Factors Affecting Superovulation and Embryo Transfer in Boer Goats*

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ABSTRACT : Despite many studies, results of superovulation protocols are not consistent in farm animals. In this study, 151 Boer goats were superovulated to examine the factors affecting superovulation and embryo transfer (MOET). An optimal regime for superovulation treatment was identified as a 4-day treatment with decreasing dosages of 6-7 mg Chinese FSH or 240 mg Canadian FSH. The 4-day treatment with decreasing dosages of 6-7 mg Chinese FSH was, therefore, adopted to study effects of the age of does, season and repeated treatments on superovulation and embryo transfer. The best season for superovulation and embryo transfer and pregnancy was autumn, and the best age range was 12-35 months old. Within animals there were no significant differences in the number of ovulations and the rate of transferable embryos between the first and the second superovulation. However, these parameters declined significantly for the third superovulation. No marked effects of the number of ovulations on the proportion of transferable embryos were noted. The parturition rate of the recipients receiving single embryos was not different significantly from those receiving two embryos, and the kidding rate calculated from embryos transferred did not differ significantly between recipients receiving one and two embryos. *(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 341-346)*

INTRODUCTION

Multiple ovulation and embryo transfer (MOET) is an effective means of increasing the contribution of superior females to breeding programs and it is also an essential procedure of embryo biotechnology. Despite its application in many domestic species, there are still many problems. For example, rates of ovulation and transferable embryos are still unpredictable. This causes problems not only in animal production but also in the application of embryo biotechnology. It is known that factors such as breeds (Terawaki et al., 2002), nutrition (Smith, 1991; Downing et al., 1995; Nolan et al., 1998; Yaakub et al., 1999; O'Callaghan et al., 2000; Armstrong et al., 2001), season (Rosnina et al., 1992; Pintado et al., 1998; Hansen et al., 2001; Mitchell et al., 2002; Chagas et al., 2003), photoperiod (Mutiga et al., 1984), ovarian status (Gonzalez-Bulnes et al., 2000; 2002), gonadotrophin preparation (Armstrong et al., 1983; Rosnina et al., 1992; Pintado et al., 1998; Lopes da, 2001; Gonzalez-Bulnes, 2000) and repeated superovulation (Boland et al., 1982; Al-Kamaliet al., 1985; Torres et al., 1987; Cognie, 1999; Magarey et al., 2003) affect superovulation as well as the quality of embryos produced. It was reported that few prepubertal heifers (Armstrong et al., 1997; Maneesh et al., 2000) and lambs (Armstrong et al., 1997; Ptak et al., 1999; 2000a, b; 2003) responded to superovulation treatments. Studies have been conducted on superovulation of goats (Armstrong et al., 1983; Tan et al., 1987; Mishra et al., 2003; Mishra et al., 2004), but further investigations are needed to optimize the protocol. Since the introduction of Boer goats into China in the 1990s, many investigations on superovulation and embryo transfer have been conducted, in order to increase the population of this new breed. Although most of these studies have examined factors influencing MOET, most have dealt with only one or two factors, and a comprehensive study comparing several different factors in a single paper is lacking.

In the present study, 151 Boer does were superovulated and embryos obtained were transferred to more than 1,500 recipient does of native breeds, so that the factors affecting MOET success could be evaluated. The optimal regimen for superovulation was first selected, and the effects of the doe age, season and repeated treatments on superovulation and embryo transfer were then studied using the optimal superovulating regime.

MATERIALS AND METHODS

Animals

The experiment was conducted on four different farms of Shandong Province between April of 2000 and January of 2004. Fifty-two Australian Boer does or their pure bred offspring, 7 to 60 months of age, were used as embryo donors. The donors were superovulated at least 70 days post partum. Parous native does with large body sizes and sound reproductive history were chosen for recipients. All animals were housed outdoors in sheltered pens and fed with hay and concentrate, with water available *ad libitum*.

Superovulation and insemination

Intravaginal progesterone sponges (Institute of Animal Breeding & Reproduction, Nanjing Agricultural University, China) or CIDR (CIDR-G; InterAg, Hamilton, New Zealand) were placed in the vagina of the donor and on days 12-14 after implantation, the donors were superovulated with one of the following regimes: (i) 6 or 7 mg Chinese made FSH (ChFSH, Institute of Zoology, China Academy of Sciences, China) were administrated intramuscularly twice a day for 3 days in decreasing dosages; (ii) 6 or 7 mg ChFSH were injected intramuscularly for 4 days in decreasing doses; (iii) 240 mg Canadian made FSH (CaFSH, Folltropin V, Vetrepharm, Ontario, ON, Canada) were given intramuscularly in decreasing doses over a 4-day period; (iv) 160 mg CaFSH were administrated intramuscularly in constant dosages over a 4-day period, with 300 IU PMSG given with the 3rd injection of FSH. Intravaginal sponges were removed at the 5th injection of FSH for does receiving a 3-day FSH treatment and at the 7th FSH injection for does given a 4-day FSH treatment.

Estrus was checked with a vasectomized buck twice a day at 06:00 and 18:00 h from sponge withdrawal. A doe was considered to be in estrus when she stood to be mounted by the buck. At the onset of estrus, the donor does were intramuscularly injected with 250 IU hCG, and artificially inseminated with semen deposited in the uterine cervix. Inseminations were performed at 8-10 h intervals until the does refused to be mounted.

Embryo recovery and evaluation

On days 6 or 7 after estrus, morulae and blastocysts were recovered surgically from the uterine horns of the donors. Reproductive tracts were exteriorized through a mid-ventral incision after induction of local anaesthesia with Lignocaine. A blunted needle was inserted into the uterine horn through the uterine wall about 1 cm distal to the utero-tubal junction, and 10-15 ml Dulbecco's phosphate-buffered saline (PBS) containing 10% fetal calf serum were flushed through the uterus. The flushings were recovered through a paediatric Foley catheter, which was inserted in the uterine horn just proximal to the bifurcation, and its bulb inflated with air. The flushings were collected into sterile plastic tubes with plane bottoms. These collection tubes were allowed to stand in a 37°C for approximately 10 min, before examination under a dissecting microscope for embryos. Embryos collected were classified into A, B, C and D categories in accordance with the criteria reported by Lindner et al. (1983), and only the A and B embryos were considered as transferable.

Recipient synchronization

Progesterone sponges were placed into the vagina of the recipient does on the day corresponding to the donors. Vaginal sponges were removed from recipients 10-12 h earlier than from the donors of the same group. Estrus of recipients was checked with a vasectomized buck after sponge removal. The difference in the onset of estrus between donors and recipients was required to be within 24 h for embryo transfer to occur.

Embryo transfer

Embryos were transferred surgically. The reproductive tracts of the recipients were exposed via a mid-ventral incision after induction of local anesthesia with Lignocaine. All embryos were transferred into the uterine horn near the utero-tubal junction. Usually, a single embryo was transferred into the uterine horn ipsilateral to the ovary with corpora lutea of each recipient, but occasionally, two embryos were put into the uterine horn ipsilateral to the ovary with better-developed corpora lutea when the recipient had two or more ovulations. Volumes of PBS were kept to approximately 5-10 µl per transfer.

Precautions were taken to minimize adhesion in donors and recipients. In particular, bleeding was kept to a minimum, and tracts were irrigated liberally with heparinized saline to prevent formation of blood clots. After transfer, animals were kept under regular observation to determine time of return to estrus. Only the recipients that were found aborted after 2 months of embryo transfer or gave birth at the end of gestation were considered as pregnant.

Experimental design

Experiment 1 : Fifty-two donor does 12 to 35 months old were treated in the autumn with the 4 different superovulation regimes. Ovulations, proportions of transferable embryos and pregnancy per donor were compared among different treatments.

Experiment 2 : Forty-one donor does of 12 to 35 months old were treated in 3 seasons with the selected regime (Regime ii). Ovulations, proportions of transferable embryos and pregnancy per donor were compared among different treatments.

Experiment 3 : Forty-eight does of 7 to 60 months old were treated in the autumn with regime ii. Ovulations, proportions of transferable embryos and pregnancy per donor were compared among different age ranges (7-11, 12-23, 24-35, 36-47 and 48-59 months).

Experiment 4 : Seventy-five does of 12-35 months old were treated in the autumn with regime ii. Ovulations, proportions of transferable embryos per donor, and proportions of does in estrus and with cystic ovaries and adhesive genital tracts were compared among the first, second and third treatments.

Experiment 5 : Twenty-nine does of 12-35 months old

Table 1. Mean and SE for different superovulation regimes in 12-35 months old does

Regimes	Does treated	Ovulations/doe	Proportion of transferable embryos	Proportion of pregnant recipients
i	15	13.82 ± 1.84^a	0.55 ± 0.11^a	0.57 ± 0.13^b
ii	19	15.49 ± 4.48^a	0.87 ± 0.09^b	0.51 ± 0.71^b
iii	9	20.55 ± 2.92^a	0.91 ± 0.09^b	0.41 ± 0.40^a
iv	9	15.42 ± 1.91^a	0.78 ± 0.06^{ab}	0.55 ± 0.13^b

[a, b] Values in columns with a common letter in superscripts do not differ significantly (p>0.05).

Table 2. Superovulation results of Boer does treated in different seasons

Seasons	Does treated	Ovulations/doe	Proportion of transferable embryos	Proportion of pregnant recipients
Spring	5	10.40 ± 3.37^a	0.82 ± 0.13^{ab}	0.58 ± 0.08^a
Autumn	17	17.24 ± 1.83^b	0.85 ± 0.07^a	0.65 ± 0.05^a
Winter	19	14.32 ± 1.73^{ab}	0.66 ± 0.07^b	0.44 ± 0.04^b

[a, b] Values in columns with a common letter in superscripts do not differ significantly (p>0.05).

Table 3. Effects of the age on superovulation of Boer goats

Months of age	Does treated	Ovulations/doe	Proportion of transferable embryos	Proportion of does with cystic ovaries	Proportion of does in estrus	Proportion of pregnant recipients
7-11	10	10.72 ± 2.49^a	0.45 ± 0.086^b	0	0.60^a	0.41 ± 0.09^a
12-23	14	14.93 ± 2.34^{ac}	0.88 ± 0.06^a	0.071^a	0.93^a	0.60 ± 0.55^b
24-35	11	18.46 ± 2.64^{bc}	0.81 ± 0.07^a	0.091^a	1.00	0.60 ± 0.62^{bc}
36-47	5	12.25 ± 3.09^{ac}	0.92 ± 0.09^a	0	1.00	0.49 ± 0.05^{ac}
48-59	8	16.63 ± 3.10^{bc}	0.82 ± 0.08^a	0	1.00	0.50 ± 0.07^{ac}

[a, b, c] Values in columns with a common letter in superscripts do not differ significantly (p>0.05).

that had been treated in the autumn with regime ii were selected, and the relationship between the number of ovulations and the proportions of transferable embryos and pregnancy was analyzed.

Experiment 6 : Transferable embryos were collected from donors of 12-35 months old that were superovulated in the autumn with regime ii and were transferred to 55 recipients in the same season. Effects of the number of embryos implanted per transfer on parturition and kidding rates of recipients were examined.

Statistical analysis

Means of ovulations, proportions of transferable embryos and recipient pregnancy were calculated from individual donors and analyzed by analysis of GLM. One factor was analyzed each time using the univariate module, and variables were compared with LSD. Results are presented as the mean±SE. Proportions of does in estrus, with cystic ovaries and with adhesive tracts, and the parturition rate of recipients and the kidding rate calculated from embryos were analyzed by Chi-square test (SPSS, Inc. San Rafael, CA).

RESULTS

Experiment 1: Optimizing superovulation

Higher ovulations were obtained with regime ii and regimen iii (Table 1). Rates of transferable embryos from regimens ii and iii were significantly higher than that of regime i. The pregnancy rate of regimen iii was significantly lower than other regimens. Therefore, regimen ii was selected for further investigations.

Experiment 2: The effect of season

The result shows that autumn was the best season for superovulation and embryo transfer (Table 2).

Experiment 3: Donor age

The highest ovulation number was obtained from does, 24-35 months old. Embryos recovered from the does aged 12-35 months produced the highest pregnancy (Table 3), and there was no significant difference between the does aged 24-59 months in the proportion of transferable embryos.

Experiment 4: Repeated treatments

No significant difference was observed in ovulations per doe and proportions of transferable embryos between does treated for the first or second time, but these values decreased significantly for does treated a third time. The proportion of does in estrus after superovulation was not significantly different among the 1st, 2nd and 3rd treatments. The data also showed that repeated treatments increased the incidence of adhesion of reproduction tract.

Experiment 5: Relationship between the number of

Table 4. Results of repeated superovulations

Times of treatments	Does treated	Ovulations/doe	Proportion of transferable embryos	Proportion of does with cystic ovaries	Proportion of does in estrus	Proportion of does with adhesions*
1	57	15.07±1.12[a]	0.762±0.04[a]	0.018[a]	0.96[a]	0
2	14	15.07±2.25[a]	0.714±0.08[a]	0.056[a]	0.94[a]	0.14[a]
3	4	7.50±4.22[b]	0.532±0.31[b]	0	100	0.50[a]

[a, b] Values in columns with a common letter in superscripts do not differ significantly ($p > 0.05$).

* Ratio of does with adhesions out of the does treated.

Table 5. Effects of the number of ovulations on proportions of transferable embryos and pregnancy

Ovulations	Does treated	Proportion of transferable embryos	Proportion of pregnant recipients
<10	10	0.76±0.067[a]	0.53±0.071[a]
10-20	8	0.77±0.074[a]	0.58±0.080[a]
20-30	8	0.70±0.074[a]	0.65±0.080[a]
>30	3	0.86±0.122[a]	0.67±0.13[a]

[a, b] Values in columns with a common letter in superscripts do not differ significantly ($p > 0.05$).

ovulations and the proportions of transferable embryos and pregnancy

It can be seen from Table 5 that the number of ovulations had no significant effect on proportions of transferable embryos and recipient pregnancy. Although the proportion of pregnant does tend to increase with the number of ovulations, the difference was not statistically significant.

Experiment 6: Effects of the number of embryos transferred on pregnancy and kidding

No statistically significant difference in the kidding rate calculated from transferred embryos and the parturition rate of recipients was observed between recipients receiving single embryos and those receiving two embryos per transfer (Table 6).

DISCUSSION

Although many investigators have studied the factors affecting superovulation in goats (Armstrong et al., 1983; Batt et al., 1993; Nowshari et al., 1995; Nuti et al., 1987; Lin et al., 2001; Li et al., 2002), those investigations studied only one or two factors. The present study, using 151 donors and more than 1,500 recipients, investigated the effect of age, season and repeated treatment on success parameters of goat MOET using a selected superovulation regime. The regime selected, 6-7 mg ChFSH (regimen ii) or 240 mg CaFSH injected intramuscularly for 4 days in decreasing doses (regimen iii), yielded the highest number of ovulations and proportion of transferable embryos. It was reported previously that FSH treatment in decreasing dosages was better than that in constant dosages (Tan et al., 1987). The poorer pregnancy rate for regimen iii might be due to it was conducted in winter. In view of cost and availability, regimen ii was chosen for further study.

In the present study, the number of ovulations and the proportion of transferable embryos were significantly higher in autumn than in spring and winter, respectively. The proportion of pregnancy was significantly lower in winter than in autumn and spring. This indicated that the best season for goat MOET was autumn and that winter was not the appropriate season for goat embryo transfer in Shandong province. Although our Boer goats seemed to have lost seasonality of reproduction after a period of breeding in this area, autumn is still the best season for reproduction with better responses to hormone treatment and better quality of oocytes ovulated. Experiments on *in vitro* maturation of goat oocytes in this laboratory indicated that oocyte quality was inferior in winter to that in autumn (unpublished data). The lower pregnancy rate of recipients in winter may also be due to the cold shock during embryo handling. Detrimental effects of low temperature have been found on *in vitro* produced bovine zygotes (Azambuja et al., 1997) and on mouse embryos (Kamimura et al., 2003).

In order to accelerate genetic gain by reducing generation interval, embryo donors should be as young as possible. According to Louw and Joubert (1964), the onset of Boer goat does occurs at around 157 days, which implies that the Boer does can be superovulated after 5 months of age. In this study, although Boer does responded to gonadotrophin after 7 months of age, and produced transferable embryos, the number of ovulations, proportion of transferable embryos and pregnancy after superovulation were significantly lower than those in adult does (>12

Table 6. Effects of the number of embryos transferred on pregnancy and kidding

Number of embryos per transfer	Number of recipients	Parturition rate of recipients	Kidding rate calculated from embryos transferred	Rates of twine kidding of recipients
1	21	52.4[a]	52.4[a]	0
2	34	73.5[a]	45.6[a]	0.25

[a, b] Values in columns with a common letter in superscripts do not differ significantly ($p > 0.05$).

months). This result was similar to that reported by Revel et al. (1995) in cattle. They found that the IVF embryos from calf oocytes were similar to those from cow oocytes in the early pregnancy rate, but they suffered severe losses in late pregnancy. We also found that the non-return rates from young does of 7-11 months during the first 2 cycles after embryo transfer were similar to those from does of 12-35 months of age. Real et al. (1995) attributed the low late pregnancy rate of blastocysts obtained from calves to the immaturity of the oocytes.

There was no significant difference in the number of ovulations and proportion of transferable embryos between first and second treatments in this study. Similar results have been reported by Al-Kamali and coworkers (1985) in ewes and by Magarey (2003) in Tammar wallabies. Although the number of ovulations and transferable embryos decreased significantly at the third treatment, the number of donor does that became in estrus after the third treatment did not differ significantly in this study (Table 4). This seemed different from that reported by Ptak et al. (2003) who found that the follicular response declined with repeated stimulation. Therefore, the decrease in ovulations and transferable embryos after repeated treatments might be caused by genital tract adhesion after repeated flushings (Wang et al., 1997).

We have investigated for the first time the effect of the number of embryos transplanted per transfer on kidding. The result showed that there was no significant difference in the parturition rate and the kidding rate calculated from embryos transferred between recipients receiving a single embryo and receiving two embryos per transfer. This suggested that the efficiency of single and double embryo transfer was similar. Therefore, double embryo transfer is more profitable because it reduces the number of recipients needed.

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