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LITTER SIZE COMPONENT TRAITS IN TWO ALGERIAN RABBIT LINES

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Abstract: The aim of this study was to estimate the limiting litter size components in rabbit females from a Synthetic line (n=32) and a Local population (n=34). Ovulation rate, number of implanted and live embryos were counted by laparoscopy at 12 d after mating. Prolificacy (total newborn, number born alive and mortality) and embryonic, foetal and prenatal survival at day of birth of the 3rd gestation were measured. The analysed traits were body weight of the female at mating, ovulation rate, implanted, live and resorbed embryos, embryonic, foetal and prenatal survival, as well as total newborn, number born alive and mortality at birth. Synthetic line females had a higher ovulation rate compared to the Local population (11.03±0.23 *vs*. 8.41±0.23 *corpora lutea*; *P*<0.0001). Synthetic line displayed a higher number of implanted embryos (10.00±0.25 *vs*. 7.85±0.25 embryos; *P*<0.0001). No difference was found between groups for number of resorbed embryos. Similar embryonic, foetal and prenatal survival rates were reported between the Synthetic line and the Local population. Additionally, total newborn was higher in the Synthetic line than in the Local population (+1.46 kits; *P*<0.05). A principal components analysis was performed. The first four principal components (PC) explained more than 90% of the total variation in both lines. Total newborn, number born alive and live embryos were the main variables defining the 1st PC. Resorbed embryos and foetal survival were located in the 2nd PC. Ovulation rate and embryonic survival were the predominant variables defining the 3rd PC. The body weight of females was located in the 4th PC. The phenotypic correlation between total newborn and its components were high and positive in both lines, except for ovulation rate and total newborn, where it was moderate in Synthetic line. In conclusion, the females from Synthetic line have a higher total newborn than those from Local population, as a consequence of a higher number of released oocytes and embryos that successfully reach implantation. However, a higher uterine crowding in Synthetic line seems to limit survival of foetuses that reach term of gestation, while ovulation rate is the principal limiting factor of total newborn in Local population.

Key Words: crossbreeding, litter size, ovulation, prenatal survival, rabbits.

INTRODUCTION

Litter size is one of the most important economic traits in rabbits (Cartuche *et al.,* 2014). Litter size is limited by several components of traits such as ovulation rate, fertilisation and prenatal mortality (see review in Blasco *et al.,* 1993a). Fertilisation rate is generally high, exceeding 90 to 95% (Peiró *et al.,* 2014), and is therefore not considered a constraining component of litter size (Belabbas *et al.,* 2016). However, ovulation rate is, together with pre and postimplantation mortality, the foremost component of litter size, and is also a limiting factor for its improvement (Laborda *et al.,* 2011). Preimplantation losses are mainly related to the embryo's viability related to chromosomal abnormalities, oocyte quality or embryonic development (Fechheimer and Beatty, 1974; Pope *et al.,* 1990) and to the quality of oviduct and uterine secretions (Peiró *et al.,* 2014). After implantation, the losses are related to the placenta development and uterine capacity of the female (Argente *et al.,* 2003).

Prenatal mortality is around 30% (Mocé *et al.*, 2010). The first peak of mortality occurs between day 8 and 17 *post coitum* (66%), when the haemochorial placenta of rabbit starts and finishes its development, respectively, in order to control the foetal nutrition (Adams, 1960). A secondary peak of mortality occurs between day 17 and 24 of gestation (27%), which matches with the period of uterine enlargement (Hafez and Tsutsumi, 1966).

In Algeria, a Local population of rabbits is well adapted to heat stress and rearing in local conditions, but with low productivity (Gacem and Bolet, 2005). A Synthetic line was created in 2003 by crossing females from local Algerian population with males from the French INRA 2666 line (Gacem and Bolet, 2005), in order to create a commercial line with a good adaptation to heat stress conditions and better production than rabbit of local Algerian population. The new commercial line has a higher growth rate (Belabbas *et al.,* 2019) and a larger litter size than the local Algerian population (Sid *et al.,* 2018). Also, Belabbas *et al.* (2016) determined lower ovulation rate and number of embryos at 72 h *post coitum* in Local population than in Synthetic line. Strategies to improve the productivity of these populations adapted to heat stress are necessary to maintain the biodiversity of these genetic resources. The aim of this work was to identify and compare the limiting litter size components in both Synthetic line and Local population.

MATERIAL AND METHODS

The present study was carried out at the experimental rabbit farm of the University Blida 1 (Algeria). All experimental procedures involving animals were approved by the Scientific Committee of the Institute of Veterinary Sciences, University Blida 1.

Animals

A total of 32 females from Synthetic line and 34 females from Local population were used in this study. The Local population was generated from breeding stock received from different Algerian regions in 1988. The animals were divided into groups according to their origins, kept in closed groups and mated following a rotary intersection plan among groups. More details of this population can be found in Zerrouki *et al.* (2014). The Synthetic line, known as ITELV2006, was created as a part of an agreement to transfer biological material for experimental purposes between the Institute of Animal Breeding (ITELV, Algeria) and INRA France. The F1 was obtained by inseminating does of Local population with semen of bucks from INRA2666, which is itself a synthetic strain (Brun and Baselga, 2005). The breeding programme was previously described by Gacem and Bolet (2005). After four generations of homogenisation, the ITELV2006 was selected for live litter size at birth and body weight at 75 d during 3 generations (Bolet *et al.,* 2012). Subsequently, the line has been maintained in discrete generations without selection and avoiding inbreeding (Ezzeroug *et al.,* 2020).

The females were housed individually in flat deck cages. They were fed *ad libitum* a standard commercial pelleted diet and water was available *ad libitum* as well from nipple drinkers. The animals were submitted to a constant photoperiod of 16L:8D light cycle during the whole experiment period.

Experimental design

The females were first mated at 18 wk of age and at 10 d after parturition. Females that did not accept males were mated again 7 d afterwards. Pregnancy testing was carried out by abdominal palpation on day 10 after mating.

A laparoscopy was performed on all does at day 12 of 3rd gestation following the surgical technique described by Santacreu *et al.* (1990). The number of *corpora lutea* in both ovaries and implanted embryos in both uterine horns were counted. Five days before birth, the nest boxes were cleaned, disinfected and put in place, containing wood chips to allow the female to build her nest. Litters were reared by their dams up to weaning (30 d of age).

Traits

The following variables were measured: body weight of females at mating (WM), ovulation rate (OR, measured by counting the number of *corpora lutea* in both ovaries), number of total implanted embryos (IE, estimated as the number of implantation sites), number of alive embryos (AE, estimated as the number of normal uterine swellings),

number of resorbed embryos (RE, estimated as the number of small uterine swellings with reduced vascular supply), number of total newborn at third parity (NTB), number born alive (NBA), percentage of mortality at birth (M, measured as [NTB-NBA]/NTB×100), embryonic survival (ES, estimated as [AE+RE]/OR×100), foetal survival (FS, estimated as AE/NTB×100), prenatal survival (PS, estimated as NTB/OR×100).

Statistical analyses

The former traits were analysed using following model: $y_{ij}=\mu+L_i+e_{ij}$, where μ is the general mean, L_i is the line effect (with 2 levels: Synthetic line and Local population) and e_{ij} is the residual effect. Number of total implanted embryos was analysed with ovulation rate as covariate. Number of live embryos, total newborn and born alive were analysed with ovulation rate and number of total implanted embryos as covariates. The GLM procedure of SAS was used for these analyses (SAS Institute, 2019).

The correlations between all recorded traits were calculated separately in each group. The CORR procedure of SAS was used for these analyses. The results are shown as least square means±standard error (LSM±SE). A principal component analysis was performed using PRINCOMP procedure of SAS (SAS Institute, 2019). This technique reduces the whole set of n correlated variables to z uncorrelated linear functions of the original measurements. The 1st principal component is the linear combinations of all variables showing the maximum variation among the samples. The 2nd, 3rd and further components are similarly linear combinations representing the next highest variation, irrespective of those represented by previous components (García-Tomás *et al.*, 2009).

RESULTS

Both lines showed similar body weight at mating (Table 1). Ovulation rate and number of total implanted embryos were 24% and 22% higher in Synthetic line than in Local population, respectively (*P*<0.0001), but both lines showed similar total implanted embryos when ovulation rate was included as covariate.

Synthetic line presented a higher number of AE (9.25 embryos) than Local population (7.52 embryos; *P*<0.0001, respectively). This difference disappeared when the ovulation rate or number of total implanted embryos were added

Table 1: Components of litter size in rabbit of Synthetic line and Local population (least square means±standard error).

Traits	Synthetic line (n=32)	Local population (n=34)	*P*-value
Weight at mating (g)	2983.15±33.43	2918.73±32.43	0.1715
Ovulation rate (ovum)	11.03±0.23	8.41±0.23	<.0001
Implanted embryos	10.00±0.25	7.85±0.25	<.0001
Implanted embryos$_{OR}$	8.92±0.22	8.87±0.22	0.8839
Alive embryos	9.25±0.29	7.52±0.28	<.0001
Alive embryos$_{OR}$	8.40±0.31	8.32±0.29	0.8791
Alive embryos$_{IE}$	8.40±0.23	8.32±0.22	0.8394
Resorbed embryos	0.75±0.21	0.32±0.21	0.1645
Embryo survival (%)	90.98±2.02	93.41±1.96	0.3924
Fetal survival (%)	93.31±2.01	95.80±1.95	0.3781
Prenatal survival (%)	88.44±2.86	81.44±2.78	0.0846
Total born (kits)	8.90±0.31	7.44±0.30	0.0016
Total bornOR (kits)	8.08±0.35	8.21±0.33	0.8257
Total bornIE (kits)	8.09±0.28	8.20±0.27	0.8202
Born alive (kits)	8.09±0.25	7.05±0.24	0.0043
Born aliveOR (kits)	7.64±0.29	7.47±0.28	0.7206
Born aliveIE (kits)	7.64±0.25	7.47±0.24	0.6717
Mortality (%)	7.51±1.77	4.21±1.72	0.1870

OR: The model included ovulation rate as covariate; IE: the model included implanted embryos as covariate.

to the model as covariates. No difference was found between groups for number of resorbed embryos or embryonic, foetal and prenatal survival.

Total newborn and number of kits born alive were higher in Synthetic line (8.90 and 8.09 kits, respectively) than in the Local population (7.44 and 7.05 kits, respectively; $P<0.01$). However, this difference did not remain significant when both were corrected by ovulation rate or number of total implanted embryos. Mortality at birth was similar between groups.

The phenotypic correlations between litter size and its components in each line are presented in Table 2. To facilitate interpretation of the correlations, a principal component analysis was performed. The first four principal components explained more than 90% of total variation in both lines (43, 25, 13 and 10%, respectively; data not shown in Figures). The first and the second principal component for the Synthetic line and Local population are shown in Figure 1A and 1B, respectively. The predominant variables defining the first principal component were total newborn, number of kits born alive and live embryos. They were far from the origin and close to the axis. High and positive correlations were found between them ranged from 0.91 to 0.65 in Synthetic line and from 0.97 to 0.82 in Local population (Table 2).

Resorbed embryos and FS were located near the second principal component but far from each other (Figure 2A, Synthetic line; Figure 2B, Local population). The correlation between them was close to −1 in both lines (Table 2). Resorbed embryos also showed negative correlation with prenatal survival (−0.70 and −0.54), total newborn (−0.54 and −0.47) and NBA (−0.48 and −0.43) for Synthetic line and Local population, respectively. While FS showed positive correlation with prenatal survival (0.71 and 0.55), total newborn (0.57 and 0.50) and NBA (0.50 and 0.47) for Synthetic line and Local population, respectively. Note also that the correlation is low and positive between total implanted embryos and resorbed embryos (0.42) and negative with FS (−0.39) in Synthetic line, but they were close to zero in Local population.

Ovulation rate and ES were located in the third principal component. Both traits showed null correlation. Ovulation rate was positive moderately correlated with total newborn in Local population (0.58), but this correlation was lower in the Synthetic line (0.38, Table 2).

The body weight of females is the predominant variable defining the fourth principal component (data not shown in Figures), and it was uncorrelated with all measured traits and for both lines. showed a positive and high correlation with prenatal survival (0.76) and a moderate correlation with total newborn (0.55) and born alive (0.55) in Local population. However, ES had a positive but low correlation with prenatal survival in Synthetic line (0.34), and no correlation was found with total newborn. Total newborn exhibited a moderate positive correlation with FS and high positive correlation with prenatal survival in both groups. Mortality at birth was moderately and positive correlated with

Table 2: Phenotypic correlations for reproductive traits in rabbit of Synthetic line (above the diagonal) and Local population (below the diagonal).

	WM	OR	IE	AE	RE	ES	FS	PS	NTB	NBA	M
WM		−0.01	−0.03	0.15	−0.20	−0.01	0.19	0.11	0.10	0.11	0.02
OR	−0.11		0.76***	0.46**	0.26	−0.23	−0.22	−0.28	0.38*	0.25	0.30
IE	−0.11	0.66***		0.53**	0.42*	0.44*	−0.39*	−0.05	0.43*	0.20	0.43*
AE	−0.14	0.61***	0.88***		−0.53**	0.18	0.55***	0.60***	0.91***	0.65***	0.66***
RE	0.10	−0.06	−0.01	−0.47**		0.25	−0.99***	−0.70***	−0.54***	−0.48**	−0.28
ES	−0.06	−0.04	0.72***	0.61***	0.04		−0.26	0.34*	0.13	0.01	0.20
FS	−0.11	0.10	0.04	0.49**	−0.99***	−0.03		0.71***	0.57***	0.50**	0.30
PS	−0.12	0.01	0.57***	0.76***	−0.54***	0.76***	0.55***		0.76***	0.62***	0.45**
NTB	−0.16	0.58***	0.85***	0.97***	−0.47**	0.59***	0.50**	0.81***		0.76***	0.65***
NBA	−0.23	0.42*	0.70***	0.82***	−0.43**	0.55***	0.47**	0.77***	0.86***		0.02
M	0.05	0.45**	0.52**	0.56***	−0.22	0.26	0.22	0.33*	0.55***	0.06	

*$P<0.05$; **$P<0.01$; ***$P<0.001$. WM: weight of the females at mating, OR: ovulation rate. IE: implanted embryos. AE: alive embryos. RE: resorbed embryos. ES: embryo survival. FS: fetal survival. PS: prenatal survival. NTB: total born. NBA: born alive. M: mortality.
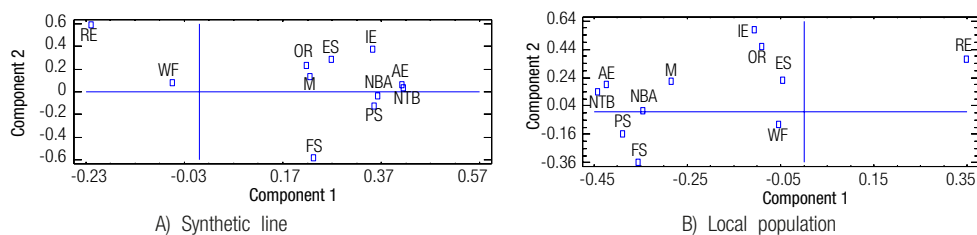
Figure 1: Projection of the traits in the plane defined by the 1st and 2nd principal component. WM: weight of the females at mating, OR: ovulation rate. IE: implanted embryos. AE: alive embryos. RE: resorbed embryos. ES: embryo survival. FS: fetal survival. PS: prenatal survival. NTB: number of total born. NBA: born alive. M: mortality.

the number of implanted and alive embryos in both groups. In the Local population, mortality had a moderate positive correlation with ovulation rate (0.45; $P<0.001$), but no correlation was found in the Synthetic line.

DISCUSSION

Litter size components

The body weight of the females was similar between both groups and slightly lower than that reported by Belabbas *et al.* (2016) and other maternal lines (Calle *et al.*, 2017). The improvement in litter size of Synthetic line could be related to a modification in the litter size components traits. Synthetic line showed a similar ovulation rate (11.03 *corpora lutea*) than those reported in other maternal lines (Ragab *et al.*, 2014 in the Spanish lines; Salvetti *et al.*, 2007 in French lines), although Synthetic line had a higher ovulation rate than Local population. Similar results were obtained by Belabbas *et al.* (2016). It is well known that folliculogenesis and oocyte maturation require the action of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Hulot *et al.*, 1985). Muelas *et al.* (2008) indicated that does with low ovulation rate showed lower LH and FSH levels than does with high ovulation rate. Therefore, the difference in ovulation rate between lines could be due to different patterns of LH and FSH secretion.

In our experiment, the number of implanted embryos in the Synthetic line was similar to those reported in French maternal lines (Brun *et al.*, 2006), but lower than those of the Spanish maternal lines (García and Baselga, 2002; Laborda *et al.*, 2012; Santacreu *et al.*, 2005). Synthetic line showed a higher number of implanted embryos than Local population, as a consequence of a higher ovulation rate.

Prenatal survival can be divided into embryonic survival and foetal survival (Mocé *et al.*, 2010). The ES in both groups was higher than those obtained in other studies (Santacreu *et al.*, 1992; Mocé *et al.*, 2005). Higher ES in
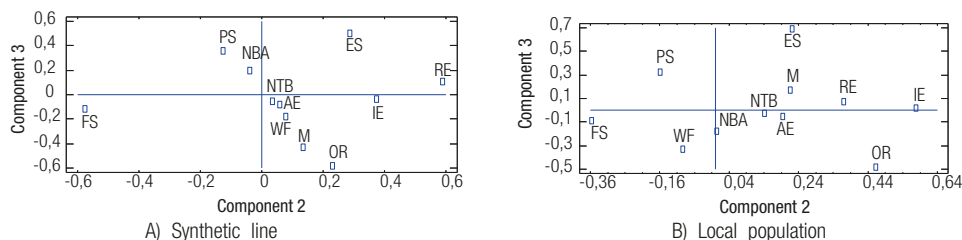
Figure 2: Projection of the traits in the plane defined by the 2nd and 3rd principal component. WM: weight of the females at mating, OR: ovulation rate. IE: implanted embryos. AE: alive embryos. RE: resorbed embryos. ES: embryo survival. FS: fetal survival. PS: prenatal survival. NTB: number of total born. NBA: born alive. M: mortality.

our Synthetic line and local Algerian population would be related to higher fertilisation rate as reported by Belabbas *et al.* (2016), but also with a better quality of oocytes (Saacke *et al.,* 2000) and more favourable oviductal and uterine environmental to embryo development (Pope *et al.,* 1990; Beier, 2000; Argente *et al.,* 2008). However, additional studies will be needed to confirm if the Synthetic line and the local Algerian population have a better quality of oocytes and uterine environmental than European maternal lines.

FS was similar between Synthetic line (93.31%) and Local population (95.80%), and higher than those estimated in other maternal lines (Mocé *et al.,* 2005; Badawy *et al.,* 2016). Higher FS in Synthetic line and Local population could mainly be related to better development of placenta (Argente *et al.,* 2003). In rabbit, after implantation, the mortality is caused by the competition between placentas, in which their development is related to the availability of uterine space (Argente *et al.,* 2006) and vascular supply (Argente *et al.,* 2003; 2008; Mocé *et al.,* 2004). In prolific species, a decrease in availability of uterine space per foetus was associated with reduction of prenatal survival (Argente *et al.,* 2008 in rabbits; Chen and Dziuk, 1993 in pigs).

In agreement with different studies, we also found higher total newborn (+1.46 kits) in the Synthetic line than in Local population (Belabbas *et al.,* 2016; Sid *et al.,* 2018). In order to compare the females at the same ovulation rate or number of implanted embryos, we have included these traits as covariables in the model. The difference for total newborn disappeared when it was corrected by ovulation rate. These results indicated that the difference between groups is mainly related to their difference in ovulation rate.

Our findings can help to better understand the critical points that condition litter size and propose strategies for their improvement in prolific species. Thus, increasing the ovulation rate is not accompanied by an increase in litter size at delivery, due to an increment in the number of immature oocytes (Laborda *et al.,* 2012 in rabbits; Rosendo *et al.,* 2007 in pig), an increase in available uterine space being more efficient.

Correlations

The differences in litter size between the lines could be explained by the different correlations that we have observed between them. Thus, total newborn had a moderate positive correlation with ovulation rate in Local population. These results agree with those reported by Blasco *et al.* (1993b) in rabbits and Ruíz-Flores and Johnson (2001) in pigs. However, the correlation between ovulation rate and total newborn was low in Synthetic line. Our result agrees with the ones obtained by Laborda *et al.* (2012), who reported that increasing ovulation rate did not increase litter size in the same way related to greater prenatal mortality. The same observations were reported in other species (pig, Rosendo *et al.* 2007; mouse, Land and Falconer, 1969). Laborda *et al.* (2011) suggested that in the females with higher ovulation rate, implantation differences may cause greater prenatal mortality in overcrowded uterine horns, and thus contribute to the lacking correlated response in litter size.

Moreover, total newborn and embryonic survival were correlated in the Local population, in agreement with the results of Laborda *et al.* (2012). However, they were uncorrelated in the Synthetic line. Lack of correlation in the Synthetic line may be related to their higher ovulation rate, which increased mortality. Indeed, in females with high ovulation rate, an increasing of ovulatory timing is observed, and later ovulating follicles may be fertilised later, inducing more heterogeneous embryo development (Torrès *et al.,* 1987; Xie *et al.,* 1990). On the other hand, Koenig *et al.* (1986) reported in pig with higher ovulation rate an increase in the proportion of immature ova, which could increase mortality.

As far as we know, this is the first time that the correlation between resorbed embryos and components of litter size has been estimated. Both lines showed similar correlation with all the traits, except for implanted embryos. Synthetic line showed negative correlation between resorbed embryos and implanted embryos. This could be due to the competition for available uterine space and vascularisation (Argente *et al.,* 2003; 2006). Nevertheless, there were no correlation in Local population.

In summary, litter size of the Local population is limited by the low ovulation rate. The process of formation of the Synthetic line, crossing the Local population with the line INRA2666, together with its subsequent genetic programme, increased the litter size due to an increase in the ovulation rate.

CONCLUSION

Synthetic line had a higher number of kits born than Local population, as a consequence of the higher number of released and fertilised oocytes that successfully reached implantation. However, the higher uterine crowding in Synthetic line seems to limit survival of foetuses that reached term of gestation, while ovulation rate is the principal limiting factor of litter size in Local population.

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