Domestic Animal Endocrinology 59 (2017) 105–115

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Glucogenic treatment creates an optimal metabolic milieu for the conception period in ewes

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 29 November 2016
Accepted 4 December 2016

Keywords:
Glycerol
NEFA
Urea
Follicle
Insulin
IGF-1

ABSTRACT

This study determined the influence of a short-term glucogenic nutritional treatment on circulating concentrations of glucose, insulin, insulin-like growth factor 1 (IGF-1), nonesterified fatty acids (NEFA), and urea, and on their correspondent levels in follicular fluid (FF) collected 12 h after the end of the treatment. After estrous synchronization with intravaginal progestagen-impregnated sponges, 20 Sarda ewes were randomly allocated into two experimental groups (GLU and WAT) and, from day 7 to day 10 (day 0 = day of sponge removal), the GLU group was gavaged with a glycogenic mixture, whereas the WAT group was gavaged with water (control group). Follicular development was stimulated by FSH administration from day 8 to 10. At day 11, ovaries were collected and follicular fluid processed. Plasma changes were assessed from day 6 to 11. In GLU group, circulating concentration of glucose ($P < 0.0001$), insulin ($P < 0.0001$), and IGF-1 ($P < 0.01$) rose significantly, whereas NEFA and urea concentrations decreased ($P < 0.0001$), as compared with controls. In particular, in FF the higher glucose concentrations found in GLU ewes compared with controls ($P < 0.0001$) were not accompanied by any increase in insulin and IGF-1 concentrations. NEFA ($P < 0.0001$) and urea ($P < 0.0001$) were lower in FF of GLU than WAT group, although NEFA clearance in the ovary proved to be less efficient than at the systemic level. No significant difference between groups was found in FF concentrations of pregnancy-associated plasma protein A (a protease regulating the levels of free IGF-1 in follicles), glutathione, and in its total antioxidant capacity. These results suggest that glycogenic mixture administration creates a suitable follicular microenvironment for the conception period in dairy ewes.

1. Introduction

Nutrition is one of the main determinants of reproductive performance in ruminants [1]. One of the key nutrients having an effect on the ovary is glucose [2,3], since it has distinct roles in follicular function: first as a nutrient to generate ATP, and second as a signaling molecule to stimulate folliculogenesis when nutritional conditions are favorable to reproduction [4]. The role of glucose is also essential in determining the quality of the oocyte [5]. In a previous study on dairy sheep, our research group showed that short-term flushing with a glucogenic mixture based on glycerol and propylene glycol improves oocyte quality, evaluated by the kinetics of their in vitro development and by the production of blastocysts [6]. In another study, the same nutritional treatment increased the ovulation rate [7]. Glucogenic precursors such as propylene glycol and glycerol and their mixture have been used in veterinary

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http://dx.doi.org/10.1016/j.domaniend.2016.12.003

practice to increase blood glucose and to reduce nutritional problems in dairy cows during the peripartum [8–10]. It has been reported that feeding glycerol as a top dress [8,11] or supplied in water [10] to transition dairy cows resulted in a positive energy status with higher concentrations of serum glucose and lower concentrations of plasma nonesterified fatty acids (NEFA) [9].

Glucose can be metabolized within the follicle by the pentose phosphate pathway to provide precursors for the synthesis of purine nucleotides and NADPH which are in turn used in various biosynthetic pathways, including those related with the antioxidant defense. Hence, glucose follicular fluid (FF) concentration can also influence follicular oxidative status [1].

In addition to the glucose-insulin system, another hormone playing a key role in the energy status and function of the ovary is the insulin-like growth factor 1 (IGF-1). Insulin-like growth factor 1 and gonadotropins are synergistic for growth and differentiation of the follicle [12,13]. The IGF system (receptors, ligands, and binding proteins) is expressed within granulosa and theca cells [12–14].

Starting from these premises, this study aimed at assessing the effect of a short-term administration of a glucogenic mixture on increasing the plasmatic and intrafollicular concentrations of metabolites and hormones which play a key role in follicular maturation and quality. In particular, we measured plasma levels of glucose, insulin, IGF-1, NEFA, and urea during the nutritional treatment and their corresponding levels in the FF as measured 12 h after the last administration of the glucogenic mixture. At the same time, the oxidative status of the FF was also investigated. Finally, to assess possible changes in IGF-I bioavailability in the FF, the concentration of pregnancy-associated plasma protein A (PAPP-A), a protease regulating the levels of free IGF-1 in dominant follicles [15,16] were determined in the FF.

This information is pivotal to better explain the effect of a rise in glycemia on the endocrine and metabolic milieu of sheep at mating and to set the basis for the formulation of short-term flushing treatment able to create the best conditions for the conception period.

2. Materials and methods

2.1. Animals

The experimental procedures with animals were approved by the Animal Care and Use Committee of the University of Sassari. Twenty Sarda ewes, 4–5 years old, were used. The ewes were penned outdoor with access to a sheltered area, at the experimental facilities of the Department of Veterinary Medicine at the University of Sassari, Italy (40°43′40.33″N, 8°33′1.33″E). These facilities meet the requirements of the European Union for Scientific Procedure Establishments. The ewes were group fed a maintenance ration at a level of 46 g of dry matter per kg of metabolic weight ($BW^{0.75}$) consisting of hay and concentrate fed twice daily.

The experiment was run during October 2014, within the natural breeding season (late August–late December) described for this breed at this latitude.

In brief (Fig. 1), synchronization was induced in all the animals with the insertion of one intravaginal progestagen-impregnated sponge (45-mg fluorogestone acetate, FGA, Chronogest; Intervet International, Boxmeer, the Netherlands) which remained in situ for 6 d. On the day of sponge withdrawal (day 0), the ewes received 125 μg of a prostaglandin analogue (cloprostenol, Estrumate, Essex Animal Health, Friesoythe, Germany) by i.m. injection. At the same time, ewe live weight (42.3 ± 0.9 kg) was determined. On day 0, the ewes were divided in two experimental groups at random. From day 7 to day 10 after sponge withdrawal, one group (GLU: n = 10; weight 42.2 ± 1.3 kg;) received, orally twice daily at 8.00 AM and at 19.00 PM, 200 mL of a glucogenic mixture, as previously described [7]. The glucogenic formulation contained 70% glycerol and 20% propylene glycol (both from Sigma Chemical Co, St. Louis, MO, USA) and 10% water. The control animals (WAT: n = 10; weight 42.3 ± 1.3 kg;) received 200 mL of water twice daily simultaneously to treatment administration. Both the glucogenic formulation and the water were administered by oral gavage using an esophageal feeding tube.

From day 8 to 10, follicular development was stimulated in all the ewes by the administration of 175 IU of FSH (Folltropin; Bioniche Animal Health, Bio 98, Milano, Italy) given every 12 h in 6 equal doses.

At day 11, 12 h after the last FSH administration, the ewes were weighed and then conducted to the slaughterhouse where they were sacrificed. After having collected the ovaries, follicles and corpora lutea on their surface were counted and FF from follicles ≥4 mm was aspirated with a 2.5-mL syringe fitted with a 22-gauge needle.

2.2. Blood and FF sampling

Plasma concentrations of glucose, NEFA, urea, insulin, IGF-1, and progesterone (P4) were determined from samples drawn from jugular vein at 8.00 a.m from day 6 to day

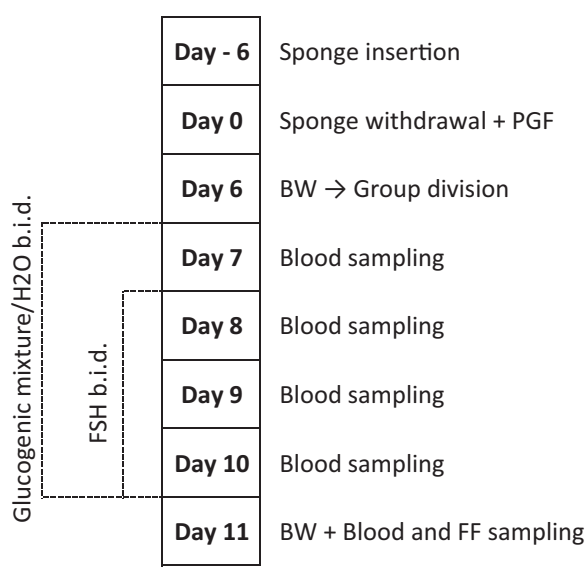

Fig. 1. Experimental protocol.