

OVULATION RATE AND EARLY EMBRYONIC SURVIVAL RATE IN FEMALE RABBITS OF A SYNTHETIC LINE AND A LOCAL ALGERIAN POPULATION

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Abstract: A higher litter size at birth has been reported in female rabbits from a Synthetic line than in those of the Local Algerian population. The aim of this work was to analyse whether this difference in litter size was due to a higher ovulation rate and/or embryonic survival rate in Synthetic line than in Local Algerian population. In total, 24 multiparous female rabbits from Synthetic line and 23 from Local population were used in this experiment. Litter size at birth was recorded up to the first 3 parties. Litter size was 20% higher in Synthetic line than Local population. At their 4th gestation, the females were euthanized at 72 h post coitum. Synthetic line females had 50% more ova and embryos than those of Local population (+4.42 ova and +3.92 embryos, respectively). Synthetic line displayed a lower percentage of normal embryos and a larger number of unfertilized oocytes than Local population (-2.81% and +0.64 oocytes, respectively), but differences were not relevant. Synthetic line showed a lesser embryonic stage of development at 72 h post coitum, showing a higher percentage of early morulae (31.50 vs. 8.50%) and a lower percentage of compact morulae (51.45 vs. 78.65%) than Local population. No relevant difference was found for early embryonic survival rate between Synthetic line and Local population. In conclusion, the difference in litter size was mainly due to a higher ovulation rate in the Synthetic line, allowing more embryos to develop in this line.

Key Words: embryonic development, fertilization, litter size, ovulation rate, Synthetic line, rabbit.

INTRODUCTION

A local rabbit breed has long been used for family production in Algeria (Gacem and Lebas, 2000). This population is well adapted to the local conditions and particularly suitable for production in hot conditions. However, its adult weight and prolificacy are too low for it to be used under intensive rabbit production systems (Zerrouki *et al.*, 2007). Attempts to introduce commercial rabbit lines in Algeria have not had the expected success (Gacem *et al.*, 2008). Recently, new strategies have been proposed in the rabbit genetic improvement programmes in hot climate countries like Egypt and Saudi Arabia (Youssef *et al.*, 2008). In this regard, new rabbit lines were developed by cross-breeding between local breeds and foreign breeding lines (Brun and Baselga, 2004). These new lines are expected to display improved production traits while remaining adapted to heat stress conditions (Gacem *et al.*, 2008). In order to develop rabbit meat production in Algeria, a Synthetic rabbit line was created in 2003 by crossing females from Local population with males from the French INRA 2666 strain at Technical Institute for Animal Production ITELV (Gacem *et al.*, 2008). In a recent study, the new Synthetic line showed 20% more litter size and higher growth rate weight than the Local population (Zerrouki *et al.*, 2014).

Litter size is a complex physiological trait in prolific species, affected by several component traits that are expressed sequentially, such as ovulation, fertilization, embryonic survival and foetal survival. Fertilization rate is usually high,

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exceeding 90 to 95% (Peiró *et al.*, 2014, in rabbits; Geisert and Schmitt, 2002, in pigs; Wilmot *et al.*, 1986, in mice), and is therefore not considered a limiting factor of litter size in prolific species. However, approximately 30 to 40% of the ova do not result in fetuses to term and one-third to one-half of these losses occur before implantation, when embryos are in the oviduct (Santacreu *et al.*, 2005, in rabbits; Ford *et al.*, 2002 in pigs; Holt *et al.*, 2004, in mice). Most of these embryonic losses are characterized by asynchronous development of the embryo with the uterine status, as reviewed by Geisert and Schmitt (2002). Ovulation in rabbit is induced by the stimuli associated with coitus. Rabbit is therefore the ideal model for such studies. Until now, it is unknown whether the difference in litter size between Synthetic line and Local population is determined in the early stages of gestation. The aim of the present study was to analyse ovulation rate, fertilization rate, early embryonic development and survival in female rabbits from the Synthetic line and the Local Algerian population.

MATERIAL AND METHODS

This experiment was conducted at the Experimental Station of the University Saad Dahleb, Blida (Algeria). All experimental procedures involving animals were approved by the Research Ethics Committee of the Department of Veterinary Sciences, University of Blida, Algeria.

Experimental animals

Rabbit does from Local Algerian population (n=23) and Synthetic line (n=24) were used in this experiment. The females were obtained from the Livestock Technical Institute (ITELV Bab Ali, Algeria). The Local Algerian population was generated from breeding stock received from different Algerian counties (Ain el Benian, Ain M'Lila, Sidi Belabes, Blida, Constantine, Djelfa, Ksar Chelala, Tiaret and Tizi Ouzou) 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. The rotation began in 1988 and closed in 2005. The Synthetic line was created as part of an agreement to transfer biological material for experimental purposes between INRA (France) and ITELV (Algeria). The first generation of rabbits was obtained by inseminating females from Local population with semen from males of the INRA 2666 strain in 2003. This latter strain was itself an experimental synthetic strain, resulting from crossbreeding between the INRA 2066 strain and V strain from the Polytechnic University of Valencia, in Spain (Brun and Baselga, 2004). The next generation of Synthetic line was obtained using the rotated plan between families from the same generation to minimize the increase in consanguinity. Synthetic line was constituted after 5 generations of intermingling (Gacem *et al.*, 2008).

The females were housed in flat deck cages and kept under controlled photoperiod with 16 hours of light: 8 hours of dark. Natural mating was performed with males from the same generation as the females. Does were fed *ad libitum* with a commercial pellet diet containing less than 16.5% crude protein and 15.5% crude fibre. Females were first mated at 18 wk of age and at 10 d after parturition thereafter. At their 4th gestation, non-lactating females were weighed and mated. At 72 h post coitum, all females were euthanized by intravenous administration of sodium thiopental in a dose of 50 mg/kg of body weight (Thiobarbital, B. Braun Medical S.A., Barcelona, Spain). Immediately after, the entire reproductive tract was removed and the ovaries were weighed. The number of *corpora hemorrhagica*, i.e. follicles with ovulation stigmas, and haemorrhagic follicles, i.e., follicles without ovulation stigmas with a presence of blood in the antral cavity, were recorded in both ovaries. The oviduct and the first one-third of the uterine horn were flushed with 5 mL of 150 mM ammonium bicarbonate solution at room temperature to recover embryos and oocytes.

Traits analysed

The traits measured were litter size up to the first 3 parities, body weight of does at 4th mating (g), ovulation rate estimated as number of *corpora hemorrhagica*, average weight of both ovaries (g), number of haemorrhagic follicles, total number of embryos recovered that included number of normal and abnormal embryos recovered, and number of unfertilized oocytes recovered. The classification of the recovered embryos was carried out by 2 operators according to the methodology followed by Peiró *et al.* (2007) using a binocular stereoscopic microscope. The embryos were classified as normal, when they presented homogenous cellular mass and intact zona pellucida. In contrast, degenerated embryos, embryos with a granular cytoplasm and irregular shape or embryos with a stage of development different from that expected, were classified as abnormal. The normal embryos were classified as

early morulae, compact morulae and early blastocysts. The following traits were calculated: percentage of normal embryos recovered [(normal embryos recovered/total number of embryos recovered) \times 100], percentage of abnormal embryos recovered [(abnormal embryos recovered/total number of embryos recovered) \times 100], fertilization rate [(total number of embryos recovered/(total number of embryos recovered+unfertilized oocytes recovered)) \times 100], embryonic survival rate [(normal embryos recovered/ovulation rate) \times 100], percentage of early morulae [(number of early morulae/normal embryos recovered) \times 100], percentage of compacted morulae [(number of compacted morulae/normal embryos recovered) \times 100], and percentage of early blastocysts [(number of early blastocysts/normal embryos recovered) \times 100].

Statistical analyses

All analyses were performed using Bayesian methodology. Litter size was analysed with the following model: $y_{ijklmn} = \mu + L_i + P_j + LS_k + S_l + p_{ijklm} + e_{ijklmn}$, where μ is general mean, L_i is the line effect (2 levels: Synthetic line and Local Algerian population), P_j is the effect of parity (3 levels: 1st, 2nd and 3rd parity), LS_k is the effect of lactation status (3 levels: nulliparous does, lactating and non-lactating does at 2nd or 3rd gestation), S_l is the season effect (3 levels), p_{ijklm} is the permanent effect of female, and e_{ijklmn} is the error. The model for body weight of does, ovulation rate, ovary weight, number of haemorrhagic follicles, embryos recovered, percentage of normal and abnormal embryos, unfertilized oocytes, fertilization rate, embryonic survival rate and percentage of early morulae, compacted morulae and early blastocysts included only the line effect. Moreover, ovulation rate and ovary weight were analysed with covariate body weight of does, and ovary weight, number of haemorrhagic follicles and number of embryos recovered were analysed with covariate ovulation rate. Bounded uniform priors were used for all unknowns with the exception of the permanent effect of female, which was considered normally distributed with mean=0 and variance= $l\sigma_p^2$, where l is an identity matrix, and σ_p^2 is the *a priori* variance of the permanent effect. Residuals were *a priori* normally distributed with mean=0 and variance= $l\sigma_e^2$. The priors for the variances were also bounded uniform. Marginal posterior distributions for all unknowns were estimated using Gibbs sampling. The Rabbit program developed by the Institute for Animal Science and Technology (Valencia, Spain) was used for all procedures. Chains of 60000 samples with a burn-in period of 10000 were used. One sample out of 10 was saved to avoid high correlations between consecutive samples. Convergence was tested using Geweke's Z criterion (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992). These parameters were obtained from the marginal posterior distributions of the differences between lines: the median of the marginal posterior distribution of the difference between Synthetic line and Local population (D_{S-L}), the highest posterior density region at 95% (HPD_{95%}), the probability of lines being different (probability of the difference between Synthetic line and Local population being greater than 0 when this difference is greater than 0 or the probability of the difference being less than 0 when this difference is less than 0; P), the guaranteed value of a difference (k) with a probability of 80% (limit of the interval [k , $+\infty$) when the difference is greater than 0 or the limit of the interval ($-\infty$, k] when the difference is less than 0), the probability of relevance (probability of the difference being greater than a relevant value; P_R). A relevant value (R) is the minimum difference which could be detected in experimental designs and having biological or economic significance. We considered one-third of the phenotypic deviation standard of each trait as a relevant value for the differences between Synthetic line and Local Algerian population, as in other studies (Blasco, 2005).

RESULTS

Descriptive statistics for reproductive traits in Synthetic line and Local population are shown in Table 1. Coefficients of variation (CV) were calculated to determine the variability of each trait within Synthetic line and Local population. The number of abnormal embryos, unfertilized oocytes, early morulae, compacted morulae and early blastocysts presented higher CV than the rest of reproductive traits in both Synthetic line and Local population. Synthetic line displayed higher CV than Local population only for body weight of does, number of haemorrhagic follicles and normal embryos. The remaining traits showed higher CV in Local population than in Synthetic line, i.e. ovulation rate, total number of embryos recovered, abnormal embryos, unfertilized oocytes, fertilization rate, embryonic survival and development, indicating considerable differences in variability of traits recorded between groups.

Table 1: Means, standard deviations (SD) and coefficients of variation (CV) for studied traits in Synthetic line and Local population.

	Synthetic line (n=24)			Local population (n=23)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Litter size ^a (kits)	8.29	1.85	22.3	6.99	1.53	21.9
Body weight (g)	3417	407	11.9	3279	257	7.8
Ovulation rate (ovum)	13.29	1.90	14.3	8.74	2.47	28.2
Ovary weight (g)	0.53	0.16	30.5	0.38	0.11	28.9
Haemorrhagic follicles	1.62	1.41	86.7	0.44	0.59	135.6
Total embryos recovered (embryos)	11.62	2.14	18.4	7.69	1.87	24.3
Normal embryos (%)	95.31	6.06	6.4	98.11	4.26	4.3
Abnormal embryos (%)	4.69	6.06	129.3	1.89	4.26	226.3
Unfertilized oocytes recovered	1.50	1.28	85.7	0.87	1.50	172.6
Fertilization rate (%)	88.63	8.89	10.1	91.18	15.01	16.5
Embryonic survival (%)	83.69	12.89	15.4	87.84	15.80	18.1
Early morulae (%)	31.50	29.49	93.6	8.50	15.86	186.5
Compacted morulae (%)	51.45	23.30	45.3	78.65	24.44	31.1
Blastocysts (%)	17.05	16.68	97.8	12.84	23.21	180.7

^a: Synthetic line had 72 data and Local population had 69 data.

Features of the estimated marginal posterior distributions of the differences between Synthetic line and Local population (D_{S-L}) for each trait are presented in Table 2. For all the traits analysed, Monte Carlo standard errors were small and lack of convergences was not detected by the Geweke test. For litter size estimated up to 3 parities, Synthetic line showed a higher value than Local population ($D_{S-L}=1.32$ kits, $P(D_{S-L}>0)=1$) and guaranteed value of difference with a probability of 80% ($k_{80\%}$) was very high (1 kits). In practice, we were interested not only in determining whether Synthetic line and Local population were different but whether this difference was relevant. A difference can be relevant or important from either an economic or a biological point of view. We considered one third of the standard deviation of the trait as a relevant difference (R) between Synthetic line and Local population. For example, R would be 0.7 kits for litter size, which means that litter size in Synthetic line was 20% greater than in Local population. The probability of the difference being greater than R was high ($P_R=0.93$), indicating a strong evidence for showing that difference was relevant. Body weight was higher in Synthetic line than in Local population ($D_{S-L}=140$ g, $P(D_{S-L}>0)=0.91$), but P_R did not allow us to infer that the difference was relevant (0.65). Synthetic line females released 50% more ova than those of the Local population ($D_{S-L}=4.42$ ova, $P(D_{S-L}>0)=1$). The probability of the difference being higher than R was equal to 1. Moreover, the difference for ovulation rate was not much reduced when using body weight of doe as covariate ($D_{S-L}=4.23$ ova and $P_R=1$). The ovary of females from Synthetic line was 40% heavier than in Local population ($D_{S-L}=0.15$ g, $P(D_{S-L}>0)=1$), and this difference was relevant ($P_R=0.99$). The difference for ovary weight remained relevant after correction by body weight ($D_{S-L}=0.12$ g, $P_R=0.98$). However, when ovary weight was corrected by ovulation rate, the difference was no longer relevant ($P_R=0.52$). The ovary had a larger number of haemorrhagic follicles in Synthetic line than in Local population ($D_{S-L}=1.19$, $P(D_{S-L}>0)=1$). The difference after correction by ovulation rate remained relevant ($D_{S-L}=1.40$, $P_R=0.96$). Synthetic line showed 50% more embryos at 72 h of gestation than Local population ($D_{S-L}=3.92$ embryos, $P_R=1$). The difference between Synthetic line and Local population decreased to 0.78 embryos when this trait was corrected by ovulation rate, and evidence was low for inferring that difference was relevant ($P_R=0.55$). Synthetic line presented a lower percentage of normal embryos ($D_{S-L}=-2.81\%$, $P(D_{S-L}<0)=0.96$) and a larger number of unfertilized oocytes than Local population ($D_{S-L}=0.64$ oocytes, $P(D_{S-L}>0)=0.93$), but the differences were not relevant ($P_R=0.70$ and 0.63, respectively). Fertilization rate and embryonic survival were lower in Synthetic line than in Local population. However, the differences were not relevant ($P_R=0.26$ and 0.39, respectively). Synthetic line females presented a less advanced embryonic stage of development. Indeed, Synthetic line showed higher percentages of early morulae ($D_{S-L}=23.14\%$, $P(D_{S-L}>0)=1$) and lower percentages of compacted morulae ($D_{S-L}=-27.36\%$, $P(D_{S-L}<0)=1$) than Local population. Considering a relevant value of 8% for percentage of early and compacted morulae, the probability of these differences being

Table 2: Features of the estimated marginal posterior distributions of the differences between Synthetic line and Local population for litter size and traits measured at 72 h post coitum.

	D_{S-L}	P -value	HPD _{95%}	$k_{80\%}$	R	P_R
Litter size ^a (kits)	1.32	1	0.59, 1.99	1.03	0.7	0.93
Body weight (g)	140	0.91	-66, 355	48	100	0.65
Ovulation rate (ovum)	4.42	1	3.22, 5.65	3.92	0.7	1
Ovulation rate _{BW} (ovum)	4.23	1	2.99, 5.35	3.72	0.7	1
Ovary weight (g)	0.15	1	0.07, 0.23	0.11	0.05	0.99
Ovary weight _{BW} (g)	0.12	1	0.05, 0.18	0.09	0.05	0.98
Ovary weight _{OR} (g)	0.05	0.82	-0.06, 0.16	0.01	0.05	0.52
Haemorrhagic follicles	1.19	1	0.55, 1.84	0.92	0.5	0.98
Haemorrhagic follicles _{OR}	1.40	1	0.42, 2.39	0.98	0.5	0.96
Total embryos recovered (embryos)	3.92	1	2.85, 5.18	3.42	0.7	1
Total embryos recovered _{OR}	0.78	0.89	-0.50, 1.98	0.27	0.7	0.55
Normal embryos (%)	-2.81	0.96	-5.91, 0.36	-1.50	-2	0.70
Abnormal embryos (%)	2.80	0.96	-0.44, 5.82	1.46	2	0.70
Unfertilized oocytes recovered	0.64	0.93	-0.14, 53	0.28	0.5	0.63
Fertilization rate (%)	-2.47	0.71	-9.95, 4.77	0.59	-5	0.26
Embryonic survival (%)	-3.89	0.81	-12.40, 4.62	-0.22	-5	0.39
Early morulae (%)	23.14	1	8.83, 36.97	16.82	8	0.98
Compacted morulae (%)	-27.36	1	-40.73, -11.48	-21.17	-8	0.99
Blastocysts (%)	4.16	0.76	-7.75, 16.39	-0.87	8	0.26

^a: 72 data in Synthetic line and 69 data in Local line. Ovulation rate_{BW}: ovulation rate corrected by body weight of does. Ovary weight_{BW}: ovary weight corrected by body weight of doe. Ovary weight_{OR}: ovary weight corrected by ovulation rate. Haemorrhagic follicles_{OR}: total number of haemorrhagic follicles corrected by ovulation rate. Total embryos recovered_{OR}: total number of embryos recovered corrected by ovulation rate. D_{S-L} : median of the marginal posterior distribution of difference. P : probability of the difference being >0 when D_{S-L} >0 and probability of the difference being <0 when D_{S-L} <0. HPD_{95%}: highest posterior density region at 95%. $k_{80\%}$: limit of the interval [k , +∞) when D_{S-L} >0 and (-∞, k] when D_{S-L} <0. R: relevant value defined as one-third of standard deviation of the trait. P_R : probability of relevance (probability of the difference being greater than R).

greater than R was high in both traits ($P_R=0.98$ and 0.99 , respectively). There was no relevant difference between Synthetic line and Local population for percentage of early blastocysts ($P_R=0.26$).

DISCUSSION

Producing meat rabbit under industrialized conditions implies that breeders should have highly specialized lines in terms of numerical productivity. However, this productivity could be compromised by heat stress in hot climate countries. Recently, breeding programmes have proposed the development of new meat rabbit lines in these countries by crossbreeding between local breeds and foreign synthetic lines (Brun and Baselga, 2004), which seems promising in terms of good production and adaptation under hot conditions (Youssef *et al.*, 2008). In this regard, a Synthetic rabbit line was created in Algeria by crossbreeding between a Local population and the INRA 2666 strain (Gacem *et al.*, 2008). After several generations of crossbreeding and homogenization, a difference of more than one kit at birth was found between Synthetic line and Local breed (Zerrouki *et al.*, 2014). In agreement with this result, we also found 20% greater litter size in Synthetic line than in Local population. However, it was unknown whether this difference is related to ovulation or early stage gestation during or after implantation. The present study was focused on the first stage of gestation, i.e. the first 72 h post coitum.

Ovulation rate in Synthetic line was high (13.29 ova) and similar to those in other maternal rabbit lines (Ragab *et al.*, 2014 in the Spanish lines; Salvetti *et al.*, 2007 in French lines). Synthetic line females released roughly 50% more ova than those of the Local population. Ovulation rate in Local line was within the range of those reported in previous studies (Belabbas *et al.*, 2011). Gonadotropic hormones LH and FSH have an important role in ovulation rate, allowing

or not the ovulation of all follicles at the time of mating (Hulot *et al.*, 1985). Therefore, difference in ovulation rate could be due to different LH and FSH secretory patterns between Synthetic line and Local population. Ovary weight in Synthetic line was 40% higher than in Local population, due to higher ovulation rate in this line. Moreover, Argente *et al.* (2003) reported a positive relationship between ovary weight and ovulation rate in rabbit. A larger number of haemorrhagic follicles was found in Synthetic line. García-Ximénez and Vicente (1992) also found that the presence of haemorrhagic follicles did not affect ovulation rate, but negatively affected fertilization and embryo survival. These finding would be in agreement with the lower fertilization rate and embryo survival found in Synthetic line than in Local population, although these differences were not relevant. A high number of haemorrhagic follicles may be due to an unbalanced hormonal profile or deficiencies in follicular development, affecting steroidogenic activity or LH receptors, an inadequate neuroendocrine reflex at hypothalamus-pituitary system as a consequence of oestrogen insensitivity or a low bioavailability of steroids (Vicente *et al.*, 2012).

Synthetic line presented 50% more embryos than Local population at 72 h of gestation. This difference disappeared when the total number of embryos recovered was corrected by ovulation rate. The percentage of normal embryos was high in both groups (95% in Synthetic line vs. 98% in Local population). These values were in the same range as those reported by Peiró *et al.* (2007) and García *et al.* (2010). However, comparing both groups, Synthetic line showed a lower percentage of normal embryos (95 vs. 98%) and a higher percentage of abnormal embryos (5 vs. 2%). It appears that an increasing ovulation rate is accompanied by an increase in number of abnormal embryos due to decreasing quality of oocytes, which is in agreement with other studies (Angel *et al.*, 2014; Peiró *et al.*, 2014). In fact, it has been shown that embryonic losses, number of oocytes and degenerated embryos are increased as ovulation rate rises (Angel *et al.*, 2014). Accordingly, Synthetic line displayed larger numbers of unfertilized oocytes than Local population, although the difference was not relevant. At 72 h of gestation, Synthetic line presented less advanced embryo development than Local population. This difference in early embryonic development could be associated with lower embryonic survival rates in Synthetic line than Local population. Moreover, embryo survival rate presented a high value in both Synthetic line and Local population, i.e. more than 85%. Early survival rate in this study was comparable to that reported by Argente *et al.* (2003) and García *et al.* (2010). Difference in embryonic development between lines and strains has been reported by several authors (Bolet and Theau-Clément, 1994; Peiró *et al.*, 2007). All these studies clearly show that it is possible to modify the embryonic stage of development in early gestation period by genetics, although it is not clear which mechanisms are involved. Difference in the embryonic stage of development could be principally related to timing of ovulation or oviductal and uterine fluid compositions (Hunter *et al.*, 2004). Torres *et al.* (1987) demonstrated that a high ovulation rate increases ovulatory timing and later ovulating follicles may be fertilized later, inducing more heterogeneous embryo development within the litter (Xie *et al.*, 1990). The oviduct synthesizes and secretes many proteins in many species including rabbit (García *et al.*, 2010), swine (Buhi and Alvarez, 2003), sheep and cattle (Nancarrow and Hill, 1995) which influence the gene expression of the developing embryos. Several proteins play an important role in embryo development and embryogenesis regulation (Insulin-like growth factor 1, Herrler *et al.*, 1998; oviductin, Buhi and Alvarez, 2003; TIMP metalloproteinase inhibitor 1, Hwang *et al.*, 2000; uteroglobin, Riffo *et al.*, 2007; leptin, Zerani *et al.*, 2005). The asynchrony associated with the advanced uterine environment could cause the death of less developed embryos (Wilde *et al.*, 1988; Xie *et al.*, 1990). Accordingly, a higher ovulation rate in Synthetic line would increase ovulation timing, and embryo development stage would consequently be reduced due to an increment in heterogeneity in embryonic development within the litter and asynchrony with the uterine environment.

CONCLUSION

In conclusion, the Synthetic line presented a higher ovulation rate and number of embryos recovered at 72 h post coitum than the Local population, which could explain the difference in litter size at birth between the 2 genotypes. However, neither fertilization rate nor embryo survival at 72 h post coitum were significantly modified by this cross-breeding. The embryo development was less advanced in the Synthetic line, which evidenced the possibility of its modification genetically, as previously demonstrated in many species by modification of oviduct and uterine environments.

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