# Effects of GnRH agonist immunization on vaginal electrical resistance, FSH, LH, and ovaries in prepubertal female sheep

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**ABSTRACT**: The study is aimed at the investigation of the effects of GnRH agonist (alarelin) immunization on the vaginal electrical resistance (VER) and secretion of FSH and LH in sheep. Forty-two prepubertal female sheep were assigned to six groups ($n$ = 7). Animals in experimental groups I (EG-I), II (EG-II), and III (EG-III) were twice subcutaneously injected with 200, 300, and 400 μg of alarelin antigens, respectively. Animals in experimental groups IV (EG-IV) and V (EG-V) were four times subcutaneously injected with 200 and 300 μg of alarelin antigens, respectively. Animals in the control group (CG) were subcutaneously injected with 2.0 ml of control liquid. Serum concentrations of FSH and LH were detected using ELISA. VER was measured by the electrical resistance detector. The follicle vertical diameter, follicle transverse diameter, theca folliculi externa thickness (FET), theca folliculi interna thickness (FIT), and follicle wall thickness (FWT) were calculated through microscope images using ImagePlus software. The results showed that the serum FSH concentration in EGs started to increase on day 14 and reached peak levels on day 35, with a maximum in EG-V. Serum LH concentration in EGs decreased from day 7 and reached the minimum levels ($P < 0.05$) on day 21 or 28. Serum FSH in EG-III–EG-V was higher than that in EG-I ($P < 0.05$) and CG ($P < 0.01$) in days 28–60. VER decreased after injection of alarelin antigen, the values reached the minimum levels on day 28 in EG-I and EG-II, on day 35 in EG-III–EG-V ($P < 0.05$), respectively. In days 45–60, VER values in EG-III–EG-V were lower than those in CG ($P < 0.01$). Ovarian weights in EGs were higher than those in CG; the values of FET, FIT, FWT, follicle vertical diameter (FVD), and follicle transverse diameter (FTD) in EG-III and EG-V were greater than those in CG, EG-I, EG-II, and EG-IV. In conclusion, alarelin active immunization can decrease VER, induce the synthesis and secretion of FSH, and promote follicle development in prepubertal female sheep. VER had a positive correlation with serum LH, and a negative correlation with FSH.

**Keywords**: gonadotropin-releasing hormone agonist; gonadotropins; vaginal electrical resistance; follicle development; ewe

The common oestrus detection method in the cattle breeding herds is to observe animals in standing oestrus. However, not all ovulating animals display standing oestrus (van Eerdenburg et al., 1996). In addition, identification of oestrus is practically difficult (Million et al., 2011). Reproductive efficiency of cattle in the hot arid regions is poor with animals showing suppressed behavioural signs of oestrus, delayed postpartum oestrus, silent oestrus, and poor conception rates. Circulating concentrations of steroid hormones are known to be low (Gupta and Purohit, 2001) in these animals.

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Vaginal electrical resistance (VER) fluctuates with the stage of the oestrous cycle, it is the highest during the luteal phase, and declines during the follicular phase (Zuluaga et al., 2008). Measuring VER is one means for detecting oestrus in animals. VER has been used as a method for predicting ovarian status without visual oestrus detection in numerous animals. During the past three decades, much attention has been paid to monitoring VER as a facile method for understanding the oestrous cycle in various domestic animals (Bartlewski et al., 1999; Gupta and Purohit, 2001). It was studied in cattle (Rorie et al., 2002), buffaloes (Gupta and Purohit, 2001), sows (Dusza et al., 1996), and ewes (Bartlewski et al., 1999), but the results to date have been inconclusive. A number of studies have reported large within- and between-animal variations in absolute VER measurements at the time of oestrus.

A decline in vaginal electrical impedance to < 40 Ω has been closely associated with the onset of oestrus in sheep (Bartlewski et al., 1999) and is apparently due to increasing oestradiol and decreasing progesterone concentrations at this time. The decline degree of VER was in a negative correlation to conception rate (Rorie et al., 2002; Saldarriaga et al., 2007). VER was associated with the size of the ovulatory follicle and chance of pregnancy in a group of oestrus-synchronized *Bos indicus* cross cattle (Zuluaga et al., 2008). The obvious relationships between VER fluctuations and changes in plasma concentrations of circulating hormones are found in sows (Dusza et al., 1996). During oestrus, the concentration of circulating oestrogen increases and progesterone decreases. This induces oedema of the vaginal interstitium and a change in electrolyte concentrations of the vaginal luminal fluid. This results in a relative reduction in the electrical resistance within the vagina of cattle during oestrus and an increase when the tissue oedema resolves during dioestrus (Lewis et al., 1989). Monitoring of VER changes can provide a more reliable indication of the preovulatory LH surge than detection of oestrus (Dusza et al., 1996). VER changes have not been investigated in prepubertal lambs, especially when ewes are actively immunized with gonadotropin releasing hormone agonist (GnRHa). Moreover, its actual mechanism for regulating VER is unclear.

Gonadotropin-releasing hormone and its analogues (GnRH-A) can increase the binding affinity to GnRH receptors (GnRHR) (Guo and Gao, 2005) and stimulates the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the pituitary, which can improve the synthesis and release of steroid hormone (such as oestradiol) and regulate the mammalian reproductive process (Crawford et al., 2009).

Injecting exogenous gonadotrophin in cows at day 15–16 during the oestrous cycle could promote the follicle development and superovulation (Padula and Macmillan, 2005). Dhaliwal et al. (2002) demonstrated that GnRH agonist treatment increased the number of oocytes recovered in the superovulated heifers and ewes. Our previous studies demonstrated that alarelin (a GnRH agonist) active immunity in female rabbits could increase serum FSH and LH levels in the experimental group compared to control group, enhance primary follicle vertical diameter, primary follicle transverse diameter, increase the nucleoli and mitochondria, broaden the zona pellucida and microvilli of the oocytes, as well as promote the development of ovary and follicles as previously reported (Wei et al., 2011; 2012a). However, so far, whether or not the follicle development is associated with the VER in animals has not been investigated.

Based on the results of our previous studies (Wei and Zhang, 2008; Wei et al., 2012b), the present study aims to determine VER and its correlation with ovarian development as well as serum hormones (FSH and LH) in prepubertal female sheep that were actively immunized against GnRH agonist (alarelin).

## MATERIAL AND METHODS

### Animals and experimental design

Forty-two prepubertal female sheep (*Ovis aries*), 5–6 months of age and with a body weight of 24.21 ± 2.51 kg were randomly assigned to 6 experimental groups (EG, $n$ = 7 per group). Preparation of the alarelin antigen emulsion was performed according to our previous report (Wei and Zhang, 2008; Wei et al., 2011). The concentration of alarelin was 100 μg/ml. Animals in experimental group I (EG-I), experimental group II (EG-II) and experimental group III (EG-III) were subcutaneously injected with 200, 300, and 400 μg of alarelin antigens, twice (on days 0 and 14), respectively. Animals in experimental group IV (EG-IV) and experimental group V (EG-V) were subcutaneously injected with

200 and 300 μg of alarelin antigens, four times (on days 0, 7, 14, and 21) respectively, to enhance the immune response. Animals in the control group (CG) were subcutaneously injected with 2.0 ml of a solvent (or control liquid, which was prepared using the same methods and reagents as alarelin antigen preparation, only without adding alarelin), twice (on days 0 and 14). The experiment was conducted over a period of 70 days on the basis of GnRH antibody duration in our preceding study (Wei and Zhang, 2008). Animals were fed hay and a commercial concentrate diet *ad libitum*. All experimental procedures on animals were conducted following the Regulations for the Administration of Affairs Concerning Experimental Animals of Gansu province, the P.R. China.

## Collection of samples and measurement of ovary weights

Blood samples were collected from the jugular vein on days 0, 7, 14, 21, 28, 35, 45, 60, and 70 following alarelin immunization. Serum for measurement of FSH concentration was separated through centrifugation and stored at –20°C until analysis. The animals were heavily sedated by injecting 0.2 mg/kg xylazine intramuscularly on day 70, then euthanatized by exsanguination from the common carotid for each ewe. The ovaries were harvested aseptically from each ewe and immediately weighed using an electronic balance. The ovarian index was calculated. The ovarian index in each ewe was equal to the average weight of both right and left ovaries divided by her body weight on day 70. Then the ovaries were fixed in 10% formaldehyde (Yuexin Co., Guangdong, P.R. China).

## Measurement of vaginal electrical resistance (VER)

A commercially available ovulation detector (Draminski, Olsztyn, Poland) was used to determine VER on days 0, 7, 14, 21, 28, 35, 45, 60, and 70 following alarelin immunity. The portable device consists of a battery-operated main unit with a digital display and a stainless steel detachable probe. The probe was disinfected daily before use and tested in a sodium chloride solution for calibration as recommended by the manufacturer. The vulvar area of each ewe was cleaned with a paper towel and the probe was introduced in the vagina by spreading the vulva to avoid contamination. The depth of probe insertion in the vagina was that the probe just touched the external orifice of cervix uteri, or about 10–12 cm. The probe was rotated and moved back and forth 2–3 times and then held in place during 3 min or until the readings on the display stabilized. After each VER determination, the probe was wiped with a clean paper towel to remove contamination and placed into a bromogeramine solution (1.0%) until the next animal was in place for examination. Before each subsequent measurement, the probe was thoroughly rinsed with water and shaken to remove any excess water. All VER readings were taken by the same operator throughout the experiment. The average value of three measurements in each ewe was used for statistical analyses.

## Measurements of serum hormones

According to the manufacturer's instructions (Cusabio Biotech Co., Ltd. Wuhan, P.R. China), serum concentrations of FSH and LH were detected using FSH and LH detection kit for sheep (ELISA), respectively. The minimum limit of detection was 0.03 ng/ml. The intra- and inter-assay CV was less than 8%. The sensitivity was 0.002 ng/ml and the correlation coefficient of the standard curve was 0.9995. The detailed operation steps were reported in our initial research (Wei et al., 2012a).

## Histological observations and measurements of follicle indicators

The tissue samples fixed in 10% formaldehyde were embedded with paraffin wax, sliced (5 μm), and stained with hematoxylin and eosin (H&E). The sections were observed under the light microscope (Leica, Tokyo, Japan). Microscopy images of mature follicles were photographed. Eight sites in each section (3 sections in each group, totalling 360 sites for each group), containing the images of the follicle with maximum diameter were measured. The data of follicle vertical diameter (FVD), follicle transverse diameter (FTD), follicle-wall thickness (FWT), theca folliculi externa thickness (FET), and theca folliculi interna thickness (FIT) were measured using Images Advanced 3.2 and Image-Pro Plus 2.0 (MOTIC Co., Hong Kong, P.R. China).

The correlations between the expression levels of VER and ovarian indexes (such as average ovarian weights, FVD, FTD, FET, FIT, and FWT) and serum hormone concentration (FSH and LH) were analyzed using the Pearson's correlation method. The data for the correlation analyses were combined from all experimental groups. The effect within groups was ignored.

### Statistical analyses

The data were presented as the means ± SD. Statistical analyses were performed using SPSS software (Version 18.0, 2009). After a square root transformation of the data, all variables complied with the assumptions for a One-Way ANOVA. Pearson's model was utilized to analyze the correlations between VER values and ovarian indexes and hormones. When significant differences were identified, supplementary Tukey's post-hoc tests were performed to investigate pairwise differences. $P < 0.05$ was considered significant.

## RESULTS

### Changes of vaginal electrical resistance (VER)

As shown in Figure 1, the vaginal electrical resistances (VERs) in all groups changed in a similar trend. VER in the control group (CG) reduced from day 14, and reached the minimum levels on day 28, then increased gradually. Compared to CG, VERs in the experimental groups (EGs) decreased obviously from the first injection of alarelin antigen. VERs in EG-III and CG reached the lowest levels on day 28 ($P < 0.05$). VERs in EG-I, EG-II, EG-III, and EG-IV reached the lowest levels on day 35 ($P < 0.01$). VER in EG-V was lower than that in EG-I and EG-II ($P < 0.05$) on days 45 and 60. From the minimum levels onward, VERs in EGs rose gradually, but they were still significantly lower than those in CG on days 45 and 60 ($P < 0.01$).

### Serum concentrations of FSH and LH

The serum concentrations of FSH in EG-I to EG-III reached the peaks on days 28, 28, and 35 respectively, with a maximum in EG-III. The serum concentrations of FSH in EG-IV and EG-V reached the peaks at day 60. Serum FSH concentrations in EG-IV and EG-V were higher than those in EG-I ($P < 0.01$), EG-II ($P < 0.01$), and CG ($P < 0.01$) from day 45 to day 70 (Figure 2). Especially, FSH concentrations in EG-IV and EG-V on day 60 were higher than those in CG and EG-I ($P < 0.01$), as well as EG-II and EG-III ($P < 0.05$).

Serum concentration LH in CG was stable during the experiment. Compared to CG, LH concentration in all EGs decreased from day 0 and reached the minimum levels ($P < 0.05$) on day 21 or 28 after the first alarelin antigen injection (Figure 3).

Table 1. Measurements of ovaries and follicles in prepubertal female sheep immunized against GnRH agonist (mean ± SD; g, μm)

| Group | AOW | FVD | FTD | FET | FIT | FWT |
|---|---|---|---|---|---|---|
| CG | 0.24 ± 0.07$^{a}$ | 375.1 ± 39.1$^{a}$ | 253.3 ± 21.6$^{a}$ | 41.7 ± 9.8$^{a}$ | 41.4 ± 9.7$^{a}$ | 82.9 ± 14.6$^{a}$ |
| EG-I | 0.36 ± 0.08$^{b}$ | 398.1 ± 17.6$^{a}$ | 254.1 ± 14.8$^{a}$ | 41.7 ± 9.0$^{a}$ | 41.9 ± 6.2$^{a}$ | 85.4 ± 11.8$^{a}$ |
| EG-II | 0.41 ± 0.09$^{b}$ | 390.3 ± 33.6$^{a}$ | 252.7 ± 13.8$^{a}$ | 42.4 ± 9.6$^{a}$ | 42.1 ± 5.4$^{a}$ | 85.4 ± 12.8$^{a}$ |
| EG-III | 0.42 ± 0.06$^{b}$ | 414.1 ± 17.8$^{b}$ | 301.9 ± 28.4$^{b}$ | 46.6 ± 9.8$^{b}$ | 45.2 ± 9.1$^{b}$ | 90.5 ± 16.4$^{b}$ |
| EG-IV | 0.45 ± 0.04$^{c}$ | 396.1 ± 27.6$^{a}$ | 276.1 ± 19.4$^{a}$ | 43.8 ± 8.9$^{a}$ | 42.2 ± 9.9$^{a}$ | 87.0 ± 16.2$^{a}$ |
| EG-V | 0.48 ± 0.10$^{c}$ | 409.6 ± 18.1$^{b}$ | 296.8 ± 19.8$^{b}$ | 46.6 ± 9.8$^{b}$ | 44.1 ± 8.9$^{b}$ | 92.5 ± 16.9$^{b}$ |

AOW = average ovarian weight of left and right ovaries, FVD = follicle vertical diameter, FTD = follicle transverse diameter, FET = theca folliculi externa thickness, FIT = theca folliculi interna thickness, FWT = follicle wall thickness

$^{a–c}$compared to control group, the same superscript letters in the same column mean that there was no significant difference ($P > 0.05$). The different superscripts mean that there were significant differences between groups, of which adjacent superscripts (such as ab, bc) indicate the difference was significant ($P < 0.05$), while interval superscripts (such as ac) show the difference was highly significant ($P < 0.01$)

observed under optical microscope (×100)

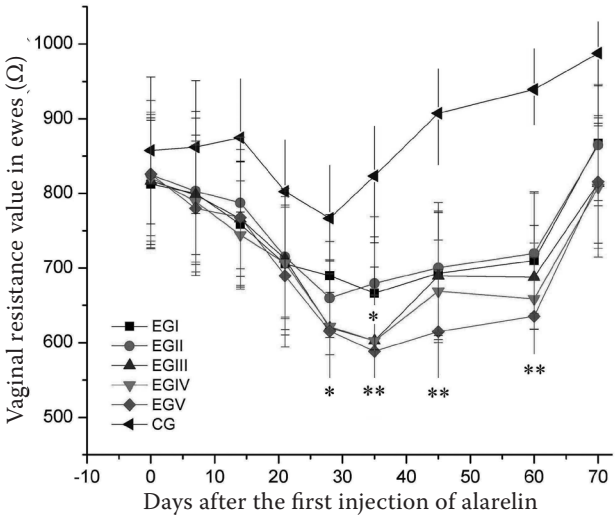


Figure 1. Vaginal electrical resistance (Ω, mean ± SD) in prepubertal female sheep immunized against GnRH agonist (alarelin)

Compared to CG, VERs in experimental groups decreased obviously from the first injection of alarelin antigen. VERs in EG-I and EG-II reached the minimum levels on day 28 ($P < 0.05$). VERs in EG-III, EG-IV, and EG-V reached the minimum levels on day 35 ($P < 0.01$). From the lowest levels onward, they were still significantly lower than those in CG on days 45 and 60 ($P < 0.01$), although VERs in EGs rose gradually

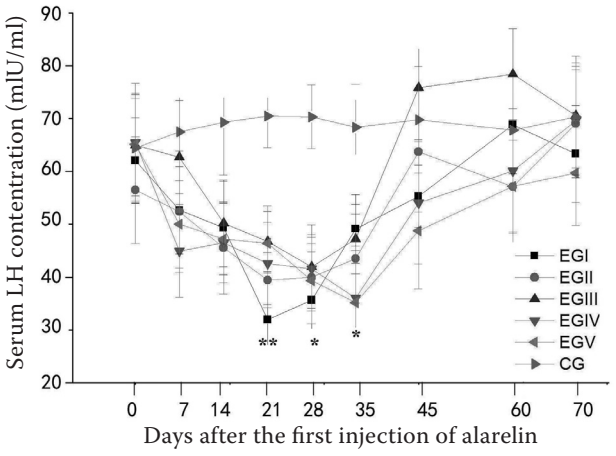


Figure 3. Concentration of serum luteinizing hormone (mIU/ml, mean ± SD) in prepubertal female sheep immunized against GnRH agonist (alarelin)

When compared to CG, LH concentration in all EGs decreased and reached the minimum levels ($P < 0.05$) on day 21 or 28 after the first injection of alarelin antigen

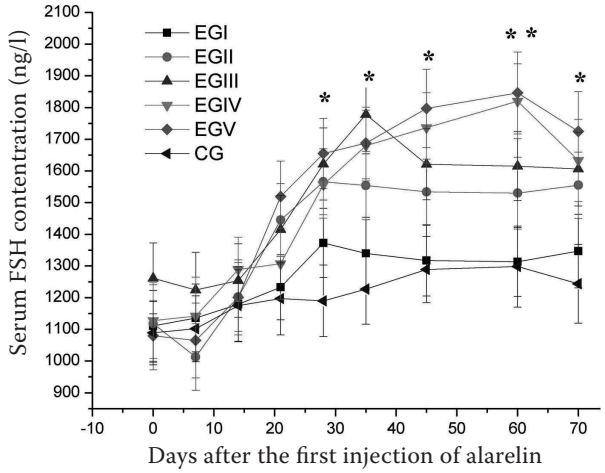


Figure 2. Concentration of serum follicle stimulating hormone (ng/l, mean ± SD) in prepubertal female sheep immunized against GnRH agonist (alarelin)

Serum FSH concentrations in all groups increased after the first injection of alarelin antigen. However, serum FSH concentrations in EG-IV and EG-V reached the peaks on day 60, and were higher than those in EG-I ($P < 0.01$), EG-II ($P < 0.01$), and CG ($P < 0.01$) on days 45–70

## Measurements of ovary weights and follicle indicators

Compared to CG, the ovarian weights in five EGs increased along with the alarelin immunization dosages (Table 1). The highest value was calculated in EG-V ($P < 0.01$). Values of FVD and FTD in EG-III and EG-V were greater than those in EG-I, EG-II, EG-IV, and CG ($P < 0.05$), with a maximum of EG-III. The values of FET, FIT, and FWT in all groups changed in similar to FVD and FTD.

## Correlations between VER, follicular indicators, and hormone concentrations

As listed in Table 2, Pearson's correlation analyses demonstrated that significant negative correlations between VER and AOW ($r = –0.814$, $P < 0.05$), FVD, FTD, FET, FIT, FWT, and FSH were calculated. There was a positive correlation between VER and serum LH ($r = 0.596$, $P > 0.05$), while correlations between LH and FVD, FTD, FET, FIT, as well as FWT were negative. In contrast, the serum FSH

Table 2. Pearson's correlation among vaginal electrical resistance (VER) and reproductive characteristics in prepubertal female sheep immunized against GnRH agonist

| Items | VER | AOW | FVD | FTD | FET | FIT | FWT | FSH |
|---|---|---|---|---|---|---|---|---|
| AOW | –0.814* | – | – | – | – | – | – | – |
| FVD | –0.707 | 0.791 | – | – | – | – | – | – |
| FTD | –0.287 | 0.658 | 0.847* | – | – | – | – | – |
| FET | –0.337 | 0.7 | 0.847* | 0.987** | – | – | – | – |
| FIT | –0.371 | 0.592 | 0.887* | 0.933** | 0.945** | – | – | – |
| FWT | –0.549 | 0.81 | 0.934** | 0.944** | 0.965** | 0.919** | – | – |
| FSH | –0.523 | 0.853* | 0.684 | 0.739 | 0.792 | 0.717 | 0.778 | – |
| LH | 0.596 | –0.614 | –0.39 | –0.204 | –0.204 | –0.021 | –0.393 | –0.146 |

AOW = average ovarian weight of left and right ovaries, FVD = follicle vertical diameter, FTD = follicle transverse diameter, FET = theca folliculi externa thickness, FIT = theca folliculi interna thickness, FWT = follicle wall thickness, FSH = follicle-stimulating hormone, LH = luteinizing hormone

*correlation is significant at the 0.05 level (2-tailed)

**correlation is significant at the 0.01 level (2-tailed)

concentration had positive correlations with all ovarian indexes. Furthermore, FVD, FTD, FET, FIT, and FWT had positive correlations with each other ($P < 0.05$ or $P < 0.01$).

## DISCUSSION

Gonadotropin-releasing hormone (GnRH), synthesized in the hypothalamus, is a key regulator of reproduction in mammals. Released from the hypothalamus in a pulsatile pattern, it travels via the portal vasculature to the anterior pituitary, where it stimulates the release of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These gonadotropins enter the circulation and regulate both steroidogenesis and gamete maturation in the gonads (Conn et al., 1994). In other words, GnRH pulsatile secretion stimulates steroidogenesis and gamete maturation in ovaries indirectly.

Gong et al. (1996) have demonstrated that continuous infusion of heifers with GnRH agonist can suppress the secretion of LH and FSH. This suppression resulted in the inhibition of ovarian follicle development beyond 4 mm in diameter.

The present study has demonstrated that FSH levels were increased, LH levels were decreased, and ovarian follicles were greater in prepubertal female sheep after immunization against GnRH agonist. These results are different from those by Conn (1994) and Gong et al. (1996). Immunization against GnRH agonist had a different effect on FSH and LH levels. Try to explain these differences. For example, the present study examined the effect of GnRH agonist in the immature female sheep, whereas other studies examined the effect of GnRH agonist in mature females. Ovaries in immature female sheep and seasonally anoestrous sheep are similar. Thus the findings in seasonally anoestrous animals can help to explain the results in the present study.

The present study demonstrated that vaginal electrical resistance (VER) was significantly lower in immunized prepubertal female sheep than in the control group. GnRHa (alarelin) active immunization could reduce VERs in prepubertal female sheep. Electrical resistance does not only reflect changes in the mucus, but also in the mucosa and deeper layers in the vagina (Rezac, 2008).

The sodium ion content in the uterine mucus maybe affects the electrical resistance of mucus in the vagina. Previous studies showed that the cervical mucus secretion increased 10–20 times during the oestrus peak stage, under the influence of oestrogen, meanwhile, sodium chloride (NaC1) in the mucus increased, leading conductivity of the mucus rose. Furthermore, VER declined (Million et al., 2011). However, whether or not the sodium content in the mucus affects VER has not been

proved. It has not been determined whether the sodium content in the mucus at different distances from the cervix uteri in the vagina is significantly different or not.

Determination of VER can be utilized to estimate the phase of the oestrous cycle (Yamauchi et al., 2009). A decrease in VER at oestrus, when a mature follicle was present, and rise in VER subsequently to ovulation was clearly observed (Meena et al., 2003). A decrease in VER at oestrus may be due to increased hydration and congestion of the vaginal mucous membranes (Gupta and Purohit, 2001; Purohit and Gupta, 2005). The decrease in vaginal impedance in the follicular phase of the oestrous cycle is probably triggered by oestradiol. VER values may be associated with the secretion of oestrogen. A significant negative correlation between VER and plasma oestradiol level during perioestrus was found in pigs (Dusza et al.,1996) and cattle (Narasimha et al., 1989). The potential role of oestradiol in the initiation of VER decrease is also supported by experiments carried out on ovariectomized cattle (Metzger et al., 1972). These changes were not measured in the present experiment, whether similar results would be found in the prepubertal female sheep needs to be explored in the future.

The overall VER values observed in the present study were relatively greater than the VER values reported previously by Wehner et al. (1997), Bartlewski et al. (1999), Scipioni and Foote (1999), and similar to the VER values reported by Gupta and Purohit (2001), Zuluaga et al. (2008), Hockey et al. (2009). These differences during the different oestrous phases may be the result of changes in intravaginal probe designs. Other factors such as depth of probe insertion in the vagina, dorsal or ventral position of the probe within the vagina, pressure against the mucous membrane, pathological conditions of the reproductive tract, and technician have also been shown to influence results (Kitwood et al., 1993). The depth of probe insertion in the vagina has an influence on VER. In the present study, the depth of probe insertion was that the probe point just touched the external orifice of cervix uteri in the vagina. Therefore, the detected VER values referred to the whole vagina.

Injection with GnRH agonist inhibited the secretion of FSH and LH and ovarian development in heifers (Gong et al., 1996). In the present study, the serum concentrations of FSH in all EGs increased gradually and reached the peaks on days 28, 35, and 60, respectively, with a maximum in EG-V. The results demonstrated that GnRH agonist immunization can promote the synthesis and secretion of FSH. Our results were consistent with the reports of Guo and Gao (2005), Junaidi et al., (2009), and Gong et al. (2011), but unfamiliar with other reports (Zamaratskalia et al., 2007; Li et al., 2009). Whether the efficacies of GnRHa are associated with the oestrous and/or ovulation of ewes and injection dosage of alarelin antigen needs to be further explored.

Follicular diameter is an indicator of ovulatory capacity (Sartori et al., 2001) and it has been associated with fertility. In the current study, the ovarian weight was the greatest in EG-V ($P < 0.01$) on day 70. Values of FVD and FTD in EG-III and EG-V were larger than those in EG-I, EG-II, EG-IV, and CG, with a maximum of EG-III. This is not in agreement with previous reports which described the follicular diameter at the time of ovulation was associated with an increase in fertility (Vasconcelos et al., 2001; Perry et al., 2005; Lopes et al., 2007). Whether the changes of ovarian indicators are associated with ovulation and fertility of the prepubertal female sheep needs to be explored in the future.

Pearson's correlation analyses demonstrated that the negative correlations between VER and AOW, FVD, FTD, FET, FIT, FWT, and FSH were calculated. There was a positive correlation between VER and serum LH while correlations between the LH and FVD, FTD, FET, FIT, as well as FWT were negative. In contrast, the serum FSH concentration had positive correlations with all ovarian indexes. Furthermore, FVD, FTD, FET, FIT, and FWT had positive correlations with each other.

## CONCLUSION

GnRHa (alarelin) active immunization could significantly affect VER, FSH, LH, and follicle development in prepubertal female sheep. VER had a positive correlation with serum LH, and negative correlations with AOW, FVD, FTD, FET, FIT, FWT, and FSH.

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