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KHALIFA R.M.1, EL-ALAMY M.A., BESHIR M.A.

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VASOECTOMIZED BUCK GAVE BETTER REPRODUCTIVE RESULTS IN ARTIFICIAL INSEMINATION TECHNIQUES IN RABBITS THAN GnRH OR hCG

KHALIFA R.M.¹, EL-ALAMY M.A., BESHIR M.A.

Department of Animal Production, Faculty of Agriculture, Suez Canal University,
P.O. Box 41522 ISMAILIA, Egypt.
e-mail rkhalifa@faci.suez.eun.eg

¹To whom correspondence should be addressed.

ABSTRACT

This study aimed to evaluate ovulation stimulators and time of insemination with artificial insemination (AI) in Californian (CL) and New Zealand White (NZW) rabbits. A 2x3x4 factorial arrangement was used. Three ovulation stimulators, 50 IU hCG, 20 µg GnRH and a vasoectomized buck (VB) were used. Four different intervals between the use of ovulators and insemination were tested, 5 h before, zero, 5 and 10 h after induction of ovulation method. About 5 million motile spermatozoa of freshly diluted semen were used for AI. Thirty NZW and 35 CL does were mated naturally as controls. There were no significant differences between breeds for conception rate (CR), litter size (LS) or litter weight (LW). Using VB as the ovulation inducer gave the best response among the three methods. The averages of CR, LS and LW at birth were 66.7 %, 5.4 kits and 326.5 g., respectively. The means were not different from the control except for LS ($P < 0.05$). The values for the control group were 57.0 %, 6.4 kit and 347.4 g., respectively. The poorest results were 39.6 % CR in the hCG group and 238.0 g. LW in the GnRH group. The averages of CR, LS and LW in the 0 time group were 67.9 %, 5.5 kit and 292.2 g., and were not significantly different from the control group. However AI after 10 hours gave significantly ($P < 0.01$) poorer results (36.1% CR, 3.3 kits LS and 187.1 g. LW). All possible interactions were statistically insignificant. The results of the present experiment indicate that using AI at 0 time with VB as the ovulation inducer gave better reproductive performance than AI at other times using GnRH or hCG. Using VB in AI in rabbits has no side effects such as those that may follow the use of hormonal treatments.

INTRODUCTION

Interest in artificial insemination (AI) is increasing in all countries where intensive rabbit raising is practiced. The AI technique is important for controlling reproduction and to make breeding progress through more intensive sire selection. It also reduces buck numbers, cages and feeding costs. The AI technique in rabbits needs an ovulation stimulator or inducer because female rabbits are induced ovulators. GnRH or hCG are the hormones which are widely used for this purpose. Khalifa, (1994) successfully used vasoectomized bucks (VB) in AI. Ovulation occurs 10-13 hours after copulation or after other stimuli (Hafez 1970, Adams 1972). In the available literature, there is a lack of comparative studies among results of AI in rabbits using different methods of ovulation induction under the same environmental conditions.

This work aimed to evaluate three methods of ovulation induction (GnRH, hCG and VB) in combination with different four times of insemination (5 hours before, 0 , 5 and 10 hours after ovulation induction). The results were compared with those of natural mating. at the same period under the same environmental conditions.

MATERIALS AND METHODS

The work was conducted at the experimental farm of the Faculty of Agriculture Suez Canal University, Ismailia, Egypt.

Experimental design:

A 2 x 3 x 4 factorial arrangement was used, consisting of two breeds, New Zealand White (NZW) and Californian (CL), three ovulatory inducers, vasoectomized buck (VB), GnRH and hCG and four different times of insemination relative to ovulation induction method. Each subclass was replicated with six does. A natural mating group which contained 30 NZW does and 35 CL was included as a control.

Animals, Housing and Feeding:

The does used had kindled once or twice and their age averaged 7 months. The bucks used were fertile and tested for semen quality. Rabbits were separately housed in individual galvanized metal cages. Throughout the study the photoperiod was maintained at 16 hours light : 8 hours dark. Each animal received a concentrated commercial diet ad-libitum. This diet contained 16.1% crude protein, 2.39% crude fat and 12.8% crude fiber. The ingredients were yellow corn, hay, wheat bran, soya bean meal, gluten, molasses, table salt, minerals and vitamin mixture. Fresh water was available continuously via automatic nipples.

Induction of ovulation:

The natural mating or AI was carried out 12±2 days postpartum. The three methods of induction of ovulation evaluated were:

- Subcutaneous injection with 50 IU human chronic gonadotropin (hCG) (Profasi, Serono-Rome 125 VIA Casilino- 00176 Rome, Italy).
- Subcutaneous injection with 0.2 ml (20 µg) gonadotropin releasing hormone (GnRH) (Fertagyl, Intervet International B. V. Boxmeer, Holland).
- Copulation one time by a vasoectomized buck (VB).

These doses of hormones in this study were used because its abundant use in rabbit AI (Tawfeek and EL-Gaafary 1991, Khalifa 1994, Alvarino et al 1996, and Remois et al 1996).

Semen collection, extension and AI.

Semen was collected by artificial vagina from 5 NZW and 5 CL bucks. Ejaculates with more than 70% initial motility were used. The average of total motile spermatozoa per ejaculate was 58.3 ± 3.0 and 38.4 ± 1.8 million for NZW and CL bucks, respectively. Ejaculates were pooled and diluted with egg yolk tris extender to maintain a concentration of about 5 million motile spermatozoa per ml. The components of 100 ml extender were 1.5 g citric acid, 1.25g fructose, 3.028 g tris (hydroxy methyl amino methane), 20 ml egg yolk, 0.1g of the antibiotic sygmamycin and distilled water. The bucks used for semen collection and AI were also used for natural mating. One ml fresh extended semen containing about 5 million motile spermatozoa was deposited near the os cervices. The four different times of AI were 5 hours before or 0, 5, or 10 hours after ovulation induction. In natural mating and VB groups, the doe was mated one time only when her vulva was red and no forced mating was used. All

does were palpated for pregnancy 10 days after AI or natural mating. Conception rates (CR), litter size (LS) and litter weight (LW) at birth were recorded.

Statistical analysis:

Data were analyzed using General Linear Models Procedure of SAS (1988) according to the following fixed model $Y_{ij} = \mu + T_i + e_{ij}$ where

Y_{ij} = the observation on the ij th rabbit doe , μ = the overall mean of the trait under consideration, T_i = the fixed effect of the i th treatment where there are 13 treatments per breed including 4 mating types (control, hCG, GnRH, and VB) and 4 studied times for each mating type except the control ($i = 1,2,3,\dots,26$), and e_{ij} = the random deviation of the ij th observation assumed to be independently and randomly distributed (NID 0, σ^2_e). The following restriction was imposed on the model: $\sum T_i = \phi$.

Data on conception rates were analyzed using the categorical model procedure “CATMOD” of SAS (1988) which partitions the variation among the response functions into the above mentioned source of variations using Chi-square tests. It tests the hypothesis that the distribution is the same across different groups.

The main effect model was applied since all the possible interactions were statistically analyzed and found not significant. They were added to the error term to increase the validity of the testing. A t-test analysis was used to make comparisons between each two treatments and each one versus the control for all traits except conception rate where single degree of freedom contrast statements were used.

RESULTS AND DISCUSSION

The results of conception rate (CR), litter size (LS) and litter weight (LW) at birth as affected by breed are presented in Table 1. The NZW breed had numerically higher values for LW than the CL breed, however the averages of CR and LS were the same .

Table 1: Means (\pm SEM). for conception rate (CR), litter size (LS) and litter weight (LW) at birth in New Zealand White (NZW) and Californian (CL) rabbits.

Characteristics	No. does used	CR (%)	LS (number)	LW (grams)
<i>Breed</i>				
NZW	102	54.2 \pm 16.8	5.2 \pm 0.4	303.7 \pm 27.3
CL	107	54.2 \pm 17.8	5.2 \pm 0.4	287.1 \pm 26.9
Overall	209	54.2 \pm 16.2	5.2 \pm 0.3	295.2 \pm 14.3

The CR, LS and LW of natural mating group averaged 57.0 \pm 3.0 %, 6.4 \pm 0.3 kits and 347.4 \pm 13 g., respectively (Table 2). The corresponding averages for the AI groups were 52.8 \pm 17.8 %, , 4.7 \pm 0.2 kits and 269.8 \pm 17 g., respectively. The LS and LW were higher ($P < 0.01$) for natural mating. The LW is affected by LS which, in turn, depends on the number of ova shed, fertilized and grown till birth. The present results indicate that there may have been more fertilizable ova shed or more motile spermatozoa in the insemination dose with natural mating. In the present study only about 5 million motile spermatozoa were used in the AI group compared to an entire ejaculation in the natural mating group. Motile spermatozoa in the ejaculate averaged 58.3 \pm 3.0 and 38.4 \pm 1.8 million, respectively for NZW and CL bucks. Cergoli et al, (1995) produced 7.67 kits at birth when they used 60 million

spermatozoa. Alvarino et al, (1996) compared 60, 30 and 20 million spermatozoa / doe and reported 70.5, 80.0 and 54.0 % CR, and 8.2, 7.7 and 8.1 kits per litter, respectively. In general, most studies in the literature report the same trend whereas natural mating resulted in higher averages of LS and LW than AI (Khalifa 1994, Alabiso et al. 1996). Other studies are needed to investigate the decrease of LS when AI is used.

Table 2: Means (\pm SEM) for conception rate (CR), litter size (LS) and litter weight (LW) at birth in both breeds after AI using three different methods of ovulation induction and natural mating.

Characteristics	No. does used	CR (%)	LS (number)	LW (grams)
Type of mating.				
AI, With GnRH	48	52.1 \pm 13.0 ^{ac}	4.3 \pm 0.5 ^c	238.0 \pm 31 ^c
hCG	48	39.6 \pm 16.5 ^a	4.3 \pm 0.6 ^c	244.8 \pm 38 ^c
VB*	48	66.7 \pm 11.7 ^c	5.4 \pm 0.4 ^b	326.5 \pm 27 ^a
Total	144	52.8 \pm 17.8 ^{ac}	4.7 \pm 0.2 ^c	269.8 \pm 17 ^c
Natural mating	65	57.0 \pm 3.0 ^{ac}	6.4 \pm 0.3 ^a	347.4 \pm 13 ^a

* VB = Vasoectomized Buck.

^{ac} means in the same column with different superscripts are significant (p<0.01).

^{abc} means in the same column with different superscripts are significant (p <0.05)

With regard to the AI group, the results in Table 2 shows the greater responses in the VB group for LS (P< 0.05) and LW (P< 0.01) than the other two groups, GnRH or hCG. For CR, the VB response was greater than for the hCG group, but was not different from the GnRH group. The comparison between VB group and natural mating results were insignificant except in LS (P< 0.05). Using VB to induce ovulation is like natural copulation especially the presence of the male around the female, insertion and friction of penis in the vagina. Also the pressure on the hips from mounting and contact with the perineal and pudendal regions These actions may stimulate release of more fertilizable ova than the use of GnRH or hCG. The other explanation may be due to the secretions of the female reproductive tract which could be different according to the three ovulation methods and consequently affect transport and fertilizability of the sperm cells. Hawk and Conley (1975) reported that a physiologic balance of ovarian steroids is necessary for the initiation and maintenance of a cervical population of spermatozoa following AI. An imbalance of progesterone and estrogen can affect the transport and consequently fertility of spermatozoa by altering either the quantitative and qualitative characteristics of the cervical secretions or the motility of the reproductive tract.

Regardless of breed and ovulation inducer, CR, LS and LW results differed (P < 0.05) among the four times of insemination and the natural mating as shown in Table 3. The averages of CR (67.9 \pm 14.9 %) and LS (5.5 \pm 0.5 kit) were higher in the group inseminated at the time of ovulation induction followed by the group inseminated five hours later. Litter weights were highest in the five hour group but were not significantly different from the 0 hour group.. The averages of CR, LS and LW from the 0 time group were not different from natural mating group. However AI after 10 hours gave significantly poorer results for all characteristics. These results could be normal, whereas freshly ejaculated semen was used in AI and the period between induction of ovulation method and ovulation is enough for sperm to become fertilizable or enough for sperm capacitation and acrosome reaction occurrence.

Hafez (1970) and Adams (1972) reported that in rabbits ovulation occurs 10-13 hours after copulation or after other stimuli. Chen et. al.(1989) reported that, rabbit bucks differ significantly in the capacitation time of their spermatozoa so these authors could not determine a precise time of insemination relative to ovulation. In the present study pooled semen samples from the same bucks in all AI groups were used, consequently the fertilizability differences due to males could be ignored and the most suitable time of insemination could be detected.

The results of the present experiment indicate that using VB as an ovulation inducer at the time of AI gave better reproductive performance than AI at other times using GnRH or hCG. Using VB with AI in rabbits has no side effects such as those that may follow the use of hormonal treatments. To increase the success of using VB with AI in rabbits, the number of motile spermatozoa per insemination, the number of ova shed after different ovulation induction methods, and economical studies are suggested for study.

Table 3: Means (\pm SEM) of conception rate (CR), litter size (LS) and litter weight (LW) at birth in both breeds after AI on four different times of ovulation methods and natural mating

Characteristics	No. does use	CR (%)	LS (number)	LW (grams)
Time of mating				
* AI. at -5 hours	36	55.6 \pm 18.4 ^a	4.7 \pm 0.5 ^c	276.5 \pm 36 ^b
0	36	67.9 \pm 14.9 ^{ac}	5.5 \pm 0.5 ^a	292.2 \pm 33 ^a
5	36	55.6 \pm 12.2 ^{ab}	5.2 \pm 0.5 ^b	323.3 \pm 35 ^a
10	36	36.1 \pm 11.3 ^b	3.3 \pm 0.7 ^c	187.1 \pm 45 ^c
Total	144	52.8 \pm 17.8	4.7 \pm 0.2	269.8 \pm 17
Natural mating	65	57.0 \pm 3.0 ^a	6.4 \pm 0.3 ^a	347.4 \pm 13 ^a

* -5, 0, 5, 10 hours time of AI relative to time of ovulation induction methods.

^{ac} means in the same column with different superscripts are significant (p<0.01).

^{abc} means in the same column with different superscripts are significant (p <0.05)

REFERENCES

- ADAMS, C.E. 1972: Induction of ovulation and AI techniques in the rabbit. *Vet. Res.*, 90: 194-197
- ALABISO, M., BOMANNO, A., ALIEATAM, L., Leto, G. and Todaro, M. 1996: Productive results of does inseminated with 5 times of 10 times diluted semen. *6th World Rabbit Congress, Toulous Vol 2:115-120.*
- ALVARINO, J.M.R., LOPEZ, F.J., DALARCO, J.A., BUENO, A. and TORRES, R. 1996: Effect of semen concentration on rabbit artificial insemination with fresh or 24 hours stored semen. *6^{ab} World Rabbit Congress, Toulous Vol 2: 33-35.*
- CERGOLI, J.M., MAKEK, Z., HERAK., M. and Tomaskovic, A. 1995: Stimulation of ovulation with luteal in doe rabbits before artificial insemination with liquid semen of different life span. *A. Breeding Abstract, 63, 10, 5904.*
- CHEN, Y., SIMKIN, M.E., YANG, X. and FOOTE, R.H. 1989: Fertility of fresh and frozen rabbit semen inseminated at different times is indicative of male differences in capacitation. *Biol. of Reprod.*, 41: 848-853.

- HAWK, H.W. and CONLEY, H. H. 1975: Involvement of the cervix in sperm transport failures in the reproductive tract of the ewe. *Biol. Reprod.* 13. 322-326.
- HAFEZ, E.S.E. 1970: Reproductive and breeding techniques for laboratory animal. 4th Ed. Lea & Febiger-Philadelphia.
- KHALIFA, R.M. 1994: Mating frequency and artificial insemination in rabbit using vasoectomized buck.. *World Rabbit Sci*, 2 (1), 1-5.
- RÉMOIS, G., LAFGUE-HAURET, P., BOURDILLON, A. and ROUILÈRE, H. 1996: Reproductive Results of does inseminated with 5 times or 10 times diluted semen. 6th World Rabbit Congress, Toulouse vol. 2: 115-118.
- SAS. 1988: Statistical Analysis System. *SAS user's guide: Statistics version 6 Edition*, Cary, N.C. 27512-8000: SAS Institute, Inc., Box. 8000, USA.
- TAWFEEK, M. T. and EL-GAAFARY, M.N. 1991: Evaluation of AI technique as compared to natural mating in association with some productive and reproductive traits in rabbits. *Egyptian j. of Rabbit Sci.*, 1(1), 13-20.