

INFLUENCE OF DOE EXPOSURE AND SEASON ON REACTION TIME AND SEMEN QUALITY OF MALE RABBITS

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ABSTRACT

The objective of the present research was to evaluate the influence of doe exposure on reaction time (RT) and five semen attributes of male rabbits in winter and spring. Fifteen-month-old selected sexually experienced fertile New Zealand White male rabbits (n=18) were randomly allocated to one of two treatments: (1) doe exposure; and (2) no doe exposure (control). Doe exposure was done with prepubertal replacement does separated by an adjacent wire-mesh wall; females were changed in an every other week schedule. Semen collection lasted 14 weeks, 7 in winter and 7 in spring; collection was twice a week using an artificial vagina and a teaser female, and two ejaculates were obtained at each collection. In total, 728 ejaculates were analyzed statistically. Analyses of variance were under a mixed model where fixed effects were treatments, season and ejaculate, rabbit was the random effect. No significant ($P \geq 0.05$) interaction of treatment with season was found. Doe exposure improved ($P \leq 0.05$) RT and four of the five semen attributes: RT was shorter (-0.9 sec) and volume (+0.21 ml), sperm motility (+17.4 percent units), concentration (+49.7 x 10⁶) and total live normal motile sperms (+44.5 x 10⁶) were higher. Spring showed better ($P \leq 0.05$) RT and semen attributes than winter. First ejaculate was better ($P \leq 0.05$) than the second except for sperm motility, which in the second ejaculate was 15.3 percent units higher than in the first one. There was a significant effect ($P \leq 0.05$) of the interaction treatment x ejaculate on total live normal motile sperms (TLNMS): bucks with doe exposure had a higher TLNMS in the first ejaculate (126.3±9.2 x 10⁶) than in the second (107.8±9.2 x 10⁶), while in bucks isolated from females the second ejaculate showed higher TLNMS (84.5±9.2 x 10⁶) than the first (58.4±9.2 x 10⁶). Doe exposure can enhance sexual behaviour and semen attributes of male rabbits in winter and spring.

Key words: Sexual stimulation, Sexual performance, Semen characteristics, Rabbits.

INTRODUCTION

Good sex drive of male rabbits and high quality semen are required year-round to achieve the maximum number of suitable doses for artificial insemination (AI); however, both are reduced during unfavourable seasonal periods which may compromise the efficiency of an AI program, then it is important to search for natural alternatives that can enhance male reproduction attributes during such periods.

Sexual stimulation techniques that improve male sexual performance have been reported in several farm animal species (Mader and Price, 1984; Gonzalez *et al.*, 1991; Rosa *et al.*, 2000; Bailey *et al.*, 2005). In pigs, sexual performance and sperm output have shown to be enhanced when boars are housed in proximity of gilts or sows (Hemsworth *et al.*, 1981) or having social contact with females (Kunavongkrit *et al.*, 2005). However, in male rabbits, there is little information on the influence of sexual stimulation on sexual performance and quantity and quality of collected semen using an artificial vagina for AI.

The aim of the present study was to evaluate the influence of long-term continuous doe exposure on reaction time and semen attributes of male rabbits under a semi-intensive semen collection rhythm in winter and spring.

MATERIALS AND METHODS

Location, animals, treatments and experimental design

The trial was conducted at 'Conejos Centro de Investigación Científica del Estado de México A.C.', a rabbit research center located in San Miguel Coatlinchan, Texcoco, México, at 19° 27' N, 98° 53' W and 2220 m above sea level. Mean annual temperature is 15°C and there are 645 mm of rainfall per year. Fifteen-month-old selected sexually experienced mature New Zealand White male rabbits (n=18) of proven fertility were randomly assigned to one of two treatments: (1) continuous doe exposure; and (2) no doe exposure (control). For the doe exposure treatment, prepubertal replacement females were used and changed for new ones on an every other week schedule. These females were separated from males by an adjacent wire-mesh wall, and the cages were located in front of exposed females. Bucks with no doe exposure were kept in a batch of cages completely isolated from females.

Rearing management of bucks, semen collection and evaluation

Rabbits were housed in a unit with natural ventilation and thermal insulation. A 54 x 60 x 40 cm wire cage in a flat-deck system with automatic watering and j-feeders was used for each buck. Daily lighting regime was 8L:16D. Feed offered was a commercial pelleted diet of 16.7% crude protein, 3.8% fat, 16.7% crude fiber and 2313 kcal of digestible energy per kg, at a daily rate of 120 g per buck. Semen collection was twice a week (Mondays and Fridays) and in every collection two successive ejaculations from each buck were obtained. Semen collection lasted a total of 14 weeks, seven in winter (fourth week of January to the second week of March) and seven in spring (third week of March to first week of May). Semen collection was with an English type artificial vagina and supported with a teaser female. First ejaculation was persuaded between 8:00 to 10:00 h, the second about 4 hours later. The samples were kept into a water bath at 31°C for evaluation. Variables measured on bucks were live weight (to the nearest gram) at the time of semen collection and reaction time (RT), which was the time interval from the introduction of the teaser doe into the male's cage to ejaculation. It was measured in seconds using a stopwatch. Semen quality was measured by ejaculate volume and sperm motility, concentration, live normal and total live normal motile sperms. Once the gel material was removed, the gel-free sperm volume was measured directly on the transparent graduated collector tube at 0.2 ml intervals. A drop of semen was placed on a warmed slide at 37°C and sperm motility was assessed between 0 and 100% using a light microscope at x100. Sperm-cell concentration per ml was measured by counting the number of spermatozoa present on both sides of a Neubauer improved bright-line haemocytometer (Marienfeld, Germany) after the semen had been diluted 1:99 in formalin and then multiplying by a dilution factor. The number of sperms per ejaculate ($\times 10^6$) was calculated by multiplying volume by concentration per ml. The percentage of live normal spermatozoa was assessed by staining an aliquot of each ejaculate with eosin/negrosin stain and counting 200 sperms cells at x100 under oil immersion. Total live normal motile sperms (TLNMS) was the product of sperm concentration times motility times live normal in the ejaculate.

Statistical Analysis

Reaction time and semen attributes were subjected to analysis of variance under the following mixed linear model:

$$Y_{ijklm} = \mu + T_i + S_j + E_k + M_l + T*S_{ij} + T*E_{ik} + S*E_{jk} + b_l X_{ijklm} + e_{ijklm}.$$

Where: μ is the overall mean; T_i is the fixed effect of the i^{th} treatment (I = with or without doe exposure); S_j is the fixed effect of the j^{th} season of semen collection (j = winter, spring); E_k is the fixed effect of the k^{th} ejaculate number (k = first, second); M_l is the random effect of the l^{th} male (l = 1, . . . ,

14) $\sim NI(0, \sigma_m^2)$; T^*S_{ij} , T^*E_{ik} , and S^*E_{jk} are the fixed effects of the corresponding first order interactions; b_1 is the regression coefficient of the linear effect of the covariable buck live weight at the time of semen collection (X); and e_{ijklm} is the residual $\sim NI(0, \sigma_e^2)$. Variables expressed in percentage were transformed to arc sine. Reaction time, concentration per ejaculate, and percentage of live normal motile sperms were log10 transformed and analyses of variance were done with both transformed and original data and the corresponding significance tests for the fixed effects were compared. If covariate or any first order interactions that were not significant ($P \leq 0.05$) were removed from the model. Means for main effects and for significant first order interactions were calculated by least squares. Statistical calculations were done with SAS (SAS, 2006) PROC MIXED and LSMEANS.

RESULTS AND DISCUSSION

Data from two bucks with doe exposure and two of no doe exposure were removed from all analysis; two of them died due to pneumonia, a third one gave semen samples with urine contamination and the fourth failed to yield a second ejaculate. Thus, statistical analyses were based on a total of 728 ejaculates. Analyses with transformed and original data gave same response pattern; therefore, results of the analyses of variance with the original data are presented.

Treatment x season and number of ejaculate x season interactions were not significant ($P \geq 0.05$) on RT and semen attributes analyzed, therefore were removed from the model. Treatment x number of ejaculate were not significant ($P \geq 0.05$) on RT, volume and percentage of live normal sperms, therefore were removed from the model. Live male weight at semen collection showed effect ($P \leq 0.05$) on RT, ejaculate volume and TLNMS, then values were adjusted to a standard live weight of 3620 g. The effect of treatment, season and number of ejaculate on the variables analyzed is shown in Table 1.

Doe exposure influenced ($P \leq 0.05$) RT and four of the five semen attributes analyzed; RT was shorter (-0.9 s) and volume (+0.21 ml), sperm motility (+17.4 percent units), concentration (+49.7 $\times 10^6$) and TLNMS (+44.5 $\times 10^6$) were higher in bucks with than without doe exposure. The better sexual performance and semen quality achieved by bucks with the female stimuli found in the present study agrees with the response shown by males in several farm animal species with the presence of females (Hemsworth *et al.*, 1981; Mader and Price, 1984; Gonzalez *et al.*, 1991; Rosa *et al.*, 2000; Bailey *et al.*, 2005; Kunavongkritt *et al.*, 2005).

Table 1: Reaction time and semen characteristics in male rabbits with and without doe exposure in two seasons and ejaculates (Least Square Means \pm Standard Error)

Factor	N	Reaction ¹ Time (s)	Ejaculate ¹ volume (ml)	Sperm motility (%)	Concentration per ejaculate (10 ⁶)	Live normal sperms (%)	Total live ¹ normal motile sperms (10 ⁶)
Doe exposure							
With	364	12.7 \pm 0.69	0.69 \pm 0.07	78.0 \pm 2.9	190.0 \pm 18.3	77.5 \pm 1.3	116.5 \pm 13.4
Without	364	13.6 \pm 0.69	0.48 \pm 0.07	60.6 \pm 2.9	140.3 \pm 18.3	78.2 \pm 1.3	72.0 \pm 13.4
P value		0.001	0.007	0.0001	0.03	0.06	0.002
Season							
Winter	364	13.3 \pm 0.02	0.57 \pm 0.02	63.7 \pm 1.8	157.9 \pm 7.3	79.8 \pm 0.8	84.6 \pm 5.6
Spring	364	13.0 \pm 0.02	0.58 \pm 0.02	74.8 \pm 1.8	172.4 \pm 7.3	75.5 \pm 0.8	103.9 \pm 5.6
P value		0.02	0.968	0.0001	0.02	0.001	0.0006
N. of ejaculate							
First	364	12.6 \pm 0.12	0.61 \pm 0.02	61.6 \pm 1.8	174.3 \pm 7.22	78.5 \pm 0.8	92.4 \pm 5.6
Second	364	13.7 \pm 0.12	0.53 \pm 0.02	76.9 \pm 1.8	155.9 \pm 7.22	77.2 \pm 0.8	96.1 \pm 5.6
P value		0.0001	0.0001	0.0001	0.01	0.10	0.499

¹Means adjusted by live weight at semen collection

The positive stimulatory effect on sexual behaviour and semen quality of bucks exposed to does could be mediated by olfactory cues (pheromones signalling). In small mammals pheromones detected by the olfactory system could be the path to stimulate male and female sexual behaviours and to increase

the level of some reproductive hormones (Novotny, 2003). However, under the conditions in which the present study was carried out, other possible sensory cues that could have mediated the stimulatory effect were: the physical close proximity of males and females that allowed limited contact; the viewing of the process of semen collection of other bucks; direct contact with the teaser does at the time of semen collection; and the every other week change of females.

The higher volume found in bucks exposed to females suggests that the efficiency of accessory sex gland secretion and epididyme function probably were enhanced; while, the higher sperm motility might be due to favourable changes in the seminal fluid composition but this need to be confirmed. Exposure to estrual females, viewing copulating conspecifics and periodical change of females have been reported to enhance male sexual performance in several species (Mader and Price, 1984; Rosa *et al.*, 2000; Silvestre *et al.*, 2004).

Sexual interaction or the mere presence of females has been associated with an increase in the plasma levels of testosterone and related to a rapid elevation of LH concentration in a variety of species (Hemsworth *et al.*, 1981; Gonzalez *et al.*, 1991; Rosa *et al.*, 2000). In the present study, males probably responded to the exposure of females by showing long-term increased testosterone production; however, this was not determined and requires further investigations including the role of other hormones related to sexual stimulation.

Season determined differences ($P \leq 0.05$) in all analyzed variables except for volume that was similar in both seasons. Bucks had better performance in spring than in winter in terms of shorter RT (-0.3 s), higher sperm motility (+11.1 percent units), concentration (+14.5 $\times 10^6$) and TLNMS (+19.3 $\times 10^6$) but lower live normal sperms (-5 percent units). The low levels of sperm production found in winter are similar to those reported by Nizza *et al.* (2003). The reduction in the quality and quantity of semen in winter may be due to a decrease of gonadotrophin hormones and testosterone secretion associated with a decrease in testicular weight. In wild rabbits, gonadotrophin hormone release has shown to increase with testicular weight during spring and summer months and decline during fall, reaching the lowest levels during the winter solstice (Boyd and Myhill, 1987). Provision of adequate environmental conditions, particularly in housing and nutrition, may have acted synergistically with doe exposure to enhance sexual performance of males even during the winter period.

The first ejaculate showed a shorter RT (-1.1 s) and higher volume (+0.08 ml) and concentration (+18.4 $\times 10^6$) than the second; while the second showed a higher sperm motility (+15.3 percent units) than the first. Live normal sperms and TLNMS were similar in both ejaculates. The higher concentration in the first than in the second ejaculate was also found by Castrovilli *et al.* (1995). Treatment \times number of ejaculate interaction was significant ($P \leq 0.05$) on TLNMS: bucks exposed to does showed higher TLNMS in the first ejaculate ($126.3 \pm 9.2 \times 10^6$) than in the second ($107.8 \pm 9.2 \times 10^6$) whereas in bucks without females TLNMS was lower in first ejaculate ($58.4 \pm 9.2 \times 10^6$) than in the second ($84.5 \pm 9.2 \times 10^6$).

Variation among bucks in RT and semen attributes was large; this is in agreement with the findings of Theau-Clement *et al.* (1995) who pointed out that the cause of differences in sexual behaviour and semen characteristics could be genetic make-up.

CONCLUSIONS

Strategic exposure of male rabbits to does can enhance sexual behaviour and semen characteristics in winter and spring, therefore, this biostimulation procedure is recommended in rabbit production systems mainly if an AI program is applied. Further studies with different breeds, time of doe exposure and rotation schemes of females may increase the knowledge on the specific pathways by which this biostimulation improves reproduction in male rabbits even in critical seasonal periods.

REFERENCES

- Bailey J.D., Anderson L.H., Schillo K.K. 2005. Effects of novel females and stage of the estrous cycle on sexual behaviour in mature beef bulls. *J. Anim. Sci.*, 83, 613-624.
- Boyd I., Myhill D. 1987. Seasonal changes in condition, reproduction and fecundity in the wild European rabbit (*Oryctolagus cuniculus*). *J. Zoo.*, 221, 223-233.
- Castrovilli C., Tamburini A., Carrara C. 1995. Livelli proteici della dieta, linee genetiche e caratteristiche del materiale seminale nel coniglio. In: *Proc. 49° Conv. SISVet., Italy, 1037-1038*.
- Gonzalez R., Orgeur P., Poindron P., Signoret J.P. 1991. Female effect in sheep. I. The effect of sexual receptivity of female and the sexual experience of rams. *Reprod. Nutr. Dev.*, 31, 97-102.
- Hemsworth P.H., Winfield C. G., Chamley W.A. 1981. The influence of the presence of the female on the sexual behaviour and plasma testosterone levels of the mature male pigs. *Anim. Prod.*, 32, 61-65.
- Kunavongkrit A., Suriyasomboon A., Lundeheim N., Heard T.W., Einarsson S. 2005. Management and sperm production of boars under different environmental conditions. *Theriogenology*, 63, 657-667.
- Mader D.R., Price E.O. 1984. The effect of sexual stimulation on the sexual performance of Hereford bulls. *J. Anim. Sci.*, 59, 294-300.
- Nizza A., Di Meo C., Taranto S. 2003. Effect of collection rhythms and season on rabbit semen production. *Reprod. Domest. Anim.*, 38, 436-439.
- Novotny M.V. 2003. Pheromones, binding proteins and receptor responses in rodents. *Biochem. Soc. Trans.*, 31, 117-122.
- Rosa H.J., Juniper D.T., Bryant M.J. 2000. The effect of exposure to oestrous ewes on rams sexual behaviour, plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes. *Appl. Anim. Behav. Sci.*, 67, 293-305.
- SAS 2006. SAS/STAT User's Guide Statistics (Release 6.03). *SAS Inst. Inc., Cary, NC, USA*.
- Silvestre M.A., Salvador L., Sanchez J.P. Gómez E.A. 2004. Effect of changing female stimulus on intensive semen collection in young Murciano-Granadina male goats. *J. Anim. Sci.*, 82, 1641-1645.
- Theau-Clément M., Michel N., Bolet G., Esparbié J., Bolet G. 1995. Effect of artificial photoperiods on sexual behaviour and sperm output in the rabbit. *Anim. Sci.*, 60, 143-149.

