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Effect of maternal undernutrition on the hypothalamic-pituitary-gonadal axis function in sheep offspring

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Introduction

The fetal programming hypothesis demonstrates that a stimulus or insult during sensitive periods of early life can lead to permanent alterations in fetus organ structure, physiology and metabolism. Nutrition as early as in embryonic life can have profound effects on the reproductive system of the fetus that could affect the reproductive potential later in adulthood.

Aim of the study

To examine the effects of maternal nutrient restriction imposed during different periods of gestation on the hypothalamo-pituitary-gonadal axis function in sheep offspring at different ages postnatal.

Materials and Methods

22 pregnant Chios ewes (ABW= 63.0±0.6 Kg) were allocated to 3 treatments: Control (C), R1 and R2 (Fig. 1)

Offspring

- Weighed at birth and biweekly throughout the experiment. Reared until day 45 on artificial milk *ad libitum*. *Ad libitum* feeding post-weaning.
- Blood samples collected biweekly for measurement of progesterone levels and detection of endocrine puberty.
- Three GnRH challenges (1.5 μg GnRH/kg BW) at the age of 2, 5.5 and 10 months to evaluate HPG axis responsiveness. Blood samples collected at 15 and 0 min before, and at 15, 30, 60, 120 and 180 min after the challenge.
- At slaughter (10 months of age) ovaries and testes were dissected out. Weight and number of visible follicles and corpora luteua were recorded on ovaries. Sertoli cell number as well as external and lumen diameter of seminiferous tubules were recorded histologically in testes.

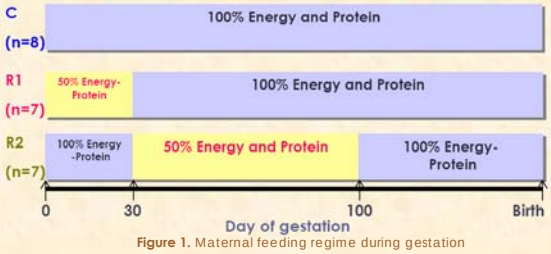

Figure 1. Maternal feeding regime during gestation

Results

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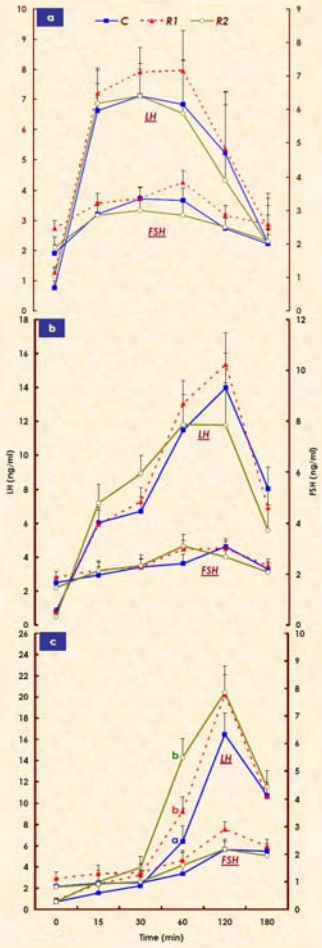

Figure 2. LH and FSH response to GnRH challenge of the female offspring at 2 (a), 5.5 (b) and 10 (c) months of age

Table 1. Medians (and quartile range) of follicles and corpora lutea recorded from female offspring's ovaries in total and according to their diameter

	Group[1] C	R1	R2	P[2]
Total follicles	1.0 (1.0) 2.00[3]	2.0 (1.0) 2.67	2.0 (1.0) 2.50	NS
Follicle diameter (mm)				
2-3	0.0[a] (1.0) 0.40	1.0[b] (1.0) 1.33	0.5[a] (1.0) 0.50	*
4-5	0.0 (1.0) 0.40	0.5 (1.0) 0.67	1.0 (1.0) 1.50	NS
6-8	1.0 (1.0) 1.25	0.5 (1.0) 0.67	0.5 (1.0) 0.50	NS
Total corpora lutea	5.0 (1.0) 5.60[3]	4.0 (1.0) 4.83	4.0 (1.0) 4.75	NS
Corpus luteum diameter (mm)				
4-7	2.0 (1.0) 1.60	1.0 (1.0) 1.33	2.5 (1.0) 3.25	NS
8-11	3.0[a](1.0) 3.80	2.5[ab] (1.0) 2.83	1.5[b] (1.0) 1.50	*
12-16	0.0 (0.0) 0.20	0.5 (1.0) 0.67	0.0 (0.0) 0.00	NS

Medians with different superscripts differ significantly (P<0.05)
*[2]NS, non-significant ; *, P<0.05*
[3]For best comprehension of the results means are shown below the corresponding medians

No effect of maternal undernutrition on the:
- **Weight at birth and subsequent growth**
- **Age of onset of endocrine puberty**
- **Preovulatory rise and the time to rise of both gonadotrophins**

of female offspring

- **FSH response at 10 months of age (Fig. 2) did not differ at each sampling time. However the area under the response curve (AUC) was higher in R1 group accompanied by a higher number of small follicles (Table 1)**
- **R2 group showed a lower number of corpora lutea (Table 1)**

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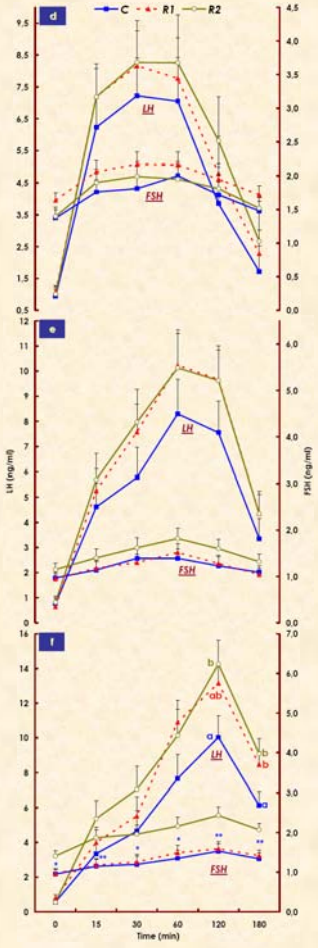

Figure 3. LH and FSH response to GnRH challenge of the male offspring at 2 (d), 5.5 (e) and 10 (f) months of age

Table 2. Testes weight, Sertoli cell count, external diameter and lumen diameter (μm) of seminiferous tubules in male offspring

	Group[1] C	R1	R2	P[2]
Testes weight (g)	287±26,7	286±26,7	280±35,3	NS
Sperm tubule				
Sertoli cell count	12,17[a]±1,14	9,85[ab]±0,75	8,51[b]±0,73	*
External diameter	215[a]±7.5	204[ab]±4.9	189[b]±4.3	*
Lumen diameter	84±5,3	84±2,7	75±4,0	NS

Means with different superscripts differ significantly (P<0.05)
*[2]NS, non-significant ; *, P<0.05*

No effect of maternal undernutrition on the:
- **Weight at birth and subsequent growth**
- **Testes weight and tubule lumen diameter**

of male offspring

- **R2 group showed a higher LH and FSH response at 10 months of age (Fig. 3) accompanied by a lower Sertoli cell count and smaller tubule diameter in testis (Table 2 and Image 1)**

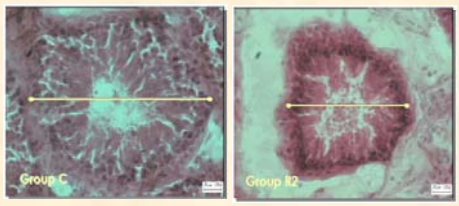

Image 1. Comparative images (20x) of representative seminiferous tubules in group C and R2. The yellow line represents the external diameter of the tubule

Conclusions

Maternal undernutrition

→ **No effect on body weight of male and female offspring**

Affected differentially the pituitary sensitivity and gonadal function with respect not only to the timing and duration of the insult imposed, but also in a sex specific manner

Female offspring
- **Early in gestation resulted in a higher pituitary response to GnRH challenge along with an increased accumulation of small follicles (2-3 mm) in the ovaries**
- **In mid gestation resulted in a significantly lower number of large (>8 mm) corpora lutea in the ovaries**
- **Did not affect the age of onset of puberty**
- **Did not affect preovulatory gonadotrophins profiles**

Male offspring
- **In mid gestation resulted in increased pituitary response accompanied by a lower Sertoli cell count and smaller tubule diameter in testis**

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