Theriogenology 108 (2018) 331–338

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Maternal nutritional restriction during late gestation impairs development of the reproductive organs in both male and female lambs

F. Hoffman [a], E. Boretto [b], S. Vitale [b], V. Gonzalez [b], G. Vidal [b], M.F. Pardo [b], M.F. Flores [b], F. Garcia [b], G. Bagnis [c], O.C.M. Queiroz [d], M.B. Rabaglino [a, b, *]

[a] National Scientific and Technical Research Council (CONICET), Argentina
[b] Department of Animal Reproduction, College of Agronomy and Veterinary, National University of Río Cuarto, Argentina
[c] Department of Animal Pathology, College of Agronomy and Veterinary, National University of Río Cuarto, Argentina
[d] Department of Animal Health and Nutrition, Chr-Hansen, Argentina

ARTICLE INFO

Article history:
Received 29 August 2017
Received in revised form
1 December 2017
Accepted 7 December 2017
Available online 15 December 2017

Keywords:
Fetal programming
Gestation
IGF system
Hypoglycemia

ABSTRACT

Maternal nutritional restrictions during late gestation could lead to fetal hypoglycemia. Glucose levels in the fetal sheep regulate circulating insulin-like growth factor 1 (IGF1) levels, which stimulate cell proliferation and differentiation of reproductive organs after binding to its own receptor or estrogen receptors. The objective of this study was to determine the effects of subnutrition of ewes during the last trimester of gestation on the serum glucose/IGF1 levels and development of reproductive organs in their lambs.

Pregnant ewes carrying singletons were randomly assigned to restricted (R ewes, n = 8) or control (C ewes, n = 8) groups (4 lambs of each gender/group) and fed with 50% or 100% of metabolic energy requirements from ~100 days of gestation to term (~147 days), respectively. Blood samples from lambs were taken on the first day after born and once at week for serum glucose and IGF1 determination. Lambs were euthanatized at 2 months of age, reproductive organs were weighted and tissue samples were collected from them for histology and to measure mRNA expression of IGF1 and its receptor (IGF1R) by qRT-PCR. Pre-partum glucose levels in R ewes were significantly lower compared to C ewes ($p < .05$). Compared to lambs born from C ewes, lambs born from R ewes showed lower serum levels of glucose and IGF1 during the first week of age ($p < .05$). At 2 month of age, these lambs had significant lower uterine and testicular weight and lower ovarian, uterine and testicular mRNA expressions of IGF1 and its receptor ($p < .05$). Histological findings showed that diameter of secondary and tertiary follicles in ovaries and number of endometrial glands in uterus of females, or number of Sertoli cells and seminiferous tubules and diameter, perimeter and tubular area in testicles of males were significantly lower in lambs born from R ewes compared to the respective organs of lambs born from the C ewes ($p < .05$). In conclusion, these results demonstrate that maternal subnutrition during late gestation affects IGF1 levels during fetal life and impairs reproductive development in the neonatal lamb, which could have permanent negative consequences in the future reproductive performance of the offspring.

1. Introduction

The concept of "fetal programming" refers to gestational stages in which fetal development may be compromised or altered, resulting in physiological changes in the fetus that could be manifested during adulthood [1]. One of the most important extrinsic factors that influences fetal programming is maternal nutrition [2]. In the sheep, due reproductive seasonality, the last stage of gestation usually happens during late autumn or winter, when the forage supply is scarce. Maternal nutritional restriction during this gestational period leads to fetal hypoglycemia [3,4] and consequently, lower insulin growth factor 1 (IGF1) levels, which is indeed regulated by glucose concentrations [5].

* Corresponding author. National Scientific and Technical Research Council (CONICET), Argentina.
E-mail address: brabaglino@ufl.edu (M.B. Rabaglino).

https://doi.org/10.1016/j.theriogenology.2017.12.023
0093-691X/

Circulating IGF1 interacts with insulin-like receptor 1 (IGF1R) and estrogen receptor alpha (ESR1) to regulate cell proliferation, differentiation, and functions in diverse organs including the uterus, through autocrine and/or paracrine mechanisms [6]. Also, IGF1 and its receptors are locally expressed on the ovary and uterus [7]. For males, mRNA expressions of IGF1 and IGF1R have been measured in bovine fetal testicles [8], and mRNA expression of ESR1 has been measured in the efferent duct, epididymis and testes of ovine fetuses [9].

Some studies demonstrated the detrimental effect of maternal malnutrition during mid or late gestation in the development of fetal ovaries [10,11] and testicles [12–14] in the sheep. However, the effects of maternal subnutrition on the fetal uterus are still unknown. We hypothesized that subnutrition of ewes during late gestation will affect the circulating levels of glucose and IGF1 in the fetus, impairing the physiological development of reproductive organs during the fetal and neonatal life. The objectives of this study were to determine the effects of maternal subnutrition during the last trimester of gestation on the serum glucose/IGF1 levels of the lambs during the postnatal period and development of their reproductive organs at two months of age. Degree of reproductive development was estimated by comparison of reproductive organs' weight, histology and mRNA expression of IGF1, IGF1R, ESR1 and estrogen receptor beta (ESR2) in those organs at 60 days of age -when the histological and structural development of the reproductive organs in the lamb has been completed and it's similar to the adult [15,16]- between lambs born from nutritionally restricted ewes or from controls ewes.

2. Methodology

2.1. Animals

All procedures were approved by the National University of Río Cuarto Ethical Committee of Research in animals (CoEdI). Pregnant Hampshire Down ewes (n = 16), of similar age and body condition scores at around 90 days of gestation were housed in individual pens in an enclosed barn at the National University of Río Cuarto (UNRC). After the adaptation period (2 weeks), ewes were randomly assigned to the control group (C ewes, n = 8) or restricted group (R ewes, n = 8). Ewes were daily fed with a diet based on alfalfa hay, ground corn grain and mineral and vitamins mix at100% (C ewes) or 50% (R ewes) of their metabolizable energy requirements according to the National Research Council (NRC) of 2007 [17] until the end of gestation (144–148 days). Each group had the same number of pregnant ewes with female (n = 4/group) or males (n = 4/group) fetuses, determined by ultrasound at ~70 days of gestation [18]. After delivery, lambs were properly identified, weighted, and kept with the mother until euthanasia, when they were weighted again.

2.2. Sampling

For ewes, blood samples from the jugular vein were collected from day 0 (assignation of ewes to each group) until delivery once a week. For lambs, the first sample was taken on the first or second day of life and then once a week until euthanasia at 2 months of age. Blood was collected in glass tubes, placed in ice and taken to the laboratory for centrifugation at 3000 rpm for 15 min for serum separation, which was stored at −20 °C until analysis.

For tissue samples collection, lambs were euthanized at 60 days of age. The uterus of each lamb was dissected away from the broad ligament, oviduct and cervix and weighed. The ovaries, testes and epididymides were also dissected and weighed. One of the ovaries, one testis, one epididymis and 1 cm^3 section of the middle portion of each horn were immersion fixed in 4% buffered paraformaldehyde overnight, then transferred to 70% reagent alcohol until paraffin embedded for histology. The other ovary, testis, epididymis and another section of the uterine horn were snap-frozen in liquid nitrogen and then stored at −80 °C until they were processed for mRNA extraction.

2.3. Measurement of blood metabolites

Glucose levels were determined at the moment of blood collection using a glucose meter (ACCU-CHEK® Performa), once a week for both ewes and lambs.

For the ewes, IGF1 levels were measured weekly until the first day after parturition, while estradiol (E_2) levels were determined on the week prior to delivery (week −1). For the lambs, measurements of IGF1 were made every 10 days until 40 days of life. Estradiol concentration was determined at ~50 days of age.

The determination of IGF1 and E_2 was performed by radioimmunoassay (RIA) in the IBYME laboratory (CONICET-Argentina). A previous extraction with HCl-ethanol and cryoprecipitation was done for IGF1, while E_2 was extracted with ethyl ether [19].

2.4. Histology

Histological sections of ovarian, testicular and uterine tissues were cut at 5 μm and stained with hematoxylin and eosin following a standard methodology. Histological examination and measurements were performed using the Fiji software [20]. Diameters of ovarian follicles were measured in at least 10 histological sections per animal at 4X. Uterine endometrial gland and glandular cells were counted at 40X, considering those that were in the ventral zone of the uterine horn and when at least half of the gland was observed. Gland cell counting was performed taking 10 glands at random (no overlapping or mitosis cells were counted). For the testicular sections, Sertoli cells were counted at 100X, taking 45 random fields per section. Cells with basal, leptochromatic (lax chromatin), ovoid or pyriform nuclei and with an eccentric nucleolus were considered. Seminiferous tubules were counted at 40X in 10 random fields per section, and they were considered if at least half of the tubule was observed. Determinations of diameter, perimeter, and tubular area were done selecting 10 transverse sections of seminiferous tubules per photo at 20X. For tubular diameter determination, two perpendicular measurements were taken by tubule and then averaged, because the tubules are not totally spherical. For each animal, the diameter, perimeter and tubular area were determined in at least 10 histological sections.

2.5. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Messenger RNA was extracted from the uterine, ovarian, testicular and epididymal tissues using Trizol reagent according the manufacturer's protocol (Thermo Scientific). Isolated RNA concentration was determined with Nanodrop, and RNA was stored at −80 °C until cDNA conversion. An aliquot of the extracted RNA was converted to cDNA using M-MLV Reverse Transcriptase (Promega) following the methodology recommended by the manufacturer. The synthesized cDNA was stored at −20 °C until qRT-PCR was performed.

The genes selected for mRNA measurements were: IGF1, IFG1R, ESR1, ESR2 and ACTB (β-actin), as housekeeping gene. Relative expressions of selected genes were determined using primers (Invitrogen) and SYBR Green PCR Master Mix (Applied Biosystems). Primers were designed with Primer Express software (Applied