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Ovarian response and embryo gene expression patterns after nonsuperovulatory gonadotropin stimulation in primiparous rabbits does

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ABSTRACT

Ovarian stimulation with equine chorionic gonadotropin (eCG) is largely used in animal reproductive technologies to provide a larger number of oocytes and embryos and to improve the reproductive outcome. However, the consequences of maternal treatment with eCG on embryo gene expression patterns are not widely studied. The aim of this work was to assess the ovarian response (preovulatory follicular population, oocyte maturation, ovulation rate, and serum steroid concentrations), the early embryo survival and gene expression patterns of a panel of quality-genes involved in glucose intake, oxidative stress, apoptosis, proliferation, implantation, and fetal growth in embryos of lactating rabbits treated with eCG. A total of 34 primiparous rabbit does (*Oryctolagus cuniculus*) were randomly distributed at Day 23 postpartum into a treatment group receiving a unique nonsuperovulatory dose (25 IU) of eCG (eCG group; N = 17 does); or a control group without eCG treatment previously to artificial insemination (control group; N = 17 does). After 48 hours, 8 does of each group were euthanized and their ovarian response was studied. The rest of animals were artificially inseminated and their ovulation was induced with a GnRH analogue. Embryos were recovered 3.5 days later. The oocytes retrieved for *in vitro* maturation showed no differences in metaphase II rate in both experimental groups, although oocyte cytoplasmic maturation, in terms of cortical granule migration rate, was improved in eCG-treated does ($P < 0.05$). The mean number of preovulatory follicles was similar between groups but the ovulation rate was significantly higher in eCG-treated does compared with does not stimulated ($P < 0.05$). No differences were found in serum estradiol and progesterone concentrations of does the day of oocyte and embryo recovery, respectively. However, progesterone:estradiol ratio was slightly increased in eCG group on embryo recovery day ($P = 0.1$). The percentage of embryos recovered at the blastocyst stage was also increased in eCG-treated does ($P < 0.05$), nevertheless, there were no differences in the gene expression patterns of candidate genes *SLC2A4, IGF1R, IGF2R, SHC1-SHC, TP53, PTGS2*, and *PLAC8*; except for the transcripts of *SOD1* mRNA which were downregulated in eCG-derived embryos ($P < 0.05$). In conclusion, the administration of eCG improves ovulation rate, oocyte cytoplasmic maturation, and blastocyst formation in primiparous rabbit does inseminated on Day 25 postpartum. Although it seems not to influence the gene expression patterns studied, a lower antioxidant defense of embryos developed after the maternal eCG treatment is suggested.

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1. Introduction

Ovarian gonadotropin stimulation is routinely administered in animal reproductive technologies for estrus synchronization and multiple ovulation and embryo transfer protocols to encourage follicular growth and generate more developmentally competent oocytes. The action of eCG (equine chorionic gonadotropin) is analogous to that of follicle stimulating hormone (FSH) and luteinizing hormone (LH) enhancing final follicular development. This gonadotropin requires a single administration because it has a relatively long circulating half-life [1]. Therefore, the application of this hormone is largely used in livestock industries as a source of exogenous FSH and LH to increase preovulatory follicular population, female receptivity, and reproductive success, and also in animal laboratory research for supplying oocytes for somatic cell nuclear transfer, animal cloning, and transgenesis [2–4]. In rabbit breeding, eCG is usually applied for improving follicular development and as a method for estrous synchronization in high-yield lactating animals because lactation exerts an inhibitory effect on ovarian function, especially in primiparous does [4,5]. Moreover, gonadotropin stimulation protocols can modify peripheral estradiol (E_2) and progesterone (P_4) concentrations, as well as their ratio (P_4:E_2), and therefore, the environment in which oocytes and embryos are developed [6,7]. Indeed, the eCG administration seems to mediate cytoplasmic oocyte maturation supporting monospermic fertilization and early embryo development [8,9]. However, evidence from other studies indicates that it could reduce embryo development in rabbits [10], and implantation rate, fetal development, and fetal weight in mice [6], and also can provoke ovarian anomalies [11]. Nowadays, the effect of this hormone on embryo quality is still controversial.

Gene expression patterns in early stage embryos rely mostly on the posttranscriptional control of maternal transcripts accumulated during follicular development and oocyte maturation [12]. Consequently, synthetic gonadotropins such as eCG could induce changes in the maternal periconceptional environment that might provoke epigenetic modifications affecting expression of genes that control embryo metabolism and early embryo development. In fact, some important events such as proliferation, differentiation, implantation, or apoptosis in early embryos are mediated by specific expression and quantity of numerous transcripts. Such marks can predict the fertility prognosis, fetal growth, and the health of future offspring [13]. However, the consequences of eCG application on embryo gene expression patterns are not yet elucidated. Candidate genes such as solute carrier family 2 (facilitated glucose transporter) member 4 (*SLC2A4*) and insulin-like growth factor 1 receptor (*IGF1R*) mediate cell proliferation and differentiation from the morula to the blastocyst stage in rabbit [14,15] and mice [16,17]. The *IGF1R* and insulin-like growth factor 2 receptor (*IGF2R*) transcripts are also considered as markers for fetal growth rate [18,19]. On the other hand, an adequate nitric oxide synthase 3 (endothelial cell) (*NOS3*) production by embryos is necessary to provide an adequate blood supply to the site of blastocyst implantation [20]. This together with an upregulation of mRNA transcripts of some genes as prostaglandin-endoperoxide synthase 2 (*PTGS2*) and placenta-specific 8 (*PLAC8*) in blastocysts can be a tool to predict successful pregnancy and delivery in cattle [21]. Moreover, it is known that metabolic and endocrine changes in the periconceptional environment can induce an oxidative stress in embryos and reduce their quality [22]. It is reflected by expression of genes related with antioxidant defense as superoxide dismutase 1 soluble (*SOD1*) and apoptotic pathways associated with oxidative stress as Src homology 2 domain containing transforming protein 1(*SHC1-SHC*) and tumor protein 53 (*TP53*), which upregulation is linked with subsequent increasing of fetal losses and fetal malformations in cattle and mice [23,24].

Taking into account these premises, it should be advisable to use hormone protocols based on the understanding of how they affect the oocyte competence and the yield, quality, and gene expression patterns of early embryos routinely produced in laboratory and farm animals. However, as far as we know, there is scarce literature about the consequences of maternal eCG administration on embryo gene expression patterns [25], and no reports existing in the rabbit specie. In this sense, rabbit is considered as a valuable animal model to study such effects for economic reasons in livestock industries and also for biomedical research, because of the precise timing of ovulation which allows the knowledge of the exact embryonic age and also for the high number and size of embryos available [26].

Thus, the aim of the present work was to determine if a single dose of eCG administered before ovulation can change the relative abundance of a panel of quality genes, involved in important aspects of glucose intake, oxidative stress, apoptosis, mitogenic capacity, implantation, and fetal growth in the rabbit blastocyst. Besides, serum estradiol and progesterone concentrations, preovulatory follicular population, nuclear and cytoplasmic oocyte maturation, ovulation rate, and early embryo development were assessed to increase the understanding of the relationship between maternal periconceptional changes and early embryo responses, and to provide insight to the origin of oocyte and embryo alterations that could be of relevance for the reproductive strategies in animals and humans.

2. Material and methods

Unless otherwise stated, all the chemicals were purchased from Sigma Chemical Company (Spain). Experimental procedures were approved by the Animal Ethics Committee of the Polytechnic University of Madrid (Spain) and were in compliance with the Spanish and European guidelines for care and use of animals in research [27,28].

2.1. Animal housing and experimental design

New Zealand × California white rabbit does *(Oryctolagus cuniculus)* were held on the experimental facilities at the Animal Production Department, Polytechnic University of Madrid (Spain). Animals were housed in individual flat-deck cages under a constant photoperiod of 16 hours of light per day. A temperature of 18 °C to 22 °C and a relative humidity of 60% to 75% were maintained by a forced