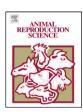


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## **Animal Reproduction Science**





# Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero

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#### ABSTRACT

The nutritional status of females during pregnancy can have profound effects on the fetus reproductive system development that could affect the reproductive potential as later as in adulthood (fetal programming). The aim of the present study was to examine the effects of maternal nutrient restriction imposed during different periods of gestation on pituitary responsiveness at different ages postnatal and on Sertoli cell number in male offspring. Pregnant ewes were fed to 100% of Metabolizable Energy and Crude Protein requirements throughout pregnancy (Control) or to 50% from 0 to 30 (R1) or from 31 to 100 days of gestation (R2). Male lambs were selected and fed to appetite throughout the study. At 2, 5.5 and 10 months of age a GnRH challenge was conducted. At slaughter (10 months) testes were removed and examined histologically. Maternal undernutrition did not affect the time of the onset of puberty, defined as the first increase in plasma testosterone concentrations ≥1 ng/ml. The LH and FSH response to GnRH challenge did not differ between groups at 2 and 5.5 months but at 10 months of age a higher (P<0.05) FSH response was found in R2 group. Testes weight did not differ between groups at slaughter. Mean Sertoli cell number was significantly lower in animals of R2 group compared with Control (P<0.01). A smaller seminiferous tubules diameter was detected in R2 group (P < 0.05), while the diameter of the lumen was similar in all groups. Collectively, these results provide clear evidence © 2008 Elsevier B.V. All rights reserved.

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for a direct effect of nutrient restriction during pregnancy on Sertoli cell number in adulthood. The lower number of Sertoli cells is the most candidate factor for the higher pituitary activity through a reduction in the suppressive effect of inhibin.

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#### 1. Introduction

The concept of fetal programming reflects the possibility of intrauterine factor acting during a sensitive period in gestation, exerting organizational effects that are apparent later in life (Barker et al., 1993).

As the reproductive system and its hormonal control systems are largely established in fetal life, adverse environmental factors may subsequently affect the function of the reproductive axis and among them maternal undernutrition is of central importance (Rhind, 2004).

In sheep maternal dietary restriction has been shown to affect lifetime reproductive performance throughout the adult life of female offspring (Gunn et al., 1995; Rae et al., 2002a) and studies have supported a direct effect on germ cell, expressed as a delay in fetal germ cell degeneration (Borwick et al., 1997) or oogonial meiosis and follicular development (Rae et al., 2001).

Results are less clear with respect to male testis development. No differences were detected for fetal testis weight or mean scrotal circumference for adult lambs in response to undernutrition (Rae et al., 2002a,b). Nutritionally mediated placental growth restriction had no effect on fetal seminiferous cords and Sertoli cell number (Da Silva et al., 2003). In contrast, Bielli et al. (2002) reported a 20% reduction in Sertoli cell number in newborn lambs, undernourished in utero. Sertoli cells could provide a target for fetal programming, since it is well accepted that their number per testis is the most important factor that determines the ceiling of sperm production and output (Orth et al., 1988). Moreover, in male sheep fetuses proliferation of Sertoli cells occurs throughout testicular fetal growth at a higher rate before day 100 of gestation rather than later and mitotic divisions are more numerous before birth than afterwards (Hochereau-de Reviers et al., 1995), so this period is of critical importance in establishing the complement of Sertoli cells that populates the adult testis.

Structural effects on gonadal development could be a result of either direct effects of nutrition on the gonads or through altered hypothalamo-pituitary function. It has been recognized that in sheep the effects of nutrition on reproductive performance could be mediated through changes in hypothalamic activity and secretion of gonadotrophin releasing hormone (GnRH) or in the pituitary response to GnRH, either of which could affect circulating gonadotrophin profiles (Rhind et al., 1989). Maternal undernutrition has been shown to influence the pituitary sensitivity to GnRH administration in male fetuses (Rae et al., 2002b) and in 2 months old lambs (Deligeorgis et al., 1996). In contrast, nutritional restriction from mating to 90 days of gestation did not affect pituitary function, with respect to gonadotrophin secretion or pituitary sensitivity in adults, suggesting that the effects on pituitary sensitivity are transitory and not expressed in adult life (Rae et al., 2002a). To date studies examining the impact of maternal undernutrition on male reproductive axis development has been mostly limited to late gestation-fetus or neonate and no information exists as to which extent the induced changes persist into adulthood, thus compromising future reproductive capacity of male animals.

Effects of undernutrition may be expressed at many stages of development, even before a certain organ, cell type or receptor has developed, through action on precursor cells (Rhind et al., 2001). In the sheep fetuses specific gonadal structures are forming between days 0 and 30 (Rae et al., 2002c), while the GnRH neuronal systems develop about 35–85 days of gestation (Caldani et al., 1995). Therefore, the present study was designed to evaluate if 50% nutrient restriction imposed during discrete periods, early (0–30 days) and mid to late (31–100 days), of gestation, programs long lasting effects on Sertoli cells and to investigate if these effects are direct or mediated through alterations on hypothalamic–pituitary axis.

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