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Feed restriction as a biostimulant of the production of oocyte, their quality and GDF-9 gene expression in rabbit oocytes

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ABSTRACT

The use of short-term feed restriction (R) without or with subsequent refeeding (F) as biostimulant of rabbit fertility was examined in this study. A total of 40 mature, non-pregnant, non-lactating New Zealand white female rabbits were allocated to five treatments. The rabbits were individually caged and fed a complete pelleted diet (16.7% CP; 13.1 CF; 2490 kcal DE/kg). Rabbits on the control (C) treatment received 150 g/d of the diet. Two groups of 8 rabbits received 70% of the control daily feed intake (105 g/d; moderate restriction; M) and the other two groups received 50% of the control feed intake (75 g/d; severe restriction; S) for 21 d. At the end of this period, one group each of M and S fed rabbits were slaughtered for oocyte recovery. Rabbits in the remaining three groups (C, MF and SF) were retained for a further 8 d before slaughter and fed the control level of the diet during this period. The effects on body weight, oocyte number and quality, GDF-9 gene expression in oocytes, and changes in serum levels of leptin and IGF-1 were recorded. Initial mean body weights were not significantly different ranging from 2.50 ± 0.33 kg (S) to 2.58 ± 0.24 kg (C). After 3 wk on treatment the C rabbits were significantly heavier (2.65 ± 0.32 kg; $P < 0.05$) than rabbits on the M (2.26 ± 0.33 kg) or S (2.10 ± 0.33 kg) treatments. Following 8 d of refeeding, the remaining group of S treated rabbits (SF) were still significantly lighter (2.40 ± 0.21 kg; $P < 0.05$) than C (2.71 ± 0.31 kg) with MF rabbits having an intermediate weight (2.50 ± 0.20 kg). The number of mature grade A oocytes recovered per ovary was significantly lower for control (3.3 ± 0.35) than the refed treatments (MF 4.0 ± 0.30; SF 4.5 ± 0.39; $P < 0.05$). Semi-quantitative PCR analysis of GDF-9 expression showed that control mature grade A oocytes had significantly lower levels of expression (1.27 ± 0.20; $P < 0.05$) than those of refed rabbits (MF 1.60 ± 0.10; SF 1.39 ± 0.01). Leptin and IGF-1 values for refed rabbits were significantly higher ($P < 0.05$) than at the end of feed restriction and the start point. It was concluded that this biostimulant method has the potential to improve the fertility of rabbits.

1. Introduction

Over last few years, important work has been done particularly by the International Rabbit Reproduction Group (I.R.R.G) to set up methods which do not require the use of hormones to increase sexual receptivity at time of insemination and subsequent reproductive potential of rabbit does. These are called "biostimulation methods" and comprehend a large spectrum of techniques including feed restriction (Mahmoud et al., 2006; Manal et al., 2010) and feeding program (Abd El-Kafy, 2006; Castellini et al., 2010).

Folliculogenesis is controlled by gonadotropins from pituitary, and autocrine/paracrine factors that are

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produced in the ovary. Some of these molecules are synthesized and secreted by the oocytes and act as morphogens that control follicle growth as well as differentiation (Knight and Glister, 2001). The transforming growth factor-(TGF-) superfamily which contains over 35 members, has been shown to be important for regulating fertility (Chang et al., 2002; Knight and Glister, 2003; Juengel et al., 2004) .Two members of this family recently identified as having a role in regulation of fertility are the related oocyte-derived family members namely, growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15, also known as GDF9B). GDF-9 gene was discovered together with GDF-3 gene using primers corresponding to conserved regions of the known family members through PCR and sequencing analyses by McPherron and Lee (1993). GDF9 was first shown to be essential for follicular development (Bernal et al., 2010) when lacking GDF9 gene mice were found to be infertile with follicular development blocked at a very early stage (Dong et al., 1996). GDF-9 protein was found in oocytes throughout the entire period of follicle development even after ovulation. The exclusive expression of GDF-9 gene in the oocyte is unique among known growth-factor-like molecules and suggested that the oocyte could at least partially, control follicle development by secreting this putative paracrine factor (Ursula and Aaron, 2000).

Over the last years, with the development of the field of molecular biology a number of investigators have examined the effects of nutrient status on gene expression patterns in various animal tissues (Khalil et al., 2008) and newly on ovaries and testes genes (Sharov et al., 2008; Bernal et al., 2010). The objective of this study was to examine the use of feed restriction as a biostimulant of the reproduction, quality and GDF-9 expression of rabbit oocytes.

2. Materials and methods

2.1. Experimental design

This study was conducted using 40 mature premiparous non pregnant non lactating female New Zealand White (NZW) rabbits with average body weight of 2.5 ± 0.30 kg. The rabbits were individually kept in galvanized wire batteries (50 cm × 50 cm × 30 cm) equipped with an automatic drinker and a manual feeder, in naturally well ventilated building, at January in Lab. animal unit, National Research Centre, Giza, Egypt, at latitude 31.2° north. Does were submitted to a 12-h photoperiod daily and the minimum ambient temperature was set at 15 ± 2 °C. Rabbits were fed on a formulated pellet ration according to NRC (1977). The ingredient composition and chemical analysis of the experimental ration is shown in Table 1.

The rabbits were allocated at random to five treatments ($n = 8$ per treatment), individually caged and fed a balanced pelleted diet (16.7% CP; 13.1 CF; 2490 kcal DE/kg). Rabbits on the control (C) treatment received 150 g/d of the diet. Two groups of 8 rabbits received 70% of the control daily feed intake (105 g/d; moderate restriction; MR and MF) and the other two groups received 50% of the control feed intake (75 g/d; severe restriction; SR and SF) for 21 d. At the end of this period, one group of both restricted fed rabbits was slaughtered for oocyte recovery (MR and SR). Rabbits in the remaining three groups (C, MF and SF) were retained for a further 8 d before slaughter and fed the control level of the diet during this period. Blood samples were collected 3 times (at the beginning; end of feed restriction; after refeeding) from the ear vein of control and treated groups and in addition body weight was determined for all groups at the same time intervals.

Table 1
composition and chemical analysis of the diet fed to rabbits.

Ingredient	%
Clover hay	30.0
Wheat bran	26.2
Barley	23.0
Soybean meal (44% protein)	16.0
Molasses	3.0
Limestone	1.0
Premix[a]	0.3
Salt	0.5
Total	100
Calculated chemical composition of diets[b]	
Crude protein (CP) %	16.72
Ether extract (EE) %	2.95
Crude fiber (CF) %	13.07
Digestible energy (DE) kcal/kg	2490

[a] Every 3 kg premix contains: vitamin A 10 M.i.u.; vitamin B6 1500 mg; copper 4000 mg; vitamin D3 2 M.i.u.; vitamin B1 100 mg; iodine 300 mg; vitamin K 1000 mg; vitamin B2 5000 mg; selenium 100 mg; vitamin E 10,000 mg; vitamin B12 10 mg; iron 30000 mg; choline 120,000 mg; biotin 50 mg; manganese 60,000 mg; nicotinic acid 30,000 mg; folic acid 1000 mg; cobalt 100 mg; pantothenic acid 10,000 mg; Zinc 50,000 mg.
[b] Calculated according to NRC (1977).

2.2. Classification of oocytes quality and quantity

The ovaries were collected from 6 slaughtered rabbits in each group (control, feed restricted and refeeding groups) and rinsed several times in warm (30–38 °C) phosphate buffer saline (PBS). The oocytes were harvested in aspiration media consisting of modified phosphate buffer saline Sigma Chemicals CO. (St. Louis, MO, USA). (pH 7.2, M 0.01) supplemented with 3% heat inactivated fetal calf serum (Grand Island, New York, USA). The oocytes were collected by slicing method (Bavister, 1989) and classified into three categories according to number of cumulus oocyte complex layers (Hadek, 1965) Class A is the largest oocytes which surrounded by completely expanded cumulus cell layers; Class B is complete and partially invested with cumulus cell layers and Class C is the smallest oocyte that have no any cumulus layers (denuded cell). Class A and B oocytes were mechanically freed from surrounding cumulus oocyte complex (COC) by centrifugation for 1 min and 3 min respectively. These mechanically denuded oocytes were collected after centrifugation and preserved in −80 °C till isolation of mRNA.