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*Full Length Research Paper*

# Effect of two follicle stimulating hormone (FSH) preparations and simplified superovulatory treatments on superovulatory response in Xinji fine-wool sheep

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**The objective of the present study was to investigate the response of Xinji fine-wool sheep to superovulation with two follicles stimulating hormone (FSH) preparations and simplified superovulatory treatments. In Experiment I, 22 adult Xinji fine-wool sheep were randomly allocated in equal number (n = 11) to two groups which were superovulated with 280 IU FSH-P and 150 mg Folltropin-V, respectively. In Experiment II, 61 adult Xinji fine-wool sheep randomly divided into four groups were treated with four different superovulatory schemes based on the dosage and number of Folltropin-V injection, respectively. The ewes in the four groups were treated with a total of 150 mg Folltropin-v (6 injection, Group A, n = 15), 120 mg Folltropin-V (3 injection, Group B, n = 18), 90 mg Folltropin-V (3 injection, Group C, n = 13) and 150 mg Folltropin-V dissolved in 30% PVP (1 injection, Group D, n = 15), respectively. The results show that the number of corpora lutea was significantly higher in ewes treated with Folltropin-V than that with FSH-P (P<0.05); higher number of transferable embryos was observed in Folltropin-V group than in FSH-P group. There is no significant difference in number of recovered embryos and transferable embryos in Groups A and B (P>0.05). However, the number of recovered embryos and transferable embryos in Groups C and D is significantly lower than those in Groups A and B (P<0.05). In conclusion, superovulation with 120 mg Folltropin-V (3 injections) is the most appropriate superovulatory treatment in Xinji fine-wool sheep.**

**Key words:** Superovulatory response, follicle stimulating hormone (FSH) preparations, Xinji fine-wool sheep.

## INTRODUCTION

Xinji fine-wool sheep are a fine wool breed in China which is developed by cooperation of research institutions from Xinjiang and Jilin provinces in 2003. Xinji fine-wool sheep wool is fine, flexible, soft and shapely and is of high demand by the textile industry. However, the quantity of wool produced by Xinji fine-wool sheep cannot meet the needs from the textile industry. Therefore, there is a need to increase the number of Xinji fine-wool sheep. Multiple ovulation and embryo transfer (MOET) can be used to produce a large number of off-spring per good quality sheep or goat in a short time (Cognie et al., 2003; Morand-Fehr and Boyazoglu, 1999; Quan et al., 2011). Superovulation is one of the crucial techniques in MOET which directly influences the quality and quantity of harvested embryos. The primary goal of superovulation in sheep and goat is to obtain the maximum number of good quality embryos from each donor. However, super-ovulatory response is high degree of unpredictability which continues to be one of the most frustrating problems with MOET in sheep and goat (Azawi and Al-Mola, 2011; Lehloenya et al., 2009; Quan et al., 2011).

There are many factors affecting superovulatory response of ewe, such as sorts and preparation of

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gonadotrophin, total dose of gonadotrophin, superovulation operation and superovulatory scheme (Ishwar and Memon, 1996; Nowshari et al., 1995; Quan et al., 2011). There are also other important factors that contribute to the variability of superovulatory response, including age, breed, reproductive history, nutritional status and season (Ammoun et al., 2006; Quan et al., 2011; Veiga-Lopez et al., 2006; Vivanco et al., 1994). Two different types of gonadotropin have generally been used for superovulation of ewe: follicle stimulating hormone (FSH) and equine chorionic gonadotropin (eCG). Because of its long half-life, a single injection of eCG is sufficient to induce superovulation. However, the residual eCG has a detrimental effect on ovulation, fertilization, embryo recovery and viability rates (Evans and Robinson, 1980; Simonetti et al., 2008). In contrast, due to its short biological half-life, FSH is usually administrated in multiple injections twice daily over three to four days, which requires more labor and expense; therefore, the superovulation protocol need to be simplified. Furthermore, difference in superovulatory response may be caused by different FSH preparations (Kanitz et al., 2002; Quan et al., 2011). To our knowledge, in this respect, no studies have been reported in Xinji fine-wool sheep. The aim of the present study was to investigate the response of Xinji fine-wool sheep to superovulation with two FSH preparations and simplified superovulatory treatments.

## MATERIALS AND METHODS

This study was conducted during the natural breeding season (autumn 2009 and 2010) and the procedures were approved by the ethical committee of the Xinjiang Academy of Animal Science. Non-pregnant adult Xinji fine-wool sheep (3 to 5 years old) were used as embryo donors and were maintained in individual pens with free access to water and fed with concentrate and hay. All the ewes were submitted to clinical-gynecological examinations prior to experiments. Oestrous synchronization of all the ewes was performed using progesterone releasing intravaginal devices that contained progestagen (Shanghai Institute of Planned Parenthood Research, China), which were inserted for 14 days. All the ewes were injected with 500 international units (IU) of pregnant mare serum gonadotropin (PMSG, Shanghai Institute of Planned Parenthood Research, China) when the devices were removed from the vagina. Estrous in ewes were detected with the aid of apronedram of high sexual vigor at 8 h intervals for 3 days.

### Experimental design

### Experiment I

22 adult Xinji fine-wool sheep were randomly allocated in equal number (n = 11) to two groups (Groups A and B). In order to induce superovulation, ewes in Groups A and B were injected with two FSH preparations, respectively on the 12th day of their estrous cycle for 3 days (daily twice) in decreasing dose. Specifically, group A were treated with a total of 280 IU FSH-P (a kind of FSH preparation, produced by The Second Hormones Factory of Ningbo, China) in decreasing dose (twice daily at 60, 50 and 30 IU per injection on days 1, 2 and 3, respectively); while group B were injected with a total of 150 mg Folltropin-V (a kind of FSH preparation, produced by Vetrepharm, Canada) twice daily at 35, 25 and 15 mg per injection on days 1, 2 and 3, respectively. When the fifth FSH injection was administered, 0.1 mg of prostaglandin $F_{2\alpha}$ (Shanghai Institute of Planned Parenthood Research, China) was injected and oestrus was detected by rams at 12 h intervals after the 24 h of prostaglandin $F_{2\alpha}$ injection. Artificial inseminations were performed at 12 and 22 h after the detection of estrus every 12 h for 2 times and 100 IU of luteinizing hormone (LH, The Second Hormones Factory of Ningbo, China) were injected at the same time. On day 6 following the second AI, embryos were collected by mid-ventral laparotomy under general anaesthesia. Ewes were anesthetized using an i.m. administration of 0.4 to 0.6 ml Sumianxin (Jilin University, Changchun, China). The ovarian response was assessed using the number of functional corpora lutea that had a good morphology. Embryos that had more than two corpora luteas were collected from the ovaries. Uterine horns were exposed and, using a Foley catheter, flushed with phosphate-buffered saline (PBS) supplemented with 1% bovine serum albumin (BSA; Sigma, USA) and antibiotics (penicillin and streptomycin). The flushing media that was recovered was scrutinized and evaluated under a stereomicroscope to identify and classify the structures (unfertilized ova and embryos) according to their morphological appearance (Lindner and Wright, 1983; Overstrm, 1996).

### Experiment II

In order to reduce the dose and times of FSH injection and simplify the superovulatory schemes, sixty-one ewes were treated with four different superovulatory schemes (A, B, C and D). All the ewes were superovulated with Folltropin-V (a kind of FSH preparation, produced by Vetrepharm, Canada) according to the superovulatory protocol described in Experiment I, except for the dose and times of FSH injection. Control ewes (Group A) received 6 injections of FSH (total dose: 150 mg). The ewes in Groups B and C received only one injection per day for 3 days. The total dose for FSH was 120 mg (60, 40 and 20 mg each time) in Group B and 90 mg (40, 30 and 20 mg each time) in Group C. The ewes in Group D were treated with a single injection of 150 mg Folltropin-V dissolved in 30% polyvinylpyrrolidone (PVP). Collection and evaluation of embryos were performed as described in Experiment I.

### Statistical analysis

Data was analyzed using the SPSS 13.0 for windows statistical software (SPSS, 2004). Differences in total corpora lutea, total recovered embryos, transferable embryos and unfertilized ova were analyzed using ANOVA and multiple comparisons.

## RESULTS

### Effect of two FSH preparations on superovulatory response in Xinji fine-wool sheep

The superovulation response of ewes treated with two FSH preparations through a number of corpora lutea, recovered embryos and transferable embryos are shown in Table 1. The number of corpora lutea was significantly higher ($P<0.05$) in ewes treated with Folltropin-V than FSH-P which are 12.47 ± 1.54 and 9.67 ± 1.93, respectively. The number of recovered embryos in Folltropin-V group was higher than that in FSH-P group which are 9.27 ± 1.86 and 7.85 ± 2.42, respectively but the

**Table 1.** Effect of two FSH preparations on superovulatory response in Xinji fine-wool sheep.

| Parameter | FSH-P | Folltropin-V |
|---|---|---|
| Number of ewes | 11 | 11 |
| Number of corpora lutea | 9.67±1.93[b] | 12.47±1.54[a] |
| Number of recovered embryos | 7.85±2.42[a] | 9.27±1.86[a] |
| Number of transferable embryos | 4.52±2.52[b] | 7.86±1.75[a] |

Values in the same line with different superscript letters (a, b) indicate significant differences at $p < 0.05$.

**Table 2.** Effect of FSH injection schemes on superovulatory response in Xinji fine-wool sheep.

| Item | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| Number of ewes | 15 | 18 | 13 | 15 |
| Total dose of FSH (mg) | 150 | 120 | 90 | 150 |
| Number of injections | 6 | 3 | 3 | 1 |
| Number of recovered embryos per donor | 9.55±2.47[a] | 10.65±2.21[a] | 6.25±2.73[b] | 5.86±2.48[b] |
| Number of transferable embryos per donor | 7.82±1.88[a] | 8.46±2.25[a] | 5.12±2.47[b] | 4.78±2.53[b] |

Values in the same line with different superscript letters (a, b) indicate significant differences at $p < 0.05$.

difference is not significant (P>0.05). High number of transferable embryos was observed in Folltropin-V group than in FSH-P group which are 7.86 ± 1.75 and 4.52 ± 2.52, respectively.

### Effect of different schemes of FSH injection on superovulatory response in Xinji fine-wool sheep

Table 2 presents the effect of FSH injection schemes on superovulation response of Xinji fine-wool sheep through a number of recovered embryos and transferable embryos. The results indicate that a total dose of 120 mg FSH (3 injections) can be as efficient as 150 mg (6 injections) in superovulation of Xinji fine-wool sheep, as there is no significant difference in number of recovered embryos and transferable embryos in Groups A and B (P>0.05). However, the number of recovered embryos and transferable embryos in Groups C and D is significantly lower than that in Groups A and B (P<0.05), which indicate that injecting with 90 mg (3 injections) or 150 mg of FSH dissolved in 30% PVP (1 injection) is unfeasible for simplifying the superovulation protocol.

## DISCUSSION

Gonadotrophin is a key factor for superovulatory response of donor, which directly affect the number of transferable embryos obtained per donor. FSH preparations produced from the pituitaries also contain other biological active substances like LH besides FSH. The amounts of FSH and LH and their activities can be different for different products (Braileanu et al., 1998; Donaldson et al., 1986). In experiment I, two FSH products (FSH-P and Folltropin-V) were used to perform the superovulation of Xinji fine-wool sheep and the results show that superovulation with Folltropin-V can obtain more transferable embryos than that with FSH-P (P<0.05) and also maintain stability of superovulation reflected by standard deviation of transferable embryos. This result is in agreement with Quan et al. (2011). The difference in superovulatory response may be caused by the different content of LH in FSH preparations. The suitable ratio of FSH/LH in FSH preparation is crucial for superovlatory response of donor, and FSH preparation of low content of LH could be better for follicle development and obtaining of more transferable embryos (Boscos et al., 2002; Donaldson et al., 1986; Nowshari et al., 1995). It was reported that Folltropin-V is a FSH preparation with low LH activity, since 80% of LH have been eliminated during its purification (Wu et al., 1988). However, superovulation with a recombinant-DNA bovine FSH was neither possible to increase the number of transferable embryos nor stabilize the superovulatory response (Wilson et al., 1993) which indicated that a small quantity of LH in FSH preparation is essential for superovulation of donor.

As a result of its short biological half-life, FSH is usually administrated in multiple injections, which not only requires more labor and expense, but also may involve errors in dose and injection times. Furthermore, multiple injections can be stressful for donors and negative for reproductive performance. For this reasons, superovulation schemes were simplified in decreasing the time of FSH injection in experiment II. The results show that 3 injections of FSH (total dose, 120 mg) can be as efficient as 6 injections of FSH (total dose, 150 mg) in superovulation of Xinji fine-wool sheep, however, superovulation protocol with total dose of 90 mg FSH (also 3 injections) or 150 mg FSH (1 injection) significantly decreased the number of transferable embryos. These results demonstrate that reducing dose and number of FSH injection in a certain extent for superovulation of Xinji fine-wool sheep can not affect embryo yield. The results are very significant to superovulation, since this superovulation scheme could reduce the expense of FSH and the stress of donors caused by multiple injections.

Our results are supported by several studies which had demonstrated that reduced dose or number of FSH injection does not affect embryo yield. Berlinguer et al. (2004) reported that the superovulatory response was not affected by decreasing the dose. It was also demonstrated that decreasing dose of FSH in superovulation of ewes is feasible and the reason may be that the decreasing-dose protocol mimics the “wave like pattern” of FSH secretion (Gonzalez-Bulnes et al., 2000; Gonzalez et al., 2002). Rexroad et al. (1991) and D’Alessandro et al. (2005) reported that reducing the number of FSH injection can not affect the superovulatory response of the donor. However, it was reported that reducing the number of FSH injection could decrease the embryo yield and the results might have been due to a short period of ovarian follicular recruitment which is a common phenomenon in superovulatory treatments that begins 24 h before the end of the progestagen treatment (Simonetti et al., 2008).

## Conclusion

In conclusion, the present study demonstrated that superovulation with Folltropin-V can obtain more transferable embryos than that with FSH-P; and 150 mg Folltropin-V (6 injections) can be as efficient as 120 mg Folltropin-V (3 injections) in superovulation of Xinji fine-wool sheep.

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