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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

# Effects of GnRH agonist on reproductive functions of female rabbits*

**Suocheng Wei[1,**], Zhuandi Gong[2], Wei Ma[3], Min Wei[1]**

[1] Life Science and Engineering College, Northwest University for Nationalities, Lanzhou 730030, China

[2] Medicine College of Northwest University for Nationalities, Lanzhou 730030, China

[3] Animal Science and Technology College of China Agricultural University, Beijing 100092, China

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**The physiological efficiency and mechanisms of action were studied of gonadotropin releasing hormone analogue (GnRH-A) on the reproductive functions of rabbits. Twenty-four female rabbits (*Oryctolagus cuniculus*) were randomly divided into four groups. Group EG-1, EG-2 and EG-3 were subcutaneously injected with 1.0 mL GnRH-A antigen at 100 μg/mL, 100 μg/mL and 50 μg/mL. EG-2 and EG-3 were re-injected on day 20. CG was a control group. Ovary and uterus samples in each animal were collected aseptically on day 70. The tissue slices were observed under optical and electron microscopy. Serum FSH and LH concentrations were detected using ELISA. GnRHR, FSH-β and LH-β genes were assayed with real time PCR. OCT, PFV, PFT, EET and UWT were measured with Motic system. The results showed that FSH and LH concentrations in EG-2 and EG-3 reached the peak on day 40. EG-2 was higher than EG-1 and CG ($P<0.01$) and EG-3 ($P<0.05$). GnRHR in EG-3 was higher than that in EG-1 and EG-2 ($P<0.05$). FSH-β in EG-1 and EG-3 were lower than that in EG-2. Meanwhile, LH-β in EG-3 was less than in EG-1 and EG-2. The homologies of GnRHR, FSH-β and LH-β sequences were 98%, 100% and 94% respectively, with those reported in National Center of Biotechnology Information (NCBI). OCT in EG-1 and EG-3 thickened with a maxium in EG-3. PFV, PFT, EET and UWT in EGs were larger than that in CG ($P<0.05$) Primary and secondary follicles in EGs increased and growed quickly. Nucleolus**

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**Corresponding author: weisc668@163.com

**and mitochondria in EGs were enlarged. *Zona pellucida* and *microvillus* of the oocytes broadened and lengthened. In conclusion, GnRH-A could increase synthesis and secretion of FSH and LH, and promote the development of ovary and follicles.**

**KEY WORDS: gonadotropin releasing hormone / GnRH receptor / follicle-stimulating hormone / luteinizing hormone /gene expression / histostructure /female rabbit**

Gonadotropin releasing hormone (GnRH) is synthesized and released at hypothalamus, which promotes the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). GnRH analogue (GnRH-A), synthesized by replacing amino acids at some sites in the natural GnRH peptide strand, may enhance its capacity of binding receptor by 100-200 times [Chabbert-Buffet 2003, Guo 2005]. Alarelin is a GnRH agonist (nine peptides). Its efficiency is 15 times stronger than that of natural GnRH [Falk *et al*. 2006].

Injection of GnRH and GnRH-A could increase the release of LH and FSH from pituitary gland and improve pregnancy rate by 12%-15% in sheep [Wei 2006, Schneider 2006, Zhang *et al*. 2009]. In dairy cows administered with GnRH-A the estrus and ovulation occurred earlier and uterus involution and calving interval following delivery was shortened. However, a sharp decline of LH plasma level was found as well as a slight decrease of FSH in dairy cow following GnRH active immunization [MarLernueei *et al*. 1984]. When the rats, rabbits and sheep were rapidly injected intravenously with GnRH or GnRH-A the LH and FSH plasma level increased remarkably, but only plasma LH level was elevated if the same dose of GnRH and GnRH-A was injected slowly [Zhang 2009]. However, Zhang *et al*. [2006] reported that the ratio of LH to FSH was regulated by GnRH. The ratio declined along with the decrease in GnRH impulse. Heterogeneous effect was caused by large dose GnRH-A, illustrating that the GnRH-A may inhibit the gonadal activity and give the anti-fertility effect. However, it is not yet clear about the exact effects and mechanism of GnRH-A immunization on reproductive performance. The aim of the present study was to explore the dynamic changes in serum LH and FSH, quantitative gene expressions of GnRHR, FSH-β and LH-β mRNAs in the pituitary, as well as microstructure and ultrastructure properties of ovary and uterus in animals resulting from the active immunizing with GnRH-A antigen, so as to provide scientific basis for regulating reproductive functions.

## Material and methods

### Experimental design

Dimethylamino-propyl Carbodiimide hydrochloride (EDC.HCL, Shanghai Biochemical Sci-Tech Development company. Shanghai, China) was used as couplets. Alarelin (ZILLION, China) was mixed in 1:1 ratio with bovine serum albumin (BSA, SIGMA, USA), and then supplemented with Freund's incomplete adjuvant (SIGMA, USA) – Wei and Zhang *et al.* [2008]. The aseptic conditions, security and physical

properties were kept according to Veterinary Biological Product Quality Inspection [Wang *et al.* 2002].

Twenty-four female rabbits (*Oryctolagus cuniculus*), aged three months, were randomly divided into four groups: experimental 1 (EG-1), experimental 2 (EG-2), experimental 3 (EG-3) and control (CG). Rabbits in EG-1, EG-2 and EG-3 were subcutaneously injected with 1.0 mL GnRH-A antigen at concentrations of 100 μg/mL, 100 μg/mL and 50 μg/mL, respectively. On day 20 from the first injection the EG-2 and EG-3 rabbits were re-injected with the initial GnRH-A dose (*i.e.* that applied on day 10). No injections were given to CG.

Ten ml blood was withdrawn from lateral vein of right hind limb on day 0, 10, 20, 30, 40, 50, 60 and 70 counting from the first GnRH-A antigen injection. Serum was harvested and stored at -20˚C. All rabbits were killed on day 70 by bleeding from jugular vein. The samples of the pituitary gland, bilateral ovaries and uterine horns were collected aseptically, fixed in 10% formaldehyde and 3% glutaric dialdehyde, embedded in paraffin wax, sliced (Reichert-Jung Ultramicrotome, REICHERT, Austria) and stained with hematoxillin and eosin (H & E).

**Serum analyses**

Serum FSH and LH concentrations were determined as prescribed in the protocol of FSH kit and LH kit (for rabbit, Northern Biotechnology Institute, Beijing, China) respectively. Briefly, (1) blank, standard and sample holes were labelled on plate. 50 μL serum and 50 μL standard solution were added into each standard hole; (2) 50 μL FITC-anti rabbit IgG were added into each hole, fully mixed and incubated at 37°C for 60 min. No liquids were added into the blank hole; (3) the supernatant liquid was removed from each hole, and holes were washed three times with well-washer (RAYTO, USA), each time for 10 s; (4) enzyme conjugate was added to each hole, fully mixed and kept at 37°C for 60 min; (5) 50 μL colour solutions A and B were added, mixed slowly and incubated at 37°C for 15 min; (6) 50 μL terminate solution was added to each hole; (7) optical density (OD) of each hole contents was read with MK-3 type enzyme-lable device (RAYTO, USA) at 450 nm wavelength.

**RNA extraction and cDNA synthesis**

Total RNA from frozen tissue of pituitary gland was extracted with a Total RNA Kit (INVITROGEN, Carlsbad, CA, USA) according to the manufacturer's prescription. Prior to RNA extraction with chloroform and isopropanol the tissues were homogenized in TRIzol (INVITROGEN, Carlsbad, CA, USA). RNA was pelleted, washed with 75% ethanol, and reconstituted in RNase-free water. GnRHR, FSH-β or LH-β cDNA were amplified using polymerase chain reaction (PCR) with GoTaq Flexi DNA Polymerase (PROMEGA, Madison, WI, USA). The concentration of total RNA was measured by absorbance at 260 nm using spectrophotometer (NanoDrop Technologies, Wilmington, USA). The quality of total RNA was assessed by formaldehyde agarose gel electrophoresis.

**RT-PCR and sequence analysis of GnRHR, FSH-β and LH-β mRNA in the pituitary**

Reverse transcription was performed using Energic Script cDNA synthesis kits (ENERGIC SCRIPT®, SHINEGENE, Shanghai, China) according to manufacturer's prescription (QIAGEN, Valencia, CA, USA). Complementary DNA (cDNA) was prepared using QuantiTech reverse transcriptase kit (ShineGene, Shanghai, China) with integrated removal of genomic DNA contamination. GnRHR, *FSH*-βand *LH*-βcDNA (0.5 μL) were amplified in 25 μL PCR with GoTaq Flexi DNA polymerase (PROMEGA, Madison, WI, USA). The primers are listed in Table 1. DNA was denatured for 30 s at 94°C, allowed to anneal for 30 s at 53°C and extended for 1 min at 72°C. DNA was electrophoresed on 2% agarose gel mixed with 3 μL ethidium bromide immersed in 1× Tris / Borate / EDTA buffer at 110 v. The cDNA was extracted from the agarose gel using a MinElute gel extraction kit (QIAGEN, Valencia, CA, USA). The nucleotide sequences of *GnRHR*, *FSH*-β and *LH*-β were blasted in NCBI.

**Table 1**. Primers and Taqman probes for real-time Taqman PCR end product for *GNRHR* (NM-001082738), F*SH*-β (FJ-887900) and *LH*-β (NM-001082695 )

| Gene | Taqman probe (5'-3') | Primers (5'-3') | Product (bp) |
|---|---|---|---|
| *GNRHR* | TTGGTCCTCTTCTCATCATGCTGGTGTGC | F-GCTCAGCCATCAACAAC<br>R-ATTCCACATCCCATCCA | 144 |
| *FSH*-b | CTTGGTGCTCTGGTTACTGCCATACACGG | F-TGTTTCCTTTTCTGTTGC<br>R-AGGTGGTATTGATGCTTAT | 82 |
| *LHB*-b | ACAGTGCTGCTGCTGCTGCTGC | F-ATGGGGACGCTCCAG<br>R-TCAGAGGAAGAGGAGGC | 144 |
| *GAPDH* | AACACAGCTTCTCCTTGATGTCACGCACGAT | F-GGTCGGAGTGAACGGATT<br>R-CTCGCTCCTGGAAGATGG | 131 |

**Real-time Quantitative PCR**

Primer information on the GnRHR, FSH-β and LH-β genes and internal control of GAPDH (glyceraldehyde's 3-phosphate dehydrogenase) gene are listed in Table 1. All gene sequences are available on http://www.ncbi.nlm.nih.gov, accession no.: NM-001082738, FJ-887900 and NM-001082695). The primers were designed using Primer 5.0 software and synthesized by TaKaRa company (Dalian, China).

**Light microscopy examination**

The ovary and uterus tissue samples fixed in 10% formaldehyde were sliced and stained with hematoxillin and eosin (H & E). The slides were examined under the optical microscope (LEICA, Japan) and photographed.

**Electron microscopy examination**

The ovary and uterus tissue samples fixed in 3% glutaric dialdehyde were sliced with Reichert-Jung Ultramicrotome (REICHERT, Austria), stained, and put in 1% toluidine blue, then doublely stained in uranyl acetate and lead citrate. The slides

were examined under the JEM-1230 electron microscope (JAPANESE ELECTRON COMPANY).

**Measurements of ovary and uterus**

Five sites in each photograph of the tissue slide (two slides for each animal, totally 60 sites per group) were measured in primary follicles. The ovarian cortex thickness (OCT), primary follicle vertical diameter (PFV), primary follicle transverse diameter (PFT), endometrial epithelium thickness (EET) and uterus-wall thickness (UWT) were measured and counted using a morphology analysis software (Images Advanced 3.2 and Image Pro Plus 2.0, MOTIC, Hong Kong, China).

**Statistical**

Experimental data were analysed using statistical software (SPSS 17.0 version; SPSS, Chicago, USA). The results are presented as means and standard deviations, and one- and/or two-way ANOVA followed by HSD (Honestly Significant Differences) test. If $P<0.05$, differences were considered significant.

## Results and discussion

**Serum LH concentration**

LH concentrations in three experimental groups (EGs) increased slightly on day 30, 45 and 60 following GnRH-A injection ($P>0.05$), but in control group (CG) it remained stable during the experiment. LH levels in EG-2 and EG-3 reached the peak on day 40 ($P<0.05$). However, LH concentration in EG-2 was significantly higher than in other groups on day 30 (*i.e*. 10 days after re-injection) and day 50 ($P<0.05$). LH level was also higher than that found prior to the first injection in the same group (Tab. 2). These results show that re-injecting with GnRH-A might enhance the synthesis and secretion of LH.

**Serum FSH concentration**

Compared to CG, FSH concentrations in EG-2 and EG-3 increased on day 30 and reached peak

**Table 2.** Serum LH concentration in female rabbits (mIU/mL)

| Group | Immunization time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
| EG-**1** | 1.48±0.16 | 1.50±0.21 | 1.48±0.24 | 1.47±0.30$^{a}$ | 1.48±0.31 | 1.48±0.31$^{a}$ | 1.48±0.28 | 1.48±0.27 |
| EG-**2** | 1.47±0.12 | 1.48±0.21 | 1.48±0.27 | 1.60±0.32$^{b}$ | 1.57±0.28 | 1.61±0.28$^{b}$ | 1.48±0.27 | 1.49±0.31 |
| EG-3 | 1.48±0.14 | 1.49±0.20 | 1.48±0.27 | 1.47± 0.29$^{a}$ | 1.56±0.29 | 1.49±0.32$^{a}$ | 1.47±0.29 | 1.46±0.27 |
| CG | 1.48±0.14 | 1.48±0.27 | 1.49±0.28 | 1.47± 0.23$^{a}$ | 1.47±0.20 | 1.46±0.32$^{a}$ | 1.48±0.30 | 1.48±0.28 |

Within columns the adjacent superscript letters (such as ab,bc) indicate that the difference is significant ($P<0.05$), while the interval superscript letters (such as ac, bd) show the differences are highly significant ($P\leq0.01$). The same meaning in following tables are.

**Table 3.** Means and standard deviations (SD) for blood serum FSH concentration (mIU/mL) in female rabbits as related to time passing from immunization

| Group | Immunization time (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
| EG-1 | 1.36±0.08 | 1.43±0.12 | 1.42±0.20 | 1.35±0.21[a] | 1.34±0.10[a] | 1.36±0.18 | 1.37±0.19 | 1.36±0.20 |
| EG-2 | 1.33±0.08 | 1.42±0.20 | 1.44±0.29 | 1.51±0.38[b] | 1.71±0.30[b] | 1.48±0.22 | 1.47±0.18 | 1.45±0.22 |
| EG-3 | 1.42±0.13 | 1.43±0.19 | 1.41±0.30 | 1.53±0.38[b] | 1.63±0.35[b] | 1.45±0.23 | 1.35±0.22 | 1.31±0.27 |
| CG | 1.37±0.21 | 1.39±0.09 | 1.39±0.22 | 1.35±0.26[a] | 1.36±0.22[a] | 1.35±0.26 | 1.41±0.24 | 1.38±0.18 |

[ab]Within columns means bearing different superscript letters differ significantly at P>0.05.

**Table 4**. Expression of GnRHR, FSH-β and LH-β mRNA in pituitary of female rabbits

| Gene | Group | EG (Ct) | | CG (Ct) | | ΔΔCt |
|---|---|---|---|---|---|---|
| | | target gene | reference gene | target gene | reference gene | |
| GnRHR | EG-1 | 28.53 | 36.07 | 23.1 | 30.07 | -0.57 |
| | EG-2 | 29.68 | 36.07 | 23.1 | 30.07 | 0.58 |
| | EG-3 | 21.14 | 36.07 | 23.1 | 30.07 | 7.96 |
| FSH-β | EG-1 | 26.23 | 33.02 | 27.11 | 30.07 | 3.83 |
| | EG-2 | 38.19 | 33.02 | 27.11 | 30.07 | 8.13 |
| | EG-3 | 32.21 | 33.02 | 27.11 | 30.07 | 2.15 |
| LH-β | EG-1 | 24.74 | 25.79 | 30.85 | 30.07 | -1.73 |
| | EG-2 | 26.01 | 25.79 | 30.85 | 30.07 | -0.46 |
| | EG-3 | 23.47 | 25.79 | 30.85 | 30.07 | -3.02 |

ΔΔCt = Ct of experimental group (target gene-reference gene) – Ct of control group (target gene-reference gene).

value on day 40. FSH values in EG-2 were significantly higher than those in EG-1, CG ($P$<0.01) and EG-3 ($P$<0.05) on day 50. There was no difference in FSH levels between EG-1 and CG during the experiment (Tab. 3). These results imply that induced synthesis and secretion of FSH by injecting the GnRH-A antigen was dose-dependent.

**Values of real time quantitative PCR for GnRHR, FSH-β and LH-β mRNA in pituitary**

GnRHR ΔΔCt in EG-3 was significntly higher than those in EG-1 and EG-2 ($P$<0.05). However, ΔΔCt values of FSH and LH in EG-3 were smaller than those in EG-1 and EG-2 (Tab. 4). These relations indicate that GnRH-A immunization of female rabbits can obviously affect the expression of GnRHR, FSH-β and LH-β mRNA in pituitary.

**Sequence of *GnRHR*, *FSH*-β and *LH*-β mRNA**

The nucleotides for GnRHR, FSH-β and LH-β mRNA amounted to 1206, 116 and 1833 bp, respectively. The homologies for GnRHR gene with NM-001082738 in Genbank, FSH-β with FJ-887900 and LH-β with NM-001082695 were 98%, 100% and 94%, respectively.

**Dimensions of ovary and follicle**

Ovary cortex thickness (OCT) in EG-2 and CG were lower than of those in EG-1 and EG-3 does ($P$<0.05), while the highest was observed in EG-3. The largest PFV and PFT were found in EG-1 ($P$<0.05) and the smallest in CG. At the same time, PFV in three EGs exceeded that found in CG ($P$<0.05). However, no significant difference was identified between EG-1 and EG-2 in PFV and PFT ($P$>0.05). These relations indicate that re-injecting with GnRH-A can decrease the OCT as well as the length and width of follicles, and promote maturation of their follicles. The injecting dose of 100 μg occurred more efficient than that of 50 μg. Re-injection of GnRH-A did not strengthen these effects (Tab. 5).

**Table 5**. Means and standard deviations (SD) for dimensions of ovary and follicle (μm) examined in female rabbits under optical microscope (×100)

| Group | OCT | PFV | PFT | EET | UWT |
|---|---|---|---|---|---|
| EG-**1** | 31.33±4.82$^{b}$ | 387.18±119.4$^{b}$ | 282.41±123.4$^{b}$ | 25.35±6.15$^{a}$ | 347.54±113.9$^{b}$ |
| EG-**2** | 26.37±5.03$^{a}$ | 371.44±80.39$^{b}$ | 279.78±69.66$^{b}$ | 16.85±2.79$^{b}$ | 345.03±52.38$^{b}$ |
| EG-**3** | 34.55±7.35$^{b}$ | 365.88±44.08$^{b}$ | 192.61±29.84$^{a}$ | 19.12±8.04$^{b}$ | 276.82±83.13$^{b}$ |
| CG | 28.99±5.82$^{a}$ | 243.89±84.39$^{a}$ | 174.98±62.46$^{a}$ | 37.40±13.30$^{a}$ | 423.53±106.4$^{a}$ |

Within columns means bearing different superscript letters differ significantly at P>0.05. OCT – ovarian cortex thickness; PFV – primary follicle vertical diameter; PFT – primary follicle transverse diameter; EET – endometrial epithelium thickness; UWT – uterine wall thickness.

**Dimensions of uterus**

Compared to CG, the EET in three EGs was found smaller (P<0.05). EETs in EG-2 were the smallest among the EGs. The UWT in all EGs was thinner compared to that found in CG (P<0.05), especially in EG-3. There were no significant differences identified among the three EGs (Tab. 5). These results indicate that the injection of GnRH-A may restrain the growth and development of uterus in rabbits.

**Ovary microstructure**

**EG-1.** As compared to CG, primordial follicles developed quickly, and secondary follicles became more abundant. Loose granular layer distributed in the secondary follicles. The *zona pellucida* clear (Fig. 1).

**EG-2.** Rich primary and matured follicles were found. More primordial and secondary follicles distributed. The *corona radiata* typical. The follicles more abundant than in other groups (Fig 2).

**EG-3.** The primordial follicles in the cortex were scarcer than in EG-2, many of them in resting state. The granular cells appeared apoptotic (Fig. 3).

**CG.** There were few matured follicles and primary follicles in CG, the follicles grew and developed incompletely. The structures were unclear (Fig. 4).

These results showed that GnRH-A could accelerate the development of ovaries in female rabbits, which may be related to the dose injected.

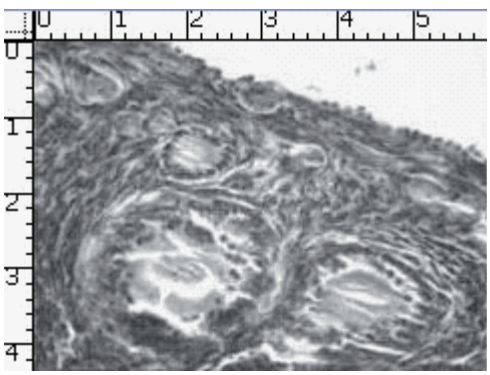

Fig. 1. Ovarian microstructure in EG-1 (×400).

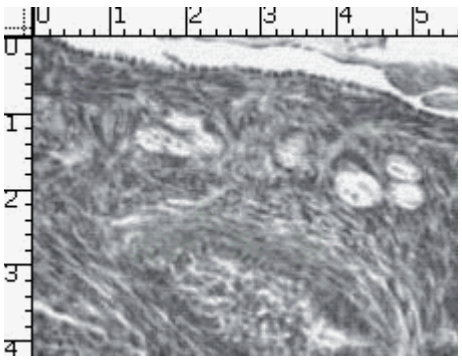

Fig. 2. Ovarian microstructure in EG-2 (×400).

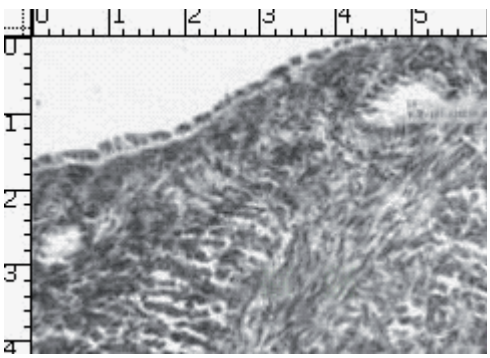

Fig. 3. Ovarian microstructure in EG-3 (×400).

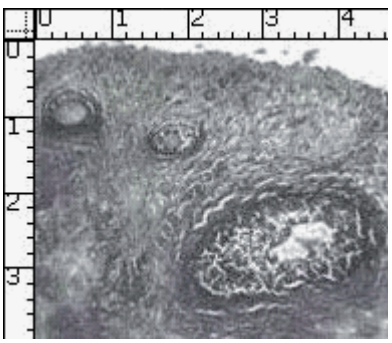

Fig. 4. Ovarian microstructure in CG (×400).

**Uterus microstructure changes**

The uterus *endometrium* in EG-1 was smooth and neat, the *microvilli* were not seen. The sparse epithelial cells flatted and arranged evenly. There were less glands and blood vessels than in CG. In EG-2 the rich epithelial cells were observed. However, they flattened and were arranged irregularly. The *endometrium* in EG-3 had irregular structure, the epithelium also flatted and arranged irregularly with few glands. The uterus in CG grew and developed normally.

**Ovary ultrastructure changes**

**EG-1.** The cytomembrane and nucleus of oocyte were intact, *zona pellucida* did not appear. The karyotheca and nucleolus enlarged and very clear, mitochondria and cristae in oocytes increased. The massive cortical granules and secretions present in cytoplasm (Fig. 5).

**EG-2.** The *zona pellucida* broadened obviously, many *microvilli* elongated, distributed evenly and reaching into *zona pellucida* regularly. Many lipid droplets, cortical granules and rough endoplasmic reticulum (RER) in the cytoplasm. The mitochondria cristae increased, the nucleolus intact, nuclear membrane very clear (Fig. 6).

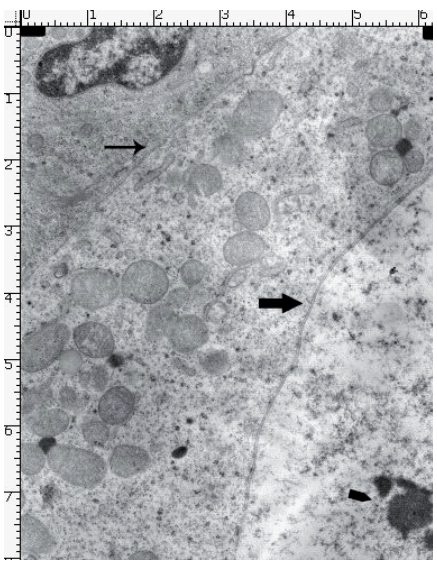

Fig. 5. The nucleolus and nuclear membrane (→) were very clear, nucleolus was lobulated (▲).

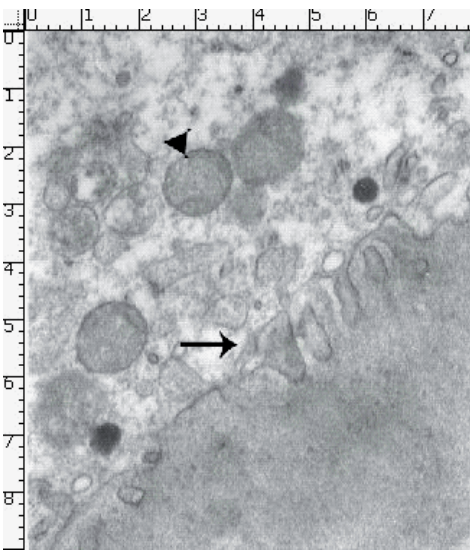

Fig 6. The *zona pellucida* broadened obviously, many lipid droplet and cortical grainules and secretions existed in the cytoplasm (▲), mitochondria and mitochondrial cristae increased, the intact nucleolus and nuclear membrane (→) were observed.

**EG-3.** The *zona pellucida* broadened obviously, the *microvillus* reached into *zona pellucida*. Many cortical granules and secretions observed clearly in the cytoplasm. The karyotheca of primary follicle complete and distinct, chromatin in the nucleus becoming concentrated (Fig. 7).

**CG.** Unlike three EGs, the quantities of mitochondria and mitochondrial cristae became less and irregularly arranged. There were few RERs, *zonae pellucidae* and *microvilli.* Many *oogonia* distributed in the follicles. The cortical granules arranged by multi-layer (Fig. 8).

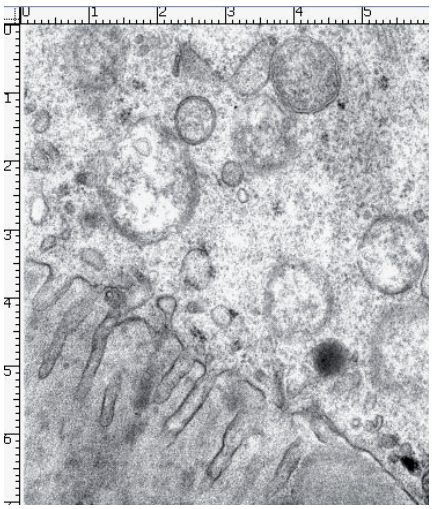

Fig. 7. The karyotheca and nucleolus of EG-3. The electron density of nuclear heterochromatin in oocytes increased.

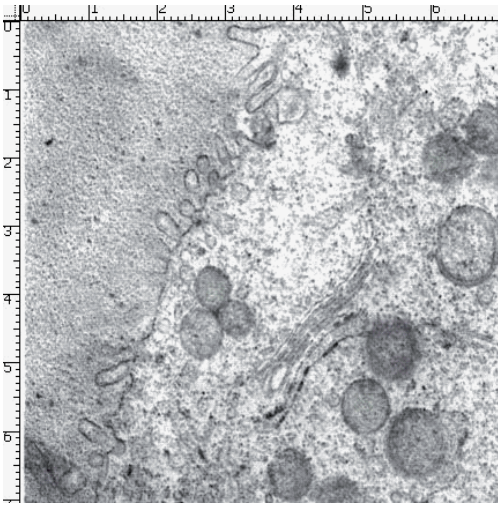

Fig. 8. There were few RER, *zona pellucida* and *microvilli*. M**any** oogonia dispersed in the follicles.

In brief, injecting female rabbits with GnRH-A may accelerate growth and development of oocytes and promote the development and maturation of ovaries and uterus.

Specific anti-GnRH antibodies can be induced when animals are immunized with GnRH  Esbenshade 1985, Ye 2003). Alarelin, as a GnRH analogue (GnRH-A), has similar function with natural GnRH. However, little is known about the mechanism of GnRH-A in regulating reproduction in animals [Guo 2005, Wei 2006]. It has been reported that FSH and LH levels can reach their peaks in female rabbits (6-16 months) after 96 hrs from injection of urinary gonadotropin (HMG), followed by a super-ovulation [Huang 2002]. However, it was also reported that GnRH immunization, due to decreasing the LH pituitary level, eventually weakens the reproductive performance in cheetahs [Bertschinger *et al*. 2006]. Gong *et al*. [1996] reported that continuous infusion with GnRH agonist could inhibit FSH and LH secretion as well as ovarian development in heifers. In the study by Esbenshade and Britt [1985], serum

LH concentration in sows on week 12 from immunization with GnRH was too low to be measured.

In the present study, serum FSH and LH concentrations began increasing on day 30 in rabbits when injected with Alarelin antigen. FSH and LH in EG-2 and EG-3 reached a peak on day 40. LH and FSH levels in EG-2 were significantly higher than those in other groups, re-injecting the antigen could promote the effect. Our results presented here are in accordance with the reports by Guo [2005], Li *et al.* [1998, 2007] and Junaidi *et al*. [2009a], but different from those by Bastings [1991], Gong *et al.* [1996], Clarkc *et al.* [1998], Zamaratskaia *et al*. [2007] and Li *et al.* [2009]. They reported that repeating medication of GnRH-A may inhibit the release of LH and FSH from pituitary. Whether the revealed efficiency of GnRH-A was associated with the estrus and ovulation stages of the experimental rabbits needs to be further explored.

FSH and LH may stimulate the growth and maturation of follicle, increase the ovary weight, promote the follicle to produce estrogen so as to promote ovulation [Zamaratskaia 2007, Li 2009], and thereby facilitate the development of the gonads and ovulation in rats, rabbits, sheep and pigs [Xian *et al.* 2002, Hapgood *et al* 2005, Zhang 2009].

The quantitative studies of GnRH-A effects on development and maturation of the follicle, ovary and uterus have rarely been reported [Dhaliwal 2002, Leung 2003, Valiente 2007, Fang 2009]. Zhang [2007] reported the epithelium of ovary cysts a became large and rich *microvilli* developed after the adult mice were injected with 20 μg estradiol. Result of our research reported here occurred similar. OCT, PFV and PFT in rabbits were measured quantitatively by using image software. The results showed that GnRH-A (Alarelin) can increase the primary follicle, PFT and PFV, as well as stimulate growing and maturation of follicles.

However, re-injection seems not to accelerate these effects. This study probably opens a new field for quantitative research of the mechanisms of reproduction in medicine and veterinary science.

Few studies reported the histomorphological changes in ovaries and other parts of genital tract when GnRH and GnRH-A were used for women and female animals [Beck 1972, Bertschinger 2006, Zamaratskaia 2007, Li *et al.* 2008, Zapletal 2008, Junaidi *et al*. 2009ab]. It may be concluded that GnRH-A active immunization promotes the synthesis and secretion of FSH and LH in female rabbits, and speeds up the development of ovary and follicle. GnRH-A may influence obviously the gene expression of GnRHR, FSH-β and LH-β mRNA in rabbit's pituitary, re-injection of alarelin had more effects.

**REFERENCES**

1. BASTINGS E., 1991 – Immunocytochemical evidence for production of lutinizing-hormone and follicle-stimulating hormone cells in separate cells in the bovine. ***Biology of Reproduction*** 45(5), 788-796.
2. BECK L.R, 1972 – Comparative observations on the morphology of the mammalian periovarial sac. ***Journal of Morphology*** 136(2), 247-54.

3. BERTSCHINGER H.T., JAGO I., NOTHING J.O., HUMAN A., 2006 – Repeated use of the GnRH analogue deslorelin to down-regulate reproduction in male cheetahs (Acinonyx jubatus). ***Theriogenology*** 66(6-7), 1762-1769
4. CHABBERT-BUFFET N., 2003 – GnRH antagonists. *Clinical Obstetrics and Gynecology* 46(2), 254-264.
5. CLARKC I..J, BROWN B.M., 1998 – Neonatal immunization against gonadotropin releasing hormone (GnRH) results in diminished GnRH secretion in adulthood. ***Animal Breeding Abstracts*** 66(9), 813.
6. DHALIWAL G..S, MURRAY R.D, REES E. M., BEECH D.J., 2002 – Quantitative unbiased estimates of endometrial gland surface area and volume in cycling cows and heifers. ***Research in Veterinary Science*** 73, 259-265.
7. ESBENSHADE K.L., BRITT J.H., 1985 – Active immunization of gilts against gonadotropin-releasing hormone: effects on secretion of gonadotropins, reproductive function, and responses to agonists of gonadotropin-releasing hormone. ***Biology of Reproduction*** 33, 569-577.
8. FALK S., WOLFGANG T., CARSTEN G., 2006 – Gonadotropin-releasing hormone (GnRH) and its natural analogues: A review. ***Theriogenology*** 66, 691-709
9. GONG J.G., CAMPBELL B.K, BRAMLE Y., 1996 – Suppression in the secretion of follicle stimulating hormone and luteinizing hormone and ovary follicle development in heifers continuously infused with a gonadotropin releasing hormone agonist. ***Biology of Reproduction*** 55, 68-74.
10. GUO GONGYU, 2005 - GnRH analogues of progress. Pract Clin Obster Gynecol, 21(11), 6-12
11. HAPGOOD J.P., SADIE H., VAN B.W, *et al,* 2005 – Regulation of expression of mammalian gonadotropin-releasing hormone receptor genes. ***Journal of Neuroendocrinology*** 17(10), 619-638.
12. HUANG LIQUAN, FANG JIANYONG, 2002 – Effect of injection with LRH-A_3 and/or progesterone on Ovulation and Conception Rate in Sows. ***Chinese Journal of Veterinary Science*** 22(4), 399-401.
13. JUNAIDI A., WILLIAMSON P.E., TRIGG T.E., CUMMINS J.M., MARTIN G.B., 2009a – Morphological study of the effects of the GnRH superagonist Deslorelin on the canine testis and prostate gland. ***Reproduction in Domestic Animals*** 44(5), 757-763.
14. JUNAIDI A., WILLIAMSON P.E., MARTIN G.B., BLACKBERRY M..A., CUMMINS., J.M. TRIGG T.E., 2009b – Dose-response studies for pituitary and testicular function in male dogs treated with the GnRH superagonist, Deslorelin. ***Reproduction in Domestic Animals*** 44(5), 725-734.
15. LA VAN DER WESTHUIZENA1, BENNET N.C., JARVIS J.U., 2002 – Behavioural interactions, basal plasma luteinizing hormone concentrations and the differential pituitary responsiveness to exogenous gonadotrophin-releasing hormone in entire colonies of the naked mole-rat (Heterocephalus glaber). ***Journal of Zoology*** 256, 25-33.
16. LEUNG P.C., CHEN C.K., ZHU X.M., 2003 – Multi-factorial role of GnRH-I and GnRH-II in the human ovary. ***Molecular and Cell Endocrinology*** 202(1-2), 145-153.
17. LI BING, SU NIANJUN, WANG FANG, *et al*, 2009 - Application of short-acting GnRH-a Alarelin in ovulation induction. ***Reproduction & Contraception*** 29(1), 55-58.
18. LI JIE, XU HUILING, 2008 – The effect of down-regulation with GnRH-a on the threshold of gonadotrophin for folliculogenesis. ***Journal of Reproduction Medicine*** 17(6):404-406
19. LI YAOHUI, ZHOU JIANPING, CHEN SHULIN, 2007 – Effect of GnRH-a on propagation property of Ra. ***Journal of Anhui Agricultural Science*** 35(66), 4857-4877.
20. LI YUANYOU, LIN HAORAN, 1998 – Regulation of GnRH on LH and FSH Secretion of Rana rugulosa. ***Acta Scientiarum Naturalium Universitatis Sunyatseni*** (natural sciences), 37(5), 92-96.
21. MARLERNUEEI C., 1984 – Effect of GnRH on conception rate in repeat-breeder dairy cows. ***Animal Breeding Abstracts*** 53, 241-245.

22. SANG RUNZI, 2005 – Application of GnRH and its anologue to animal reproduction. ***Chinese Journal of Animal Science*** (4), 55-58.
23. SCHNEIDER F., TOMEK W., GRUNDKER C., 2006 – Gonadotropin-releasing hormone (GnRH) and its natural analogues: a review. ***Theriogenology*** 66, 691-709.
24. VALIENTE C., CORRADA Y., DE LA SOTA P.E., GALASSI-GEREZ P., GOBELLO C., 2007 – Effect of the GnRH antagonist, acyline, on canine testicular characteristics. ***Theriogenology*** 68(5), 687-692.
25. WANG JUNWEI, 2002 – Veterinary biological product quality inspection: quality management and control on veterinary biological products[M]. China Agriculture Press. Beijing, pp. 181-186,
26. WEI SUOCHENG, GONG ZHUANDI, 2009 – Experimental Research on Efficacies of GnRH-A immunocastration in rabbits. ***Acta Veterinaria et Zootechnica Sinica*** 40(2), 191-196.
27. WEI SUOCHENG, ZHANG JIAN, 2008 – Preparation of GnRH-A Antigen and Researches on Efficacies of Active Immunization Castration with Different Dosage. ***Journal of Northwest Normal University*** (Natural Science Edition***)*** 19(2), 49-52.
28. WEI SUOCHENG. 2006 – The basic principle of immunization castration and its applications. ***Journal of Northwest Normal University*** (Natural Science Edition) 17(2), 49-53.
29. XIAN Y.Z., TURKSTRA J.A., MELOEN R.B., 2002 – Active immunization against gonaotropin releasing hormone in chinese male pigs: effects of dose on antibody titer hormone levels and sexual development. ***Animal Reproduction Science*** 70(3-4), 223-233.
30. XUE WEI., 2009 – SPSS Statistical Analysis and Applications. Beijing Electronics Industry Press. January, 156-223.
31. YE LIANSHUN, NU JING, JI YUAN, LIU DONGZHI, 2003 – The study on reproductive endocrine changes of New Zealand rabbits before and after superovulation. ***Journal of Southwest Normal University*** (Natural Science Edition) 28(5), 805-807.
32. ZAMARATSKAIA G., RYDHMER L., ANDERSSON H.K., CHEN G., LOWAGIE S., ANDERSSON K., LUNDSTORM K., 2007 – Long-term effect of vaccination against gonadotropin-releasing hormone, using ImprovacTM, on hormonal profile and behaviour of male pigs. ***Animal Reproduction Science*** 81(10), 662-665.
33. ZAPLETAL D., PAVLIK A., 2008 – The effect of lecirelin (GnRH) dosage on the reproductive performance of multiparous and lactating rabbit does. ***Animal Reproduction Science*** 104, 306-315.
34. ZHANG HAIQUAN, SHI ZHENSHENG, XIA GUOLIANG, 2006 – Function of gonadotropin releasing hormone and its applications. ***Chinese Journal of Veterinary Medicine*** 42(3),33-35.
35. ZHANG NIN, 2007 – Effect of estrogen on the ultrastructure of ovary bursa in the mouse. ***Journal of Jinhua College of Profession and Technology*** 7(4), 69-72.
36. ZHANG ZHONGSHU, SHANG XUEJUN, 2009 – Gonadotrophic hormone releasing hormone and its application in reproductive system. ***Chongqing Medicine*** 38(5), 604-606.
37. ZHAO XINGXU, 2002 – Veterinary Obstetrics. 3rd ed. China Agriculture Press. Beijing, pp.120-148.
38. ZHOU XU, DONG WEI, 2001 – Release of LH and FSH by pituitary cells of prepubertal gilts cultured in vitro***. Heilongjiang Journal of Animal Reproduction*** 9(1), 1-3.