Superovulation and embryo recovery in two breeds of rabbits

S Satheshkumar*
Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur 680 651, India

Received 19 April 2004; revised 2 May 2005; accepted 12 May 2005

Broiler rabbit does of two breeds, viz. New Zealand White (NSW, 6) and Soviet Chinchilla (SC, 6), were subjected to superovulatory treatment by administering PMSG (150 IU; intramuscular). At the induced oestrus, each doe was allowed to mate with two bucks and HCG (150 IU; intravenous) was administered. Equal numbers of control animals from the respective breeds were allowed double mating without any hormonal treatment. In the treatment group, high intensity oestrus was recorded in NZW and SC rabbits (66.7 and 83.3%, respectively), while the control animals exhibited moderate intensity oestrus. Both, NZW and SC rabbit does responded similarly to the superovulation treatment. Thus, the breed influence could not be appreciated in the ovulatory response. However, the ova recovery rate in NZW rabbits and percentage of fertilized embryos in SC rabbits were significantly affected in the treatment group. The dose of PMSG used might be higher and it would have caused disturbance in terms of recovery as well as the fertilization rate of the embryos. However, no breed influence could be noticed on the above parameters in control rabbits.

Keywords: breed influence, embryo quality, ova recovery, rabbit, superovulation

IPC Code: Int. Cl. 7 A61K38/24

Introduction

Rabbits with their unique reproductive physiology and good meat quality contribute much for the biomedical studies as well as the broiler industry all over the world. In India, rabbit rearing is becoming popular and coming up fast in the recent years. So with careful research, multiple ovulation and embryo transfer technology (MOET) could be applied as a breeding tool for rapid multiplication of genetically superior animals. However, commercial embryo transfer work has been hampered by high degree of variability in superovulatory response and yield of transferrable embryos. Breed of the donor animals had been reported as a source for variations in superovulatory response in many species1-4. The present investigation was aimed at comparing the influence of superovulation treatment on ovulatory response and quality of embryos between New Zealand White (NZW) and Soviet Chinchilla (SC) rabbits, which are being considered the best breeds for meat production in India.

Materials and Methods

Twenty-four healthy rabbit does of two breeds, viz. NZW (12) and SC (12), in the age group of 9-12 months and maintained under ideal and identical management conditions, were formed the material for the study. All the animals were kept in a conventional caged environment individually with 12 h of artificial light per day. They were fed commercially-available pelleted diet, containing a minimum of 17% protein with access to nipple water ad libitum. Of 12 does randomly selected from each breed, 6 were allotted for superovulation studies and the remaining 6 formed the control group. Healthy bucks of proven fertility from the same breed, maintained in the University rabbit farm, were utilized for breeding. All the does were isolated in individual cages for at least 30 d prior to experiment, to preclude the development of pseudopregnancy.

Animals in the experimental group were subjected to superovulatory treatment by administering 150 IU of pregnant mare serum gonadotrophin (PMSG-Folligon; Intervet International, Holland) intramuscularly as a single dose. These animals were closely observed for the onset of heat symptoms at regular intervals and the intensity of oestrus was scored5. At the induced heat, they were allowed to mate with the two bucks of the same breed, one after another for effective fertilization. Human chorionic
gonadotrophin (HCG-Chorulon; Intervet International, Holland) 150 IU was administered intravenously soon after the second mating to ensure better ovulation rate. All animals in the control group of both the breed were monitored regularly for oestrus signs and allowed double mating in the same manner without any hormonal treatment.

Surgical collection of embryos was performed 96 h post-coitum. Animals were fasted 12 h prior to surgery. They were administered with xylazine hydrochloride (5 mg/kg body wt), followed by ketamine hydrochloride (50 mg/kg body wt) 10-15 min later, intramuscularly for proper sedation and mid-ventral laparotomy was performed. Both the ovaries and uterine horns were exteriorised for in vitro recovery of embryos. Laparotomy incision was closed and animals were kept under antibiotic coverage.

The number of ovulations showing evidence of luteinization was counted to arrive at the number of corpora lutea on the ovaries, while those follicles of comparable size to mature follicles were taken as an ovulatory follicle. The excised genitalia were trimmed off the excess adipose tissue and embryos were flushed from oviduct and uterine horn separately with Dulbecco’s modified phosphate buffered saline. Stereoscopic binocular zoom microscope was used for the identification and collection of ova in the medium. Ova were located and transferred into fresh medium in depression slides for further morphological studies. The ovum that contained two or more equal blastomeres was considered to have been fertilized, and classified as morphologically normal eggs of transferable quality. The data were tabulated and statistically analyzed by standard procedure.

### Results and Discussion

Both, NZW and SC rabbits of treatment group evinced oestrus at an interval of 48-72 h (mean 56.0 ± 5.1 h) after PMSG treatment. Of 6 rabbits (66.7%) from both the breeds, 4 exhibited oestrus symptoms as early as 48 h, while the remaining 2 animals (33.3%) at 72 h. This observation was in agreement with the earlier findings. In the treatment group, high intensity oestrus was observed in 66.7 and 83.3% of NZW and SC rabbits, respectively; while the remaining animals exhibited moderate intensity oestrum. None of the PMSG treated rabbits showed poor intensity oestrus in the present study.

Data on the ovulation response, embryo harvest, fertilization rate and quality of embryos in NZW and SC rabbits belonging to both control and treatment groups were presented in the Table 1. Perusal of the data reveal that both the NZW and SC rabbits responded to the superovulatory treatment with 3-5-fold increase in ovulation rate than the corresponding control animals. Illera et al. recorded an average of 26.6 ovulations per doe, while a slightly higher ovulation rate (33.7/doe) was reported by Wischark et al. in NZW rabbits with a similar superovulation protocol adopted in the present study. However, the number of ovulations showing evidence of luteinization was counted to arrive at the number of corpora lutea on the ovaries, while those follicles of comparable size to mature follicles were taken as an ovulatory follicle. The excised genitalia were trimmed off the excess adipose tissue and embryos were flushed from oviduct and uterine horn separately with Dulbecco’s modified phosphate buffered saline.

### Table 1—Ovulation response, embryo harvest, fertilization rate and quality of embryos in control and treatment groups of NZW and SC rabbits

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Control group</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NZW (4.7 ± 1.4)</td>
<td>SC (6.7 ± 1.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0-9)</td>
<td>(5-9)</td>
</tr>
<tr>
<td>1</td>
<td>Mean number of ovulations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mean number of anovulatory follicles</td>
<td>2.3 ± 0.9</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1-7)</td>
<td>(1-2)</td>
</tr>
<tr>
<td>3</td>
<td>Mean number of ova harvested</td>
<td>2.5 ± 0.9 (5-5)</td>
<td>3.5 ± 0.8 (0-5)</td>
</tr>
<tr>
<td>4</td>
<td>Mean percentage of ova recovered (%)</td>
<td>47.2 ± 12.0 (33.3-83.3)</td>
<td>53.3 ± 14.0 (0-100)</td>
</tr>
<tr>
<td>5</td>
<td>Mean number of fertilized ova</td>
<td>2.2 ± 0.7 (0-5)</td>
<td>2.7 ± 0.8 (0-4)</td>
</tr>
<tr>
<td>6</td>
<td>Mean number of unfertilized ova</td>
<td>0.3 ± 0.2 (0-1)</td>
<td>0.8 ± 0.3 (0-2)</td>
</tr>
<tr>
<td>7</td>
<td>Mean percentage of fertilized ova (%)</td>
<td>86.7 ± 16.4 (50.0-100.0)</td>
<td>77.8 ± 14.0 (60.0-100.0)</td>
</tr>
<tr>
<td></td>
<td>Percentage of transferable embryos</td>
<td>92.3</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>Percentage of degenerated embryos</td>
<td>7.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

(Range within the parenthesis)

* Anovulation, ** Complete Obstruction at uterotubal junction, *** Ovaro-bursal adhesion.
higher ovulation rate in control groups were recorded in both these studies than our findings. Even though the number of ovulations was higher in NZW rabbits than the SC rabbits, but there was no significant difference in superovulation response between the breeds. Bavin et al also could not observe any breed influence on the superovulatory response between NSW and Giant Chinchilla breeds. Nuti et al also reported similar results in superovulated goats. However, contrary to our findings, Driancourt et al observed greatest line differences in HCG induced ovulation rate in rabbits. Hulot and Mariana, in their comparison study between Californian and NZW rabbits, attributed the increased number of ovulations in the former strain to a significantly higher number of healthy follicles as compared to the NZW rabbits.

All the animals in the treatment group ovulated as against the control group, in which only 91.7% animals responded to mating stimulus. One NZW rabbit of the control group did not ovulate following coitus probably because that particular animal previously exhibited only low intensity oestrum at the time of mating. Hulot et al suggested that lack of ovulation following natural mating in non-receptive does might be caused by a lack of LH discharge.

A total of 91 (NZW, 41; SC, 50) and 36 ova (NZW, 15; SC, 21) were recovered, respectively from treatment and control groups. Analysis of the present findings revealed that the embryo recovery rate was comparatively higher in the control groups than that of the superovulated rabbits but there was no significant difference. The percentage of embryos recovered in the treatment groups concur with the earlier findings.

Illera et al and Wischark et al recovered 98-91% of embryos from NZW rabbits with a similar superovulation treatment regimen. In the present study, however, a complete or partial obstruction was felt near the utero-tubal junction while flushing the oviducts of three (50.0%) PMSG treated NZW rabbits and of 16 ovulations, only two ova could be recovered from these animals. This ‘tubal-lock’ phenomenon caused an overall reduction in embryo recovery rate in this breed. Retention of ova in the Fallopian tube (tubal locking), long past the time they would normally enter the uterus, would lead to an eventual degeneration of cleaving ova. It could be assumed that the dose of PMSG (150 IU) might be higher and the altered oestrogen concentration due to excessive follicular development would have caused disturbances in recovering the embryos. However, such hindrances could not be observed in SC rabbits, but ovaro-bursal adhesion was noticed in one animal of this breed due to the development of haemorrhagic follicles.

In the present study, fertilization rate in the superovulated NZW rabbits (100%) was higher than SC rabbits (69.8%). Ishijima et al also observed 99.3 per cent fertilization rate in Japanese white rabbits. While Terblanche et al found out that fertilization rates were not affected by superovulation treatment in boer goats. Lower fertilization rate, as observed in SC rabbits in the present study, was also reported earlier by others. Similarly, Olivera-Angel et al recorded more unfertilized ova in superovulated heifers and opined that multiple ovulations that took place over a period of time resulted in the aging of sperm and reduced fertilizing ability.

In the control group, even though the percentage of fertilized embryos was higher in NZW rabbits, there was no significant difference when compared with SC rabbits. The fertilization rate of control NZW rabbits is in general agreement with the value (93.8%) reported by Agrawal et al. Although the fertilization rate in control SC rabbits was lower in the present study, the value was much higher than (22%) reported by El-Din and Fulka in the same breed.

Morphological evaluation of fertilized embryos revealed that the percentage of normal, transferrable quality embryos was higher in SC rabbits than the NZW rabbits in both groups, but statistical analysis revealed no significant difference between the breeds (Table 1). These results concur with the earlier findings. In the present study, the quality of embryos was not much affected by the superovulation treatment in both the breeds of rabbits. Moor et al observed more number of degenerated eggs in sheep, superovulated with high dose of PMSG, and ascribed the cause of embryonic abnormalities to the elevated levels of circulatory steroids.

From the above facts, it is evident that administration of 150 IU PMSG with 150 IU HCG induced satisfactory ovulatory response in both the breeds of rabbits under study and thus, breed influence could not be appreciated in the superovulatory response. But the ova recovery rate in NZW rabbits and percentage of fertilized embryos in SC rabbits were significantly affected in the treatment group. It could be assumed that the dose of
PMSG (150 IU) in the present study might be higher and the resultant altered oestrogen-progesterone concentrations would have caused disturbances in ova recovery as well as the fertilization rate of embryos in superovulated rabbits. But in the control group, no breed influence could be noticed on all the above parameters.

References