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EFFECT OF LACTATION STAGE ON LITTER SIZE COMPONENTS IN RABBITS

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ABSTRACT

The aim of this study was to evaluate the possible effect of lactation stage on ovulation rate (*corpora lutea* number, OR), implanted embryos (IE), total born (TB), and embryo (ES), fetal (FS) and prenatal survival (PS) in multiparous does, pertaining to a complete diallel cross between four Spanish maternal lines of rabbits. Sixteen genetic types were studied (four maternal lines: A, V, H and LP, currently belonging respectively, to generation 41, 37, 20 and 7, selected for litter size at weaning), and their twelve simple crosses). A total of 1592 does in their third, fourth or fifth gestations were subjected to a laparoscopy 11-12 days after mating. To estimate the lactation stage effect, in relation to the number of days between kindling and insemination, 4 classes were considered: <11, 11-12, 13-15 and >15 days of lactation. The means of all traits reflected the high reproductive capacities: 15.43 ova, 13.17 embryos, 10.97 total born, 86.67%, 83.05% and 71.98% for OR, IE, TB, ES, FS and PS, respectively. Regarding parity order, no differences between the third, fourth and fifth gestations were observed for all analyzed traits. A positive, significant and relevant relationship was found between OR and IE, and also between OR and LS, due to their physiological sense.

On multiparous and receptive rabbit does, the number of *corpora lutea* increases with the interval between birth and mating or AI (P=0.05), but lactation stage did not significantly influences the IE, TB, ES, FS and PS. In multiparous lactating rabbit does, the ovulation rate, implanted embryos and litter size could increase when the interval between kindling and mating (or insemination) increases.

Key words: Rabbits, laparoscopy, litter size components, prenatal survival, lactation stage effect.

INTRODUCTION

In polytocous species, litter size at birth is mainly determined by two parameters: ovulation rate (number of ova) and prenatal survival. Fetal survival and development can be impaired when does are simultaneously, pregnant and lactating, due to an hormonal antagonism between prolactin and gonadotropins (Fortun-Lamothe and Bolet 1995) and a negative energetic balance of rabbit does during the second half of gestation and at the end of lactation (Parigi-Bini *et al*., 1990 a,b). Nevertheless, when sufficient food is available, rabbit does seem well adapted to a lactation-reproduction overlapping. In fact, artificial insemination (AI) around 11 days *postpartum* is the most common reproductive rhythm in commercial rabbit farms, but not all females are receptive at AI day, and sometimes females are inseminated or mated at high *postpartum* intervals (more than 11 days *postpartum*).

In rabbit does, sexual behaviour and consequently productivity, varies considerably with parity order, physiological state (lactating or not), and also lactation stage (Theau-Clément, 2007). Regarding the lactation stage at mating or insemination time, the sexual behaviour of does is high on the day of birth (Roustan and Maillot, 1990), decreases at day 4 of lactation (Theau-Clément *et al*., 1990), increases at day 11 of lactation (Theau-Clément *et al*., 1998) and is the highest after weaning (Fortun-Lamothe and Bolet 1995). The influence of parity order and lactation in fertility and prolificacy in does is well documented also (Perrier *et al*., 2000; for a review see: Theau-Clément, 2007), although a higher effort has been done in the knowledge of these factors in primiparous does (Rebollar *et al.,* 2006). By contrast, the literature is scarce about the influence of the lactation stage on embryo, foetal and prenatal survival, and only some lactation periods (different to 10-12 days) were studied, usually 1-4 days after birth or 25 days of lactation (Torres *et al*., 1977; Theau-Clément *et al.,* 1990; Fortun-Lamothe 2006). Only Theau-Clément *et al.* (2000) have described the ovarian status and fertilising ability of primiparous rabbit does at different lactation stage.

The aim of this study was to evaluate the possible effect of lactation stage on ovulation rate, implanted embryos, embryo, fetal and prenatal survival, litter size in multiparous does.

MATERIALS AND METHODS

Animals and management

A complete diallel cross involving four maternal lines of rabbits (A, V, H, and LP), selected for litter size at weaning, previously described by Ragab and Baselga (2011) was carried out. Their current generation of selection was the 41st, 37th, 20th and 7th, respectively. Data were collected from January 2009 to April 2011. The experimental work was carried out in four Spanish farms with a total of 2260 rabbit does. The farms were located in León (farm 1: 800 does), Castellón (farm2: 800 does), Tarragona (farm 3: 300 does) and finally the farm of Universidad Politécnica de Valencia (UPV, farm 4: 360 does). The genetic groups involved in the experiment were the four lines (AA, VV, HH and LL) and the twelve single crosses (AV, VA, AH, HA, AL, LA, VH, HV, VL, LV, HL and LH, the first letter refers to the sire line, and the second one to the dam line; L is used to identify the LP line). Farms 1 and 2 raised all the crossbreds and purebred V line animals; the farm 3 raised females of the groups VV and HH and the farm 4 housed VV, AA and LL animals. The group VV was present in all the farms to connect data between farms.

Management was slightly different across farms. The mating was practiced, in all farms, each week (6 batches). Rabbits were fed *ad libitum* a standard commercial pelleted diet and keep under a constant photoperiod of 16L: 8D.

In all farms, the first mating of does was around 18 weeks of age. Natural mating was used in farms 2 and 3 with males of the same line, while artificial insemination was practiced in farm 1. In farm 1, before AI a visual examination has been made to evaluate does receptivity (vulva color) and only receptive ones have been inseminated (red vulva). The does were inseminated with heterospermic pools from a paternal line (line R, Estany *et al*., 1992). Semen doses were prepared 16 hours before and had a motility rate higher than 70%, abnormal sperm lower than 15% and a concentration 20-40 millions per ml. Females were inseminated with 0.5 ml of diluted pooled semen and induced to ovulate with 1µg of busereline actetate.

Traits and statistical analyses

The studied traits (calculated on only ovulating does) were: estimated ovulation rate (OR; estimated as the number of *corpora lutea* in both ovaries), number of implanted embryos (IE; measured as the total number of implantation sites), litter size (LS; measured as the total number of kits born), embryo survival (ES; estimated as IE / OR), fetal survival (FS; estimated as LS / IE), and prenatal survival (PS; estimated as LS / OR). These records were obtained from does in their third, fourth or fifth gestations that were subjected to a laparoscopy 11-12 days after mating or insemination. The surgical technique was described by Santacreu *et al*. (1990). A total of 1592 lactating does, from the sixteen genetic types were subjected to laparoscopy.

The fitted model for all traits except IE and LS was:

$$Y_{ijk} = GFYS_i + OP_j + L_k + e_{ijk} \qquad \text{(Model 1)}$$

where Y_{ijkl} is one observation of trait being analysed; $GFYS_i$ is the fixed effect of the genetic group-farm-year-season combination (129 levels); OP_j is the parity order (3 levels: 3, 4 and 5)); L_k is the lactation stage with 4 levels depending on the day of lactation (does with <11, 11-12, 13-15 and >15 days of lactation), and e_{ijk} is the residual of the model.

For IE and LS traits, the following model was used

$$Y_{ijkl} = GFYS_i + OP_j + L_k + \beta\,(OR_{ijkl}) + e_{ijkl} \qquad \text{(Model 2)}$$

where, β is the regression coefficients on OR, respectively; the other components of the model were the same as in model 1.

The interaction between GFYS and L has not been included in the model, because its interpretation would be difficult if is noted that there are 4x129=516 different classes. Moreover, the estimation of the effects of L_k would be minimal affected by considering the interaction if the distribution of the lactation stage through the GFYS is balanced. The design of our experiment has not any element that determine unbalance between both effects.

The models were solved and least squares means for fixed effects was determined using option of the GLM procedure of SAS (version 9.1.3; SAS Institute, 2002). Significance was claimed at a type I error, α=0.05.

RESULTS AND DISCUSSION

Means and standard deviations of the studied traits are presented in Table 1; values of IE and ES are in agreement with values published by other authors in maternal rabbit lines (Brun *et al.*, 1992; García and Baselga, 2002; Laborda *et al.*, 2012). Prenatal mortality (approximately 30%) was similar than those previously reported by García and Baselga (2002), this percentage is almost equally distributed before and after the 12th day of gestation (13 and 17%, respectively).

Regarding to OR, the value is higher than in previous experiments with some of the lines involved in this study (García and Baselga, 2002), and similar than those obtained by Laborda *et al.* (2011) in a rabbit line selected for ovulation rate. This is probably due to the consequence of the selection for litter size at weaning (García and Baselga, 2002) and to the inclusion of crossbred does in this study (Brun *et al.*, 1992; Ragab *et al.*, 2012a).

Results obtained in our study show the high influence of the genetic line, farm and season (represented by the GFYS combination) in all the analyzed traits (P<0.05; data not shown in tables). This combination represents apart of differences in genetic background, different breeding management procedures and also specific environmental conditions.

Regarding parity order, no differences between the third, fourth and fifth gestations were observed for all the analyzed traits (Table 2). Prolificacy results are in concordance with those observed previously by Perrier *et al.* (2000) for crossbred females of the INRA (A2066 x A1077). In fact multiparous does have high fertility and litter size levels.

A positive, significant and relevant relationship was found between OR and IE (0.69±0.02) and also between OR and LS (0.43±0.03), due to their physiological sense. In fact, by definition, the number of ova by doe (OR) is the maximum number of embryo that the female could implant (IE) and also could

Table 1: Means and standard deviations (SD) for ovulation rate (OR), number of implanted embryo (IE), litter size (LS), embryo survival (ES), fetal survival (FS) and prenatal survival (PS).

Trait	N	Mean	SD
OR	1592	15.43	2.82
IE	1592	13.17	3.14
LS	1466	10.97	3.36
ES	1592	86.67	15.95
FS	1466	83.05	18.22
PS	1466	71.98	20.51

N: number of data

Table 2: Litter size components per parity order and lactation period (lsmeans± standard errors).

Parity order	N	OR	ES	FS	PS	IE	LS
3	771	15.35±0.17	86.66±1.03	81.51±1.22	71.09±1.38	13.33±0.16	10.82±0.21
4	591	15.51±0.17	86.05±1.03	81.54±1.24	70.17±1.39	13.24±0.16	10.69±0.21
5	229	15.31±0.23	85.99±1.36	81.91±1.65	70.18±1.85	13.12±0.21	10.67±0.28
Lactation stage							
1	425	14.88±0.25^{b}	87.26±1.51	80.57±1.79	70.43±2.01	13.32±0.23	10.57±0.35
2	896	15.04±0.15^{b}	86.67±0.88	83.41±1.07	72.20±1.20	13.23±0.14	10.86±0.18
3	152	15.49±0.29^{ab}	86.85±1.69	79.28±2.01	69.65±2.26	13.27±0.26	10.58±0.35
4	119	16.17±0.30^{a}	84.44±1.76	83.35±2.08	69.62±2.34	13.10±0.27	10.88±0.36
Covariate OR						0.69±0.02*	0.43±0.03*

OR: ovulation rate; ES: embryo survival; FS: fetal survival; PS: prenatal survival; IE: number of implanted embryo; LS: litter size (total born). Values with different letters on the same column within the same group significantly differ.
*Significant (P<0.05).

give to term (LS). The same relationships have been found by Ragab *et al.* (2012b) in a study, involving the same genetic types, but not limited to lactating does. One interesting finding was the influence of the lactation stage on the ovulation rate (Table 2). The data indicate that ovulation rate increases with the increase in the interval between birth and mating or AI (P<0.05). The same trend was observed by Theau-Clément *et al.* (2000) in primiparous does. When the does were inseminated at 19 days *post partum* or 2 days after weaning, the ovulation rate increased by 0.3 and 0.4 with respect to does inseminated at 12 days *post partum*. They included in the analyses receptive and no receptive females and found that does receptivity plays an important role in the ovarian status and fertilising ability of does. In our case, all the data were from receptive multiparous does, so comparison should be made with caution. Our results showed that ES, FS and PS were not affected by the lactation period and similar values were observed for the different periods. However, the number of implanted embryos and the litter size were affected trough the effect of the lactation period on the ovulation rate, despite there were not significant differences between periods when correcting to a constant ovulation rate.

CONCLUSION

In multiparous lactating rabbit does with high reproductive potential (sexually receptive), the ovulation rate could increase when the interval between kindling and mating (or insemination) increases. The same could occur for implanted embryos and litter size through the effect of the lactation period on ovulation rate, whereas the other components of litter size do not seem to change.

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REFERENCES

Brun J.M., Bolet G., Ouhayoun J. 1992. The effects of crossbreeding and selection on productive and reproductive traits in a triallel experiment between three strains of rabbits. *J. Appl. Rabbit Res. 15:181-189.*

Estany J., Camacho J., Baselga M., Blasco A. 1992. Selection response of growth rate in rabbits for meat production. *Genet. Sel. Evol., 24, 527–537.*

Fortun-Lamothe L. 2006. Energy balance and reproductive performance in rabbit does. *Anim. Reprod. Sci., 93:1-15.*

Fortun-Lamothe L., Bolet G. 1995. Les effets de la lactation sur les performances de reproduction chez la lapine. *INRA Productions Animales, 8, 49-56.*

García M.L., Baselga M. 2002. Estimation of genetic response to selection in litter size of rabbits using a cryopreserved control population. *Livest. Prod. Sci. 74:45-53.*

Laborda P., Mocé ML., Santacreu MA., Blasco A. 2011. Selection for ovulation rate in rabbits: genetic parameters, direct response and correlated response on litter size. *J Anim Sci 88:2981-2987*

Parigi-Bini, R., G. Xiccato, and M. Cinetto. 1990b. Repartition de l'énergie alimentaire chez la lapine non gestante pendant la première lactation. *In Proc.: 5emes Journ. Rech. Cunicole Fr., Comm. no. 47, Paris.*

Parigi-Bini, R., Xiccato G., Cinetto M. 1990a. Energy and protein retention and partition in rabbit does during the first pregnancy. *Cuni-sci. 6(1):19.*

Perrier G., Theau-Clément M., Jouanno M., Drouet J.P. 2000. Reduction of the GnRH dose and inseminated rabbit doe reproductive performance. In Proc.: *7th World Rabbit Congress, 4-7 July, Valencia, Spain. A, 225-230*.

Ragab M., Baselga, M. 2011. A comparison of reproductive traits of four maternal lines of rabbits selected for litter size at weaning and founded on different criteria. *Livest. Sci. 136, 201-206.*

Ragab M., Sánchez J.P., EL Nagar A.G., Mínguez C., Desantes J, Baselga M. 2012a. Heterosis effect on litter size components in rabbits. Submitted to *10th World Rabbit Congress, 2012 September, Sharm El-Sheikh, Egypt.*

Ragab M., Vicente J. S., Lavara R., Mínguez C., Desantes J., Baselga M. 2012b. Uterine crowding in commercial breeding does. Submitted to *10th World Rabbit Congress, 2012 September, Sharm El-Sheikh, Egypt.*

Rebollar P.G., Milanès A., Pereda N., Millán P., Cano P., Esquifino A.I., Villarroel M., Silván G., Lorenzo P.L. 2006. Oestrus synchronisation of rabbit does at early post-partum by doe litter separation or ECG injection: Reproductive parameters and endocrine profiles. *Animal Reprod. Sci.*, *93, 218-230.*

Roustan A., Maillot D. 1990. Comparaison des résultats de fertilité et de productivité numérique à la naissance de deux groupes de lapines conduites en insemination artificielle et en saillie naturelle. Analyse de quelques facteurs de variation. *In Proc.: 5émes Journées de la Recherche Cunicole, 12-13 Décembre, Paris, France. Tome I, Comm 3.*

Santacreu, M. A., Viudes M.P., Blasco A. 1990. Evaluation par coelioscopie des corps jaunes et des embryons. Influence sur la taille de portée chez la lapine. *Reprod. Nutr. Dev. 30:583–588.*

Theau-Clément M. 2007. Preparation of the rabbit doe to insemination: A review. *World Rabbit Sci., 15: 61-80.*

Theau-Clément M., Boiti C., Mercier P., Falières J. 2000. Description of the ovarian status and fertilising ability of primiparous rabbit does at different lactation stage. *In Proc.: 7th World Rabbit Congress Valencia Spain, Vol A, 259-266.*

Theau-Clément M., Bolet G., Roustan A., Mercier P. 1990. Comparaison de differents modes d´induction de l´ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise à la reproduction. *In: Proc.: 5émes Journées de la Recherche Cunicole, 12-13 Décembre, Paris, France. Tome I, Comm.6.*

Theau-Clément M., Castellini C., Maertens L., Boiti C. 1998. Biostimulations applied to rabbit reproduction: theory and practice. *World Rabbit Sci., 6, 179-184.*

Torres S., Gerard M., Thibault T. 1977. Fertility factors in lactating rabbits mated 24 hours and 25 days after parturition. *Ann. Biol. Anim. Biochem. Biophys. 17(1):63.*