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ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS INFLUENCING KINDLING RATES AND LITTER SIZE AT BIRTH IN ARTIFICIALLY INSEMINATED DOE RABBITS

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ABSTRACT : A total of 713 inseminations and 431 kindling records were used for statistical analysis to determine the fixed effects of season, sexual receptivity and physiological status on kindling rates (KR) and total born per litter (TB). Rabbits were reared in México under a moderate climate located at 2240 m above sea level with an average yearly temperature of 15°C. Eighty four New Zealand White rabbits two months of age were reared in a 64-cage replacement system and bred throughout a one-year reproduction cycle. Does kept in 32 cages were inseminated regularly 4-8 days after parturition while does maintained in the other 32 cages were under a 9-13 days reproductive rhythm. Season had no influence on reproductive performance (P>0.05). Kindling rates for spring, summer, autumn and winter were 0.67, 0.67, 0.66, and 0.65 respectively, with TB values of 8.35 ± 0.26, 8.06 ± 0.25, 8.03 ± 0.27 and 7.86 ± 0.40 respectively. Does that were sexually receptive at insemination had significantly (P<0.0001) greater KR (0.87) than non-receptive (0.46) does. TB in the first category (8.23 ± 0.22) was higher (P<0.01) than in the second (7.92 ± 0.20). There was an important effect of physiological status on reproductive performance (P<0.0001). KR in non-lactating nulliparous (0.86) was higher than non-lactating multiparous does (0.68), lactating does under 4-8 days reproductive rhythm (0.53) and lactating does under 9-13 days rhythm (0.59). Nulliparous does had significantly lower TB (6.79 ± 0.14) than the other physiological statuses (8.64 ± 0.25, 8.50 ± 0.26 and 8.37 ± 0.26 respectively). Kindling rate was influenced by the interaction between receptivity and physiological status (P<0.05). The effects of the number of suckling rabbits at insemination on reproductive performance of next litter are discussed.

RÉSUMÉ : Facteurs environnementaux et physiologiques influençant le taux de mise bas et la taille de la portée à la naissance chez les lapines inséminées artificiellement.
Une analyse statistique, portant sur les données enregistrées de 713 inséminations et 431 mises bas, a été effectuée pour déterminer l'effet fixe de la saison, de la réceptivité sexuelle et du stade physiologique sur le taux de mise bas (KR) et le nombre total de lapereaux par portée (TB). Ces lapins ont été élevés au Mexique, sous un climat modéré à 2240m d'altitude avec une température moyenne annuelle de 15°C. Quatre-vingt-quatre lapines NZW sélectionnées à l'âge de 2 mois ont été élevés pendant un cycle de reproduction d'un an dans un ensemble de 64 cages avec remplacement des sujets morts ou réformés. Les lapines, logées dans 32 des cages, ont été régulièrement inséminées 4-8 jours après la mise bas, tandis que celles des 32 autres cages l'ont été 9-13 jours après la mise bas. La saison n'a pas eu d'influence sur les performances de reproduction (P>0.05). Le taux de mise bas au printemps, en été, à l'automne et en hiver a été de 0,67 ; 0,67 ; 0,66 et 0,65 respectivement avec des nombres de lapereaux nés de (TB) 8,35 ± 0,26 ; 8,06 ± 0,25 ; 8,08 ± 0,27 et 7,86 ± 0,40 respectivement. Les femelles sexuellement réceptives à l'insémination ont eu un taux de mise bas significativement (P<0.0001) plus élevé (0,87) que celui des femelles non réceptives (0,46). Le TB des premières citées (8,23 ± 0,22) est significativement supérieur (P<0.01) à celui des secondes (7,92 ± 0,20). Un effet significatif (<0.0001) du stade physiologique sur les performances de reproduction a été constaté. Le KR chez les nullipares non allaitantes (0,86) était plus élevé que chez les multipares non allaitantes (0,68), les allaitantes soumises au rythme de reproduction 4-8 jours (0,53) et les allaitantes soumises au rythme 9-13 jours (0,59). Les nullipares ont eu un TB (6,79 ± 0,14) significativement plus bas que les lapines des autres stades physiologiques (8,64 ± 0,25 ; 8,50 ± 0,26 ; 8,37 ± 0,26 respectivement). Le taux de mise bas a été influencé par l'interaction entre la réceptivité et le stade physiologique (P<0.05). Les effets du nombre de lapereaux allaités au moment de l'insémination sur les performances de reproduction de la portée suivante ont été discutés.

INTRODUCTION

Artificial insemination (AI) techniques applied to commercial rabbit meat production systems became practical after gonadotrophin releasing hormone (GnRH) proved to induce ovulation satisfactorily (PAUFLER *et al.*, 1979). The intramuscular administration of GnRH to does at the time of insemination resulted in good levels of fertility even after using this factor systematically. AI is an important tool to increase efficiency, reduce the time of services, facilitate breeding management procedures, diminish the risk of venereal diseases and to gain genetic improvements in rabbit production. However, reproductive performance in this programme in the long term has shown to be at a lower level than natural mating (NM) (BLOCHER and FRANCHET, 1990; RODRIGUEZ, 1996). Studies with AI have shown that not all does are sexually receptive at the time of insemination (ROUSTAN, 1982; THEAU-CLEMENT *et al.*, 1996) and this factor appears to strongly influence the results attained in this programme. However, there is little detailed information available on factors affecting reproductive performance in AI under different environmental conditions and management procedures. The present study was carried out in order to determine the influence of some environmental and physiological factors on kindling rate and total kits born per litter in artificially inseminated doe rabbits under Mexican conditions.

MATERIAL AND METHODS

A total of 713 inseminations and 431doe kindling records from an experiment carried out at "Conejos" Centro de Investigación Científica del Estado de México A. C. from May 1990 to December 1991 were used for statistical analysis. The rabbit research center is located in the Valley of México at 19°27' N and 98°53' W and 2240 meters over sea level. Climatic conditions include an annual average temperature of 15°C and a rainfall of 645 mm. Eighty four New Zealand White rabbits two months of age were reared in a 64-cage replacement system throughout a one-year reproduction cycle. Does kept in 32 cages were bred regularly under a 4-8 days parturition-insemination interval schedule while does maintained in the other 32 cages

were under a 9-13 day reproductive rhythm. Does were first inseminated when they reached 3.2 kg live weight. Abdominal palpation was carried out between days 10 and 14 after AI and non-pregnant does were re-inseminated as soon as possible. Nest boxes and hay were provided 4 days before the expected kindling date. Weaning of young was at 28 days of age. Breeding does were housed in a natural ventilation system provided with roof and wall insulation. The rabbits were kept individually in cages 90 x 60 x 40 cm in a flat-deck system provided with an automatic watering supply and English food type. They received 16 hours light and 8 hours dark daily throughout the experiment. A commercial pelleted diet containing 16.7% crude protein, 3.8% fat, 16.7% crude fibre and 2220 kcal digestive energy per kg of food was used. Young does were fed *ad-libitum* until they reached 3.1 kg live weight and then restricted to 120 g daily until palpation was positive. Pregnant and lactating does were fed *ad-libitum*. Does becoming pregnant during the lactation period continued on *ad-libitum* feeding after weaning. Does with negative pregnancy palpation were given 120 g of food per day until a positive palpation was determined.

Semen collection was carried out according to the method of WALTON (1945) with the help of a teaser female. Ejaculates were kept in a water bath at 31°C and used within one hour after collection. Those samples with milky and creamy appearances and motility scores greater than 75% were selected and pooled into a phosphate buffered saline solution (Dulbbecco A, Oxoid). Inseminations were heterospermic when semen of two or more males was mixed in the medium. The dilution rates used varied from 1/4 to 1/12 depending on the number of does to be inseminated in one day. Does were restrained individually in a supine position and inseminated 6 cm deep inside the vagina, with an English type Pyrex pipette giving 0.8 ml of fresh semen. Following this technique, we assured that each doe received at least ten million motile spermatozoa per dose. To induce ovulation, does were administered intramuscularly 0.2 ml of gonadotrophin releasing factor (GnRH, Conceptal, Hoechst). Immediately after inseminations were performed, each doe was placed in a cage with a vigorous male fitted with a nappy. Does that exhibited lordosis behaviour were considered to be receptive, and those which did not do so after several copulation attempts by the buck were considered to be non-receptive.

Inseminated does were grouped by season (spring, summer, autumn and winter), receptivity (receptive and non-receptive) and physiological status (non-lactating nulliparous, non-lactating multiparous, lactating under 4-8 days reproductive rhythm and lactating under 9-13 days rhythm). Kindling rate was considered as a variable of BERNOULLI (variable 0-1). This trait and total litter size at birth were analysed with the following statistical model:

$$Y_{ijkl} = \mu + S_i + R_j + P_k + (SR)_{ij} + (SP)_{ik} + (RP)_{jk} + (SRP)_{ijk} + e_{ijkl}$$

where:

Y_{ijkl} = observed trait value.
μ = overall trait mean.
S_i = fixed effect of i^{th} season group (i=1,2,3,4).
R_j = fixed effect of j^{th} receptivity group (j=1,2).
P_k = fixed effect of k^{th} physiological status (k=1,2,3,4).
$(SR)_{ij}$ = interaction between season group and receptivity.
$(SP)_{ik}$ = interaction between season group and physiologica status.
$(RP)_{jk}$ = interaction between receptivity and physiological status
$(SRP)_{ijk}$ = interaction among season group, receptivity and physiological status
e_{ijkl} = random error.

A particular analysis of variance for lactating does only was carried out to determine the fixed effects of number of rabbits suckling at insemination (1-4, 5-8 and >8 young), receptivity (receptive and non-receptive) and the interaction of kindling rate and total litter size at birth. Traits were analysed by least square analysis of data for unequal subclass numbers using the GLM procedure of SAS (1988). Results presented in tables are least square means with standard errors.

RESULTS AND DISCUSSION

After a complete one year reproductive cycle, artificial insemination of does under the 4-8 and 9-13 days reproductive rhythms were carried out on average at 6.5 and 10.8 days after parturition respectively. Mean intervals between parturition for the two reproductive rhythms were 51.2 and 60.8 days with 7.6 and 6.6 litters being produced per doe-cage per year. Average kindling rates of does under 4-8 and 9-13 days reproductive rhythms were 60.8 and 60.1% with 8.34 ± 0.17 and 8.21 ± 0.18 young at birth. The overall kindling rate and total born per litter in this study was 60.4% and 8.28 ± 0.12 were respectively. Fertility levels are much lower than the 78.7% and 86% reported by UZCATEGUI and JOHNSTON (1987) and BECHSTEDT (1989) respectively, but similar to the 57.2% observed by BLOCHER and FRANCHET (1990). The total number of rabbits born per litter is similar to the 8.2 and 8.3 reported by ANGELI *et al.* (1990) and SZENDRŐ and BIRO-NEMETH (1991) in artificial insemination programmes but differs from the values of 5.3 and 10.4 published by UZCATEGUI and JOHNSTON (1987) and BOURDILLON *et al.* (1992). Inconsistencies in reproductive performance among studies can be attributed to different breeding systems (reproductive rhythm, housing, feeding) and the genotype used. However, differences in the technology and handling of semen have also been shown to be other important sources of variation (THEAU-CLEMENT and ROUSTAN, 1980).

The low average kindling rate in this study compared with other investigations can be attributed largely to nutritional deficiencies in the long term and probably related to differences in levels of sexual receptivity. Protein and energy levels of the diet used were far below the requirements for the two reproductive rhythms. XICCATO (1996) has established requirements according to the intensity of reproduction. Does breed under intensive and semi-intensive reproductive rhythm require no less than 13.0 and 12.5% of digestible protein respectively and 2600 and 2500 Kcal of digestible energy per kg of food. Dietary deficiencies in does reared under continuous reproduction have been shown to decrease the reproductive performance in the long term as the result of negative energy balance and deterioration of body condition particularly in periods of maximum milk production and in primiparous does (PARIGI-BINI *et al.*, 1990). It is also likely that food restriction to 120 g daily when does were not pregnant at weaning may have had some influence on body condition deterioration in some of their reproductive cycles, especially if levels of lactation were

Table 1 : Inseminations and litters data distribution in relation to season, receptivity and physiological status of does.

Season	Receptivity	Number of inseminated does				Number of litters			
		L⁻N	L⁻M	L⁺4-8	L⁺9-13	L⁻N	L⁻M	L⁺4-8	L⁺9-13
Spring	R	10	10	22	22	10	9	17	17
	NR	24	23	31	27	19	8	15	8
Summer	R	18	11	30	23	18	11	24	21
	NR	20	34	25	28	14	13	7	9
Autumn	R	9	14	31	14	8	13	22	11
	NR	9	36	37	31	8	22	7	10
Winter	R	1	26	40	28	1	21	30	25
	NR	8	30	22	19	5	14	6	8

R- Receptive; NR - Non-receptive;
L⁻N - Non-lactating nulliparous; L⁻M - Non-lactating multiparous;
L⁺4-8 - Lactating 4-8 days reproductive rhythm; L⁺9-13 - Lactating 9-13 days rhythm.

high. Nutritional deficiencies in rabbits have been related to a decrease in sexual activity, fertility and embryo survival (WITTORFF *et al.*, 1988).

Raw data and the fixed effect of season, receptivity and physiological status on kindling rates and litter size at birth are shown in tables 1 and 2. Reproductive performance in autumn and winter tended to be lower than in spring and summer but no significant difference among seasons was observed (P>0.05). SITTMANN *et al.* (1964) found a seasonal trend from high conception rates in the spring to low rates in the autumn associated with changes in temperatures. HULOT and MATHERON (1981) agreed with these findings and pointed out that conception rates during autumn could increase if adequate environmental conditions are given particularly in temperature, lighting and feeding. In this study, a similar kindling rate trend was observed but seasonal variations were low. These results are similar to those reported by SINKOVICS and HAUT (1988) and Rodriguez (1996) who observed low seasonal variations in conception rates in artificial insemination programmes when compared to natural mating. There was no influence (P>0.05) of the interaction between season groups and receptivity nor between season and physiological status on reproductive performance of does.

In this study 43.3% of the total inseminations were to receptive does and 56.7% to non-receptive. Does that showed sexual receptivity had (P<0.0001) greater kindling rates (87.0%) than non-receptive (46.2%) (table 2.). These results confirm that fertility under AI varies with sexual receptivity (THEAU-CLEMENT *et al.*, 1990; THEAU-CLEMENT *et al.*, 1996). Total born per litter was higher (P<0.01) in receptive than non-receptive. THEAU-CLEMENT and ROUSTAN (1992) also observed significant differences in litter size at birth between the two groups of does. Previous studies with natural mating have shown that does accepting the male show greater number of large and pre-ovulatory follicles than those not accepting service (LEFEVRE and CAILLOL, 1978). RODRIGUEZ and UBILLA (1988), when inducing ovulation with GnRH in AI, found greater numbers of corpora lutea in receptive than non-receptive does. Receptive does present higher number of large follicles (KERMABON *et al.*, 1994) and higher oestrogen levels (UBILLA and REBOLLAR, 1995). Non-receptive does showed a low response associated with an increase in prolactin. The secretion of this hormone has been related with a reduction on the number of binding receptors to luteinizing hormone (LH) in the follicle cells (HAMADA *et al.*, 1980) and to decrease the magnitude of follicle stimulating hormone (FSH) and LH in response to GnRH when the levels of receptivity are low (RODRIGUEZ *et al.*, 1989).

There was a significant effect of physiological status on kindling rate (P<0.0001). This influence was mainly due to differences between L⁻N with the other physiological status. L⁻N had 18.2, 33.0 and 27.1% higher kindling rates when compared with L⁻M, L⁺4-8 and L⁺9-13 respectively. The higher level of fertility in L⁻N

Table 2 : Analysis of variance. Effect of season, receptivity and physiological status of does on kindling rate and total born per litter

	N	Kindling Rate	N	Total Born/litter
Season				
Spring	169	0.67 ± 0.03	103	8.35 ± 0.26
Summer	189	0.67 ± 0.03	117	8.06 ± 0.25
Autumn	181	0.66 ± 0.04	101	8.03 ± 0.27
Winter	174	0.65 ± 0.06	110	7.86 ± 0.40
P value		0.382		0.892
Receptivity				
Receptive	309	0.87 ± 0.04[a]	258	8.23 ± 0.22[a]
Non receptive	404	0.46 ± 0.02[b]	173	7.92 ± 0.20b
P value		0.0001		0.013
Physiological status				
L⁻N	99	0.86 ± 0.07[a]	83	6.79 ± 0.41[b]
L⁻M	184	0.68 ± 0.03[b]	111	8.64 ± 0.25[a]
L⁺4-8	238	0.53 ± 0.03[c]	128	8.50 ± 0.26[a]
L⁺9-13	192	0.59 ± 0.03[bc]	109	8.37 ± 0.26[a]
P value		0.0001		0.0001
R^2		0.26		0.13

[abc] - Different superscripts in the same row indicate significant differences.

Table 3 : Effect of the interaction between receptivity and physiological status of does on the proportion of does kindling.

	Physiological status			
	L^-N	L^-M	L^+4-8	L^+9-13
Receptive	0.97 ± 0.12^{ab}	0.91 ± 0.06^{a}	0.76 ± 0.04^{b}	0.84 ± 0.05^{ab}
Non-receptive	0.75 ± 0.06^{ab}	0.45 ± 0.04^{c}	0.30 ± 0.04^{d}	0.34 ± 0.04^{d}

^{abcd}- Different superscripts in the same row indicate significant differences (P<.05).

does in this study is explained by the high kindling rates obtained by the receptive does (97%) which represent 38.4% of the total and due to the good rates obtained by the 61.6% non-receptive (75%). In the L^-M group 33.1% of does were receptive at insemination and their kindling rate was high (91%) but levels of fertility in the 66.8% of non-receptive were low (45%). There were no differences (P>0.05) in reproductive performance between the L^+4-8 and L^+9-13 groups. Nulliparous does have shown higher fertility levels than lactating does (ROUSTAN and MAILLOT, 1990; THEAU-CLEMENT *et al.*, 1996). Non lactating multiparous does had 14.8 and 8.9% higher kindling rates than lactating does under the two reproductive rhythms. SZENDRÖ and BIRO-NEMETH (1991), reported higher kindling rates in non-lactating multiparous does compared with those inseminated during the lactation period. The lower conception rate in lactating does has been explained in terms of a hormonal antagonism between prolactin and gonadotrophin (REBOLLAR *et al.*, 1992; THEAU-CLEMENT and ROUSTAN, 1992). This effect is more pronounced in primiparous does with energy deficit during lactation (PARIGI-BINI *et al.*, 1990) when they simultaneously have a high energy requirements for milk production, body growth and gestation (CASTELLINI, 1995). Lactation negatively influences the reproductive functions in the rabbit by depressing sexual receptivity, ovulation rates, fertilisation and embryo development (THEAU-CLEMENT and POUJARDIEU, 1994; FORTUN-LAMOTHE and BOLET, 1995).

Kindling rates in lactating does under a 4-8 days reproductive rhythm tended to be lower than those reared under a 9-13 days rhythm, but there were no significant differences (P>0.05). Receptivity rate in L^+4-8 does was higher (51.7%) than in L^+9-13 does (45.3%) but the kindling rate in the first group (76%) was less than in the second (84%). The percentages of non-receptive does in these two groups were 48.3 and 54.7% and their kindling rates were low (30 and 34% respectively). The lowest kindling rates attained in lactating does in this study were from non-receptive does because of their low fertility levels. Low sexual receptivity during this period relates to the effect of suckling. UBILLA and REBOLLAR (1995) found low sexual behaviour in does nursing 7 to 10 young from days 7–24 days *post-partum* and detected low plasma oestradiol levels during this period. UBILLA *et al.* (1992) have suggested that high plasma prolactin levels during these days prevent the estrogenic effect on sexual receptivity. Depression of sexual receptivity during the lactation period has been reported (THEAU-CLEMENT *et al.*, 1990). ZANIRATO (1989) found conception rates of 50.0 % and 63.0 % when inseminations were carried out between 0-5 and 6-11 days after parturition. The low fertility in the early period after parturition indicates an antagonism between pregnancy and nursing. CASTELLINI (1995) indicates that days 3 to 5 after parturition are especially negative periods for reproductive activity and fertility increases as the parturition-insemination interval increases. TORRES *et al.* (1977), comparing does mated between 12 to 18 hours or 25 days after parturition found lower levels of fertility in the first group as a direct result of a decrease in ovulation rate and a greater number of oocytes remaining unfertilized.

The interaction between receptivity and physiological status on kindling rate was significant (P<0.05) as is shown in table 3. Receptive does had consistently higher kindling rates than non-receptive. However, the values for nulliparous non-receptive does (L^-N) were high and appeared to be the same as receptive L^-N does. The high fertility in L^-N non-receptive does (75%) indicates that sufficient levels of FSH and pre-ovulatory follicles in these does were present at the time of insemination but for some unclear reasons the does did not exhibit lordosis behaviour when tested with a male. It is possible that handling, insemination and testing in these does may cause a stressful condition but more research on the mechanisms involved with this behaviour is necessary. GOSALVEZ *et al.* (1989) reported that the number of pre-ovulatory follicles at 17 weeks of age was at about the same level as multiparous does. In the present study rabbits were inseminated at about 18 weeks of age.

Total kits born per litter in nulliparous does was significantly lower (P<0.0001) than the other physiological status. These results supports the litter sequence reported by ROLLINS *et al.* (1963) who observed in New Zealand White rabbits that the first litter was smaller than in the second and third litters. Reproductive performance in this study is in agreement with THEAU-CLEMENT *et al.* (1996) who observed that nulliparous were more fertile than primiparous but litter size in the former was smaller. There were no interactions among seasons, receptivity and physiological status on kindling rates and

Table 4 : Effect of number of suckling rabbits at insemination and receptivity of does on kindling rate and total born per litter.

	N	Kindling rate	N	Total born/litter
Number of suckling rabbits at insemination				
1-4	66	0.51 ± 0.05	35	8.46 ± 0.48
5-8	250	0.54 ± 0.03	133	8.41 ± 0.26
>8	99	0.61 ± 0.04	58	8.65 ± 0.35
P value		0.558		0.808
Receptivity				
Receptive	201	0.76 ± 0.03^{a}	159	8.99 ± 0.24^{a}
Non-receptive	214	0.34 ± 0.03^{b}	67	8.01 ± 0.36^{b}
P value		0.0001		0.045
R^2		0.24		0.03

^{ab} – Different superscripts in the same row indicate significant differences.

total born per litter.

The receptivity rates for does with 1-4, 5-8 and >8 young at the time of inseminations were 56.1, 48.0 and 44.4% respectively. This trend is in agreement with the performance found under a natural mating programme by TORRES *et al.* (1987) who observed that increasing the number of suckling rabbits depressed the mating acceptance.

Kindling rates and total born per litter were not influenced (P>0.05) by the number of suckling young at insemination as shown in table 4. These results are similar to those reported by SZENDRÖ and BIRO-NEMETH (1991) who found no relationship between number of young in the litter and reproductive performance when comparing groups of inseminations with 1-5, 6-7, 8-9 and 10-11 suckling young in the litter. Considering only lactating does, there was a strong effect of receptivity on kindling rate (P<0.0001) and on total born per litter (P<0.04). Receptive does were 42.4% more fertile and produced on average one kit more than non-receptive does. Kindling rates and litter size at birth were not influenced by the interaction between number of young suckling and receptivity (P>0.05).

Sexual receptivity and physiological status of does at the time of insemination represent the most important factors influencing reproductive performance in rabbits. Receptive does are more fertile and more prolific than non-receptive. Kindling rates in nulliparous does are higher than multiparous but litter size in the latter is higher. Overall, lactating does under a 4-8 and 9-13 days reproductive rhythm show lower fertility than non-lactating does. However, receptive does inseminated during the lactation period show a good reproductive performance while non-receptive does give poor results. The number of young suckling at the time of insemination had no impact on the immediate reproductive performance. Rabbit farmers taking advantage of an AI programme should not spend time identifying does in oestrous as in natural mating and instead should inseminate does at once. They must employ high management standards, provide adequate environmental conditions and provide a diet suitable for the reproductive rhythm. These can help to assure in the long term that a higher proportion of does are receptive than non-receptive at service even during critical seasons periods and when rearing a litter. In this way levels of productivity should be increased. The use of techniques such as flushing, lighting schedule, doe cage changes and doe stimulation during lactation should be considered for synchronising oestrus for AI instead of systematic use of hormones.

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