduction in negative feedback by inhibin cannot be excluded entirely, since our assay detects forms of inhibin other than dimeric inhibin. This may obscure any decrease in feedback by dimeric inhibin. Another possibility is that a reduction in testicular steroids, in particular oestrogens of Sertoli cell origin, may be responsible for the increase in FSH secretion. Support for this hypothesis is provided by several observations. First, it is well established that immature Sertoli cells are a source of oestrogen during prepubertal development (see Sharpe, 1994 for review). Second, we have shown that reduced gonadotrophic support during the last half of gestation results in a reduction in the number of Sertoli cells at birth (Thomas *et al.*, 1994). Finally, immunization of ram lambs against oestradiol or oestrone results in an increase in plasma FSH concentrations that is associated with an increase in testicular growth (Land *et al.*, 1981).

The finding that GnRH agonist pretreated lambs secreted a similar amount of testosterone despite having reduced testicular size and an attenuated LH response to GnRH stimulation was surprising. However, there is considerable evidence that Sertoli cells, under FSH control, can act to modulate Leydig cell differentiation and function during prepubertal development (see Sharpe, 1994 for review). Thus, the

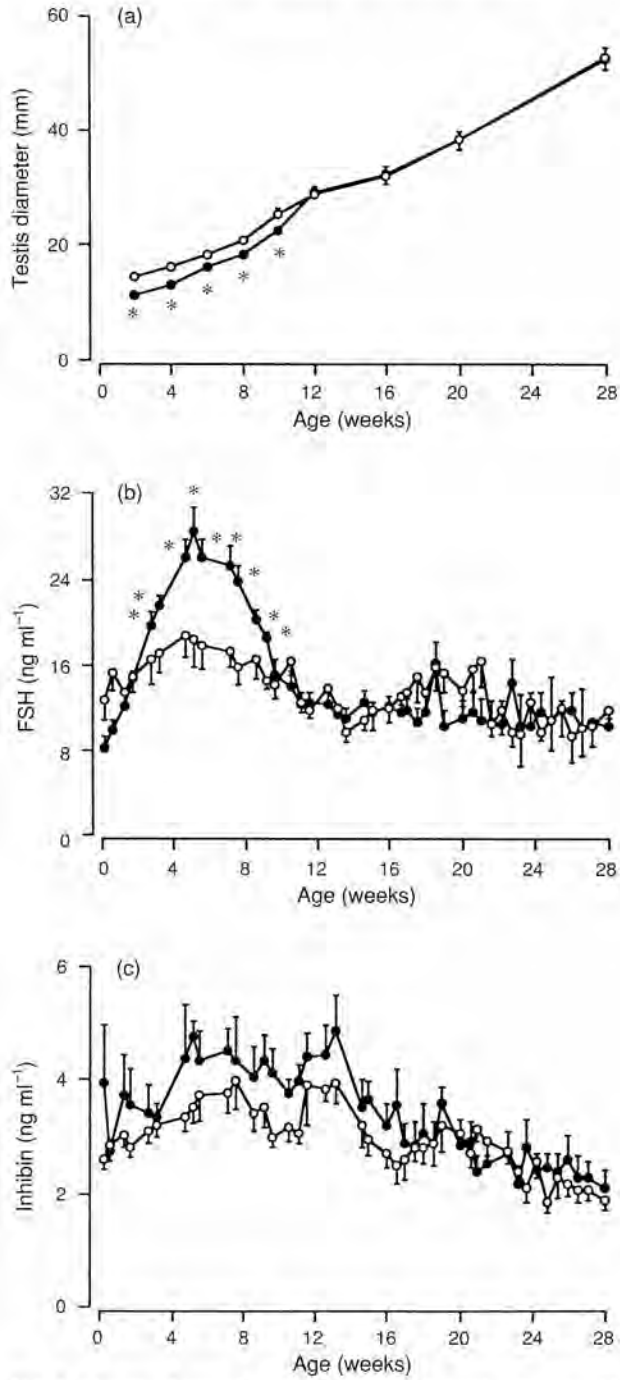

Fig. 1. Changes during the first 28 weeks of life in (a) diameter of the testis; and plasma concentrations of (b) FSH and (c) immunoreactive inhibin in control lambs (○) and lambs implanted from day 75 of gestation until birth with 3.0 mg buserelin (●). Values are means ± SEM of 12 animals per group. $^*P < 0.05$ compared with control.

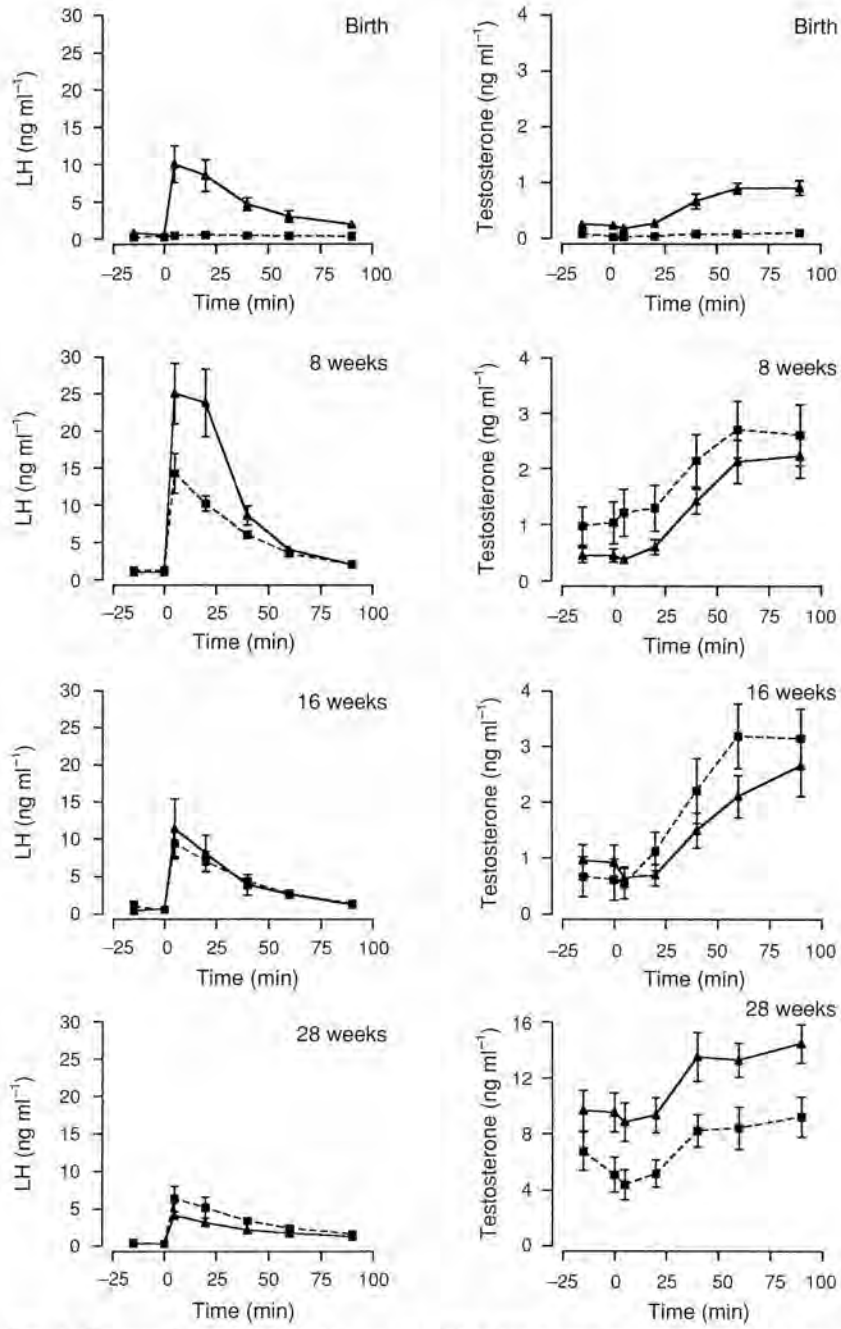

Fig. 2. Changes during the first 28 weeks of life in the plasma concentration of LH and testosterone in response to a 500 ng i.v. injection (time 0) of GnRH in control lambs (▲) and lambs implanted from day 75 of gestation until birth with 3.0 mg buserelin (■). Values are means ± SEM of 12 animals per group.

increase in FSH concentrations in GnRH agonist pretreated lambs may have caused an increase in the sensitivity of the Leydig cells to respond to stimulation by LH. For example, treatment of hypophysectomized immature rats with FSH has been shown to increase the number of testicular LH

receptors, resulting in an increase in capacity of the Leydig cells to secrete testosterone in response to LH (Selin *et al.*, 1977).

By 28 weeks of life, after the animals had attained puberty, plasma testosterone concentrations were significantly higher in control lambs than in lambs pretreated with GnRH agonist during the later stages of fetal development. This finding suggests that, although the testes of GnRH agonist pretreated lambs appear to have undergone complete compensatory growth in the prepubertal period, there may be important functional aspects which remain impaired. If these defects were to be still apparent during the rest of adult life, the result could be impaired reproductive function (for example, spermatogenesis) and behaviour. In conclusion, the present study supports the contention that impaired gonadotrophic drive during fetal life may influence reproductive potential in adult life. This issue should be addressed with priority in future studies.

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