Tissue and Cell 47 (2015) 478–488

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Potential roles of matrix metalloproteinases and characteristics of ovarian development in neonatal guinea pigs

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ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form 1 July 2015
Accepted 20 July 2015
Available online 21 July 2015

Keywords:
eCG
Neonatal guinea pigs
StAR
PCNA
MMP-2
MMP-9

ABSTRACT

The present study was conducted to investigate expression of matrix metalloproteinases (MMPs) and early ovarian development in neonatal guinea pigs. Thirty neonatal guinea pigs at 3 or 8 days of age were administrated 5 IU equine chorionic gonadotropin (eCG) or saline, and the ovaries were collected after 2 days of eCG. Serum concentrations of estradiol and progesterone were determined, and ovarian localization of StAR, MMP-2 and MMP-9 were analyzed by immunohistochemical staining. Results indicate that injection of eCG sensitized the neonatal ovary and elevated serum concentrations of estradiol and progesterone, but not enough to stimulate ovarian follicular development in the ovaries. MMP-2 and MMP-9 were both immunolocalized to the surface of granulosa cells of primary and secondary follicles, and high MMP-2 expression was accompanied by low StAR expression in eCG-treated ovaries. Collectively, we hypothesize that MMP-2, -9 and StAR are both involved in follicular atresia through their participation in cell proliferation and tissue remodeling.

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1. Introduction

Guinea pigs share some similarities in their reproductive cycle with humans, have a small ovulatory quota, and constitute a reliable animal model for studying human reproduction (Kulduk et al., 2014). However, ovarian development after birth in guinea pigs has been studied rarely, when compared with other rodents. Gonadotropins are basically used to induce multiple ovulations in animals, and equine chorionic gonadotropin (eCG) is a standard gonadotropin used to recruit additional follicles (Bó and Mapletoft, 2014). eCG plays a role similar to that of follicle-stimulating hormone (FSH) in superovulation (Mapletoft et al., 1990), but has a half-life up to 40 h (Zanetti et al., 2014; Murphy and Martinuk, 1991). eCG was effective in rats and mice, but eCG did not stimulate superovulation in adult guinea pigs (Suzuki et al., 2003). Our previous study showed that eCG played a role in adult guinea pigs similar to that of luteinizing hormone (LH); *i.e.*, eCG induced luteinized unruptured follicle (LUF) syndrome in cyclic guinea pigs (Supplement Material). Our understanding of the sensitivity of neonatal guinea pigs to eCG is lacking and the etiology of LUFs is still unknown.

Matrix metalloproteinases (MMPs), which participate in tissue remodeling (Kessenbrock et al., 2010), are also involved in morphogenesis, apoptosis, ovulation and angiogenesis (Jones, 2014; Roy et al., 2006; Vu and Werb, 2000). MMPs play a critical role in follicular development in ovaries by remodeling the extracellular matrix (ECM) in the periovulatory period (Bagavandoss, 1998; Baker et al., 2000; Cooke et al., 1999; McCaffery et al., 2000). MMP-2 and MMP-9, both with a fibronectin-like domain in the middle of the catalytic domain (Bode et al., 1999), can degrade collagens, and possess laminin and aggrecan core proteins (Nagase et al., 2006), and MMP-2 and MMP-9 were both immunolocalized in developing follicular theca and stroma in rodents (Garcia et al., 1997). The intrafollicular levels of MMP-2 and MMP-9 both increased during the progression from normal to atresia ovarian follicles in sheep (Huet et al., 1997). A higher production of MMP-2 and MMP-9 was also accompanied by a higher follicular apoptosis rate in patients (Ben-Shlomo et al., 2003; Shalev et al., 2001).

The objective of the present study was to investigate the role of eCG in neonatal guinea pigs, with a focus on expression of MMP-2 and MMP-9 in ovaries. In addition, immunolocalization of steroidogenic acute regulatory protein (StAR) and

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http://dx.doi.org/10.1016/j.tice.2015.07.005
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proliferating cell nuclear antigen (PCNA) were performed, since they are the indicators of steroidogenesis (Clark et al., 1997) and cell proliferation (Sun et al., 2014; Wildemann et al., 2003), respectively.

2. Materials and methods

Thirty neonatal female Harley-White guinea pigs (*Cavia porcellus*) at 3 days of age were obtained from the Laboratory Animal Research Center of Zhejiang Chinese Medical University (Hangzhou, China). The guinea pigs were randomly divided into 5 groups (each $n = 6$): groups D-3, D-5, P-5, D-10 and P-10. The animals in group D-3 were sacrificed at 3 days of age; the animals in groups P-5 and D-5 were administered 5 IU eCG or saline at 3 days of age, and then sacrificed at 5 days; the animals in groups P-10 and D-10 were administered 5 IU eCG or saline at 8 days of age, respectively, and then sacrificed at 10 days of age. Blood samples were collected before scarification; and ovarian samples were collected immediately after scarification. The serum was separated from blood by centrifugation at 5000 g for 10 min, and the serum concentrations of estradiol and progesterone were measured (Adicon Clinical Laboratories INC, Hangzhou, China). The ovaries were fixed in 4% paraformaldehyde at room temperature for 36 h, and then kept in 70% alcohol for histologic and immunohistochemical (IHC) analyses.

After fixation, ovarian samples were embedded in paraffin, sectioned serially at 5 μm and stained with H&E. Sections were analyzed for observations of morphologic changes in ovaries after eCG injection. The follicular stages in neonatal guinea pigs were determined according to a previous study on mouse ovaries (Flaws et al., 1997). The numbers of primordial and antral follicles were both counted: primordial follicles in guinea pigs contained only one layer of granulosa cells, while antral follicles contained an antrum.

In order to immunolocalized steroidogenesis in ovarian follicles, we performed IHC staining using monoclonal antibodies against StAR and PCNA (Santa Cruz Biotechnology Inc., TX, USA; SC25806, lot: I2308; SC7907, lot: L5346) with a strept avidin-biotin complex (SABC) kit (Boshide Biotechnology Inc., Wuhan, China; SA2002, lot: 10C09A). Ovarian expressions of MMP-2 and MMP-9 were characterized by IHC staining with antibodies to MMP-2 and MMP-9 with an SABC kit (all from Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China; lot: MAB-0244; lot: MAB-0245; lot: KIT-5910). The antibodies were diluted to 1:200 in phosphate-buffer saline (PBS) containing 1% bovine serum albumin (BSA). Heat-induced epitope retrieval (HIER) was performed by heating the slides immersed in the 10 mM sodium citrate buffer (pH 6.0) at 100 °C in a microwave oven for 8 min. The sections were mounted on slides coated with 3-aminopropyl-triethoxysilane (APES), and dried at 37 °C for 24 h. The sections were incubated with the primary antibodies overnight at 4 °C. The immunoreactivity was visualized using diaminobenzidine (DAB) (Sigma-Aldrich Corp., MO, USA) as substrate and counter-stained with hematoxylin. Normal rabbit serum, instead of primary antibody, was used as negative control.

The stained ovarian samples were observed in a BX51 electrical microscope (Olympus Corporation, Tokyo, Japan). Images were recorded by an XC10 optical imaging system (Olympus Corporation), and analyzed on OlyVIA (version 2.4, Olympus Soft Imaging Solutions GmbH, Japan).

Statistical analyses were performed on IBM SPSS Statistics 21 (Chicago, IL, USA). Differences were evaluated by one-way analysis of variance (ANOVA) with followed by Tukey's test. $P < 0.05$ was considered to be significant.

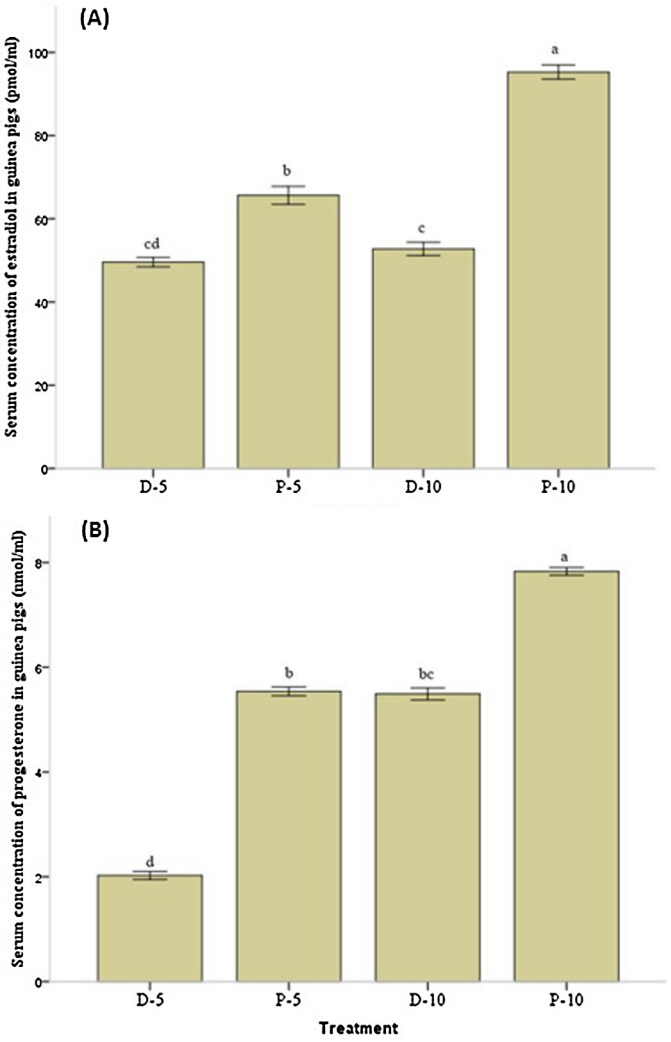

Fig. 1. Serum concentrations of estradiol and progesterone in neonatal guinea pigs after eCG injection. D-5, 5-day-old guinea pigs with saline injection at 3 days of age; P-5: 5-day-old guinea pigs after injection with 5 IU eCG at 3 days of age; D-10, 10-day-old guinea pigs with saline injection at 8 days of age; P-10: 10-day-old guinea pigs with injection of 5 IU eCG at 8 days of age. Each value is expressed as mean ± SEM. Different superscripts represent significant differences among categories ($P < 0.05$) as analyzed by Tukey test; while identical letters denote non significance ($P > 0.05$). *F*-test for homogeneity of variance, $P > 0.05$; *W*-test for normality of distribution, $P > 0.05$.

3. Results

3.1. Serum concentrations of estradiol and progesterone in neonatal guinea pigs

As shown in Fig. 1, the serum concentrations of estradiol are significantly higher in group P-5 vs. group D-5, in group P-10 vs. group D-10, and in group P-10 vs. group P-5 (all $P < 0.05$); the serum concentrations of progesterone are significantly higher in group P-5 vs. group D-5, in group P-10 vs. group D-10, in group P-10 vs. group P-5, and in group D-10 vs. group D-5 (all $P < 0.05$).

3.2. Observation of ovarian morphology in neonatal guinea pigs

Few antral follicles were observed in the ovaries of group D-3 (Fig. 2A). Specifically, primordial follicles were distributed close to ovarian walls (Fig. 2A1) and surrounded primordial follicles.

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