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Additional Information

1 **Evaluation of foetal growth, litter size and reproductive performance in rabbit after 18**
2 **generations of selection for growth rate using cryopreserved embryos**

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8 **Abstract**

9 In livestock, adverse effects on reproductive performance, health traits and robustness
10 have been demonstrated in animals selected for high production and efficiency. Using embryo
11 cryopreservation and rederivation, we compared phenotypic traits between rabbit
12 populations separated for 18 generations under growth rate selection pressure (R18 vs R36).
13 To do so, embryos from the ancestral population (R18) and the most recent population (R36)
14 were vitrified in 2000 and 2015, respectively, and rederived and grown together in a
15 randomized controlled environment in 2015. To eliminate confounding maternal and embryo
16 handling effects, traits were measured in the second generation after rederivation (R20 and
17 R38 generations). Our study suggests that selection for growth rate has no adverse effect on
18 litter size components. Thus, in the R38 generation we observed a significant increase in
19 embryo implantation (7.2 ± 0.71 vs 5.1 ± 0.79) and litter size (7.1 ± 0.29 vs 6.5 ± 0.32). Besides, the
20 foetal sac area at day 12 of gestation (2.44 ± 0.070 vs 2.07 ± 0.071 mm², for R38 vs R20,
21 respectively), and foetal placenta area (136.7 ± 6.14 vs 116.0 ± 6.31 mm², for R38 vs R20,
22 respectively) and crown-rump length of the foetus (38.0 ± 0.68 vs 35.8 ± 0.68 mm, for R38 vs
23 R20, respectively) at day 19 of gestation were higher in the R38 generation. Altogether, these
24 results show that selection for growth rate does not adversely affect components of litter size,

25 foetal growth and reproductive performance. However, the extent to which foundation
26 criteria play a role in the high prenatal and perinatal mortality rate remains unclear in paternal
27 lines.

28 **Keyword:** foetal growth, reproductive performance, paternal line, rederived population,
29 rabbit.
30

31 **1. Introduction**

32 Rabbit lines specialized for growth rate or feed efficiency and litter size are a common
33 selection objective, as in other animal farms, due to their economic interest (Blasco et al.,
34 2018). Nevertheless, the relationship between growth and maternal traits and their correlated
35 responses, unclear or not, is always positive. Although maternal effects have long been
36 acknowledged as potentially important factors in artificial selection, their magnitude of
37 response remains unclear. In mammals, the role of maternal effects is especially complex due
38 to the fact that progeny experience two distinct maternal environments (prenatal uterine and
39 postnatal nursing) influenced by numerous factors, such as the number of foetuses or litter
40 size, parity, age, breed, heat stress and nutrition (Vuguin, 2007; Wolf et al., 2011).

41 The success of selection for high prolificacy in polytocous species is related with
42 negative consequences in survival, foetal growth and birth weight in rabbit (Vicente et al.,
43 1995; Argente et al., 2003 and 2008) and pigs (Damgaard et al., 2003; Foxcroft et al., 2006;
44 Wolf et al., 2008). Argente et al. (2003) found a reduction in placental and foetal development
45 in rabbits with each additional implanted embryo at 25 days of gestation. Moreover, higher
46 intrauterine crowding has been correlated with higher foetal mortality at 18 days of gestation
47 (Argente et al., 2008). In pigs, it has been shown that selection for increased litter size entails
48 the possibility of various degrees of intrauterine growth retardation associated with impaired
49 foetal and placental growth, which can result in lower birth weights (Town et al., 2004). Thus,

50 postnatal variation in growth performance variation may be pre-programmed during foetal
51 development in the uterus. Furthermore, it is likely that these pre-programmed limitations in
52 growth performance will only finally express themselves in the late grower and not at the early
53 finisher stage of production (Du et al., 2010).

54 Some studies suggest that long-term selection for growth rate results in physiological
55 and reproductive changes (Rauw et al., 1998). For example, mice selected for increased early
56 body weight gains showed a decreased response to superovulation and oestrous
57 synchronization, and when they were used as recipients, they produced pups that were
58 significantly larger with respect to body weight and tail length compared with litters gestated
59 in females non-selected or selected divergently (Ernst et al., 2000). These authors suggested
60 that retarded pre-pubescent reproductive development results in reproductive uterine horns
61 more efficient for sustaining pregnancy, foetal development and growth. Thus, cattle breeds
62 with postnatal different growth impetus and muscularity show differences in foetal
63 development, especially in muscle tissue deposition and development (Mao et al., 2008). In
64 rabbit paternal line, the reproductive performance traits are not a selection factor, as
65 reproductive traits like parity and litter size had a negligible effect on growth rate (Piles and
66 Blasco, 2003), but is unclear whether a selection programme for growth rate affects
67 reproductive traits in rabbits. Low or null correlations between litter size at birth and postnatal
68 growth rate have been observed in rabbit (García and Baselga, 2002; Mocé and Santacreu,
69 2010; Drouilhet et al., 2013; Mínguez et al., 2016; Peiró et al., 2019) and an uncertain pattern
70 was found in pigs (Damgaard et al., 2003; Wolf et al., 2008; Zhang et al., 2016). Meanwhile, a
71 positive genetic correlation was estimated between postnatal growth rate and ovulation rate
72 in pig and rabbit (Bidanel et al., 1996; Ruiz-Flores and Johnson, 2001; Drouilhet et al., 2013;
73 Peiró et al., 2019). In rabbit, reproductive differences have been described between maternal

74 and paternal lines. Females from the paternal line showed widespread failures, such as altered
75 LH and steroidogenic patterns, low response to ovulation frequency and high losses in
76 implantation during foetal development and birth (Vicente et al.; 2012, Naturil-Alfonso et al.,
77 2015 and 2016). Paternal line males did not present normal sexual behaviour, observing low
78 libido, lower sperm production (Pascual et al., 2004; Rosell and De La Fuente, 2009) and
79 changes in seminal and sperm proteome (Lavara et al., 2011; Casares-Crespo et al.; 2018,
80 Juárez et al., 2020).

81 The aim of this study was to evaluate whether the selection programme for daily gain
82 in fattening period has changed foetal growth and prenatal survival, using two rederived
83 populations separated for 18 generations. To this end, embryos from the population R18 and
84 the most recent population (R36) were vitrified, rederived and grown together in a
85 randomized controlled environment. To rule out confounding maternal and embryo handling
86 effects, prenatal growth traits and litter size components were measured in the second
87 generation after rederivation (R20 and R38).

88

89 **2. Materials and methods**

90 The animal study protocol was reviewed and approved (code number
91 2015/VSC/PEA/00061) by Ethical Committee of the Universitat Politècnica de València before
92 initiating the study. All experiments were performed following guidelines and regulations
93 outlined in Directive 2010/63/EU EEC. Animal experiments were conducted at an accredited
94 animal care facility (code: ES462500001091).

95

96

97

98 **2.1. Animals**

99 A rabbit paternal line (R) selected at the Universitat Politècnica de Valencia was used.
100 This line was founded in 1989 from two closed paternal lines selected according to individual
101 weigh gain from weaning to end of fattening period (77 days old) during 12 and 9 generations
102 (Estany et al., 1992). Since then, the line has been selected for individual weight daily gain
103 from 28 days (weaning) to 63 days of age (end of fattening). For the present study, two
104 populations (R19V and R37V) separated for 18 generations of selection were used. R19V
105 population was rederived from 256 embryos of 25 donors belonging to 10 different sire
106 families of 18th generation and vitrified in 2000. R37V population was rederived from 301
107 embryos from 28 donors belonging to 15 different sire families of 36th generation, which were
108 vitrified in 2015. Both populations were rederived at the same time in 2015 (see details in
109 Marco-Jiménez et al., 2018). Offspring were bred in non-overlapping generations; 101 females
110 from generations 20 and 38 were used in this experiment (named R20 and R38, respectively).
111 Environmental conditions were maintained using a control system for light (16:8 light/dark
112 photoperiod), ventilation and temperature (18–25 °C) and relative humidity: 60 to 75% by a
113 forced ventilation system. Rabbits does were fed ad libitum throughout the gestation and
114 lactation period with a commercial pelleted diets (2900 kcal of digestible energy / kg, 3.5%
115 crude fat, 15.5% crude fibre and 17% crude protein dry matter). Non-pregnant rabbit does
116 were fed with 140 g/animal/day until a positive pregnancy diagnosis.

117

118 **2.2. Reproduction management**

119 One hundred and forty-two females were inseminated by males from the
120 corresponding generation (60 from R20 and 82 from R38). To control inbreeding, males and
121 females sharing a grandparent were avoided. Receptivity of does was improved with 12-15 UI

122 of eCG via intramuscular 48 hours before insemination. First insemination was performed at
123 20 weeks of age and then 10-12 days after parturition. Fourteen days post-insemination,
124 pregnancy was diagnosed by abdominal palpation and, if they were non-pregnant, females
125 were inseminated again 7 days later. Young rabbits were weaned at 28 days of age.

126 Insemination was performed after evaluation of ejaculate with 0.5 ml and 20 to 40
127 million sperm per seminal dose. Only ejaculates with more than 70% of motility rate and less
128 than 20% of abnormal sperm were used. Ejaculates were diluted with tris-citric-glucose
129 diluent to adjust the concentration (Viudes-de-Castro and Vicente, 1997). Immediately after
130 insemination, ovulation was induced by an intramuscular injection of 1 μ g of Buserelin Acetate
131 (Suprefact, Hoechst Marion Roussel, S.A., Madrid, Spain). Reproductive status of does at
132 insemination time (nulliparous, primiparous lactating, multiparous lactating and non-lactating
133 does), total litter size, liveborn and litter size at weaning were recorded for each female.

134

135 **2.3. Laparoscopy and evaluated litter size components**

136 A total of 85 does were used and 103 laparoscopies were carried out on females from
137 fourth and fifth parity (38 does from R20 and 47 does from R38). In brief, the females were
138 sedated with intramuscular injection of 5 mg xylazine/kg (Rompun, Bayer AG, Leverkusen,
139 Germany) and 3 mg/kg morphine chloride (). Five minutes later, 35 mg/kg Ketamine
140 hydrochloride (Imalgene[®], Merial, S.A., Lyon, France) was administered intravenously. After
141 laparoscopy, does were treated with antibiotics (200,000 IU procaine penicillin and 250 mg
142 streptomycin, Duphapen[®] Strep, Pfizer, S.L.), 0.03 mg/kg of buprenorphine hydrochloride
143 every 12 hours and 0.2 mg/kg of meloxicam every 24-h for 3 days. The number of corpora
144 lutea, the number of implanted embryos at 12 days (IE) and litter size at birth (LS) were
145 recorded per female. The following variables were calculated using the above data. Ovulation

146 rate (OR), defined as the number of corpora lutea, was recorded to determine ovulation rate
147 (OR), embryonic loss rate (ELR), estimated as $(OR-IE)/OR$, and foetal loss rate (FLR), estimated
148 as $(LS-EI)/LS$.

149

150 **2.4. Foetal growth. Ultrasound measurement**

151 Thirty-one pregnant does from laparoscopised females (15 from R20 and 16 from R38)
152 were examined on day 12, 19 and 26 of gestation using a portable colour Doppler ultrasound
153 device (Esaote, Spain) with a 7.5 MHz linear probe (4–12 MHz range). Does were sedated
154 according to the procedure described above and placed in a polystyrene cage where they were
155 prevented from moving. The ultrasound examination was performed from right to left with
156 the probe in sagittal orientation and, after localization of different foetal sacs, 4–6 whole
157 foetal sac examinations per doe were performed. The identifiable structures (foetal sac,
158 foetus and foetal and maternal placenta) were measured from frozen frame pictures on the
159 monitor, using the Esaote 16 ultrasound software. Measurements on different days of
160 gestation are illustrated in Fig. 1. Briefly, foetal sac (FS, A, C and E) measurements were taken
161 when the largest surface area appeared on the screen. For whole foetus measurements,
162 crown-rump length (CRL) was determined as the maximum distance from crown to tail base,
163 with the foetus on a sagittal plane (Fig. B, D and F). Placental size was difficult to assess, but
164 placental measurements were determined when the maximal placental surface with the two-
165 lobed foetal (L1FP and L2FP) and maternal placenta (MP) were visible on screen (Fig. 1A, 2A,
166 3A).

167

168

169

170 **2.5. Statistical analyses**

171 Reproductive performance was analysed with a linear general model including as fixed
 172 effects rederived generation group (R20 and R38), reproductive status of does (nulliparous,
 173 primiparous lactating, multiparous lactating and non-lactating does), month-year in which
 174 insemination was done (18 levels) and the interaction between generation group and
 175 reproductive status of the mothers. Delivery rates were analysed using a probit link with
 176 binomial error distribution, included in the generalized model described above.

177 Litter size components (ovulation rate, implanted embryos, litter size, liveborn and
 178 rates of embryo and foetal losses) were analysed by a generalized linear model including as
 179 fixed effects rederived generation group (R20 and R38) and lactating or non-lactating status
 180 and their interactions.

181 To analysis foetal sac area, crown-rump length of foetus, foetal and maternal placenta
 182 areas at days 12, 19 and 26 of gestation and, weight of liveborn kits, a mixed linear mode was
 183 used:

$$184 \quad Y_{ijkl} = \mu + P_i + R_j + PR_{ij} + CO_k + Cov X_l + e_{ijkl}$$

185 , where Y_{ijkl} was the trait to analyse, μ was the general media, P_i was the fixed effect
 186 of the rederived generation group (R20 and R38), R_j was the fixed effect of reproductive status
 187 of the doe used to analysis of weaning weight (lactating and non-lactating doe); PR_{ij} was the
 188 effect of interaction between rederived population and reproductive status of the mothers
 189 used to analysis of weaning weight, CO_k was the random effect of common litter, $Cov X_l$ was
 190 the covariate of number of implanted embryos and e_{ijklm} was the error term of the model.

191 Values were considered statistically different at $P < 0.05$. Results were reported as least
 192 square means with standard error of the mean (SEM). All analyses were performed with SPSS
 193 26.0 software package (SPSS Inc., Chicago, Illinois, USA, 2012).

194 **3. Results**

195 **3.1. Reproductive performance between rederived populations**

196 Of the different reproductive parameters evaluated, only litter size at parturition was
197 significantly different between the two rederived populations, with the litter size being larger
198 in the most recent generation R38 (6.5 0.32 vs 7.1 0.29, Table 1). However, the high mortality
199 in both groups around delivery and during the lactation period (more than 20% and 40%,
200 respectively, Table 1) make this difference irrelevant. In addition, the delivery rate was also
201 similar in both groups (Table 1).

202 Considering the reproductive status of the doe at the time of insemination, nulliparous
203 females had the highest delivery rates, the lowest litter size and the highest mortality rate
204 during lactation (Table 1). Furthermore, it is necessary to highlight that the females with 1 or
205 more deliveries that were not pregnant presented problems to gestate again, obtaining the
206 lowest delivery rate (Table 1).

207 Non-significant effects of year-month and interactions between generational group
208 and week of reproductive status were observed for all evaluated traits.

209

210 **3.2. Litter size components between rederived groups**

211 Litter size components evaluated such as ovulation rate, foetal and embryo losses were
212 not different between the rederived populations, despite the 18 generations of selection that
213 separate them (Table 2). However, a significant increase in the number of implanted embryos
214 was observed in R38 vs R20 population if the number of implanted embryos included those
215 females that did not implant any. Both rederived populations showed high rates of loss at
216 implantation and from implantation to parturition, but not significantly different (Table 2).
217 Total implantation failure was 38.6% (17) in R20, while this occurred at 23.7% (14) in the R38

218 population. Non-significant interactions between generational group and lactation status
219 were observed for evaluated traits.

220

221 **3.3. Foetal growth during gestation**

222 Early differences in foetal sac growth, foetal placenta and Crown-rump length of
223 foetuses were observed at day 12 and 19 of gestation, respectively, between both populations
224 (Table 3). At the onset of gestation, the lactation status affected the foetal sac size at 12 and
225 19 days and the foetal placenta size at 19 days. On day 26 of gestation, neither the generation
226 nor the lactation status affected any trait. The bodyweight of the liveborn kits was significantly
227 different between generations, but was not influenced by the lactating status. The highest
228 birth weight was obtained in the offspring of R38 population (57.2 ± 3.47 vs 69.3 ± 3.89 for R20
229 and R38 from 48 and 42 liveborn kits, respectively. Data not shown in tables).

230

231 **4. Discussion**

232 To the best of our knowledge, this is the first in-depth study to evaluate the effect of
233 selection for growth on reproductive parameters or traits using populations rederived (re-
234 established) from cryopreserved embryos. Although rarely used in selection experiments,
235 cryopreserved populations offer the advantages of optimizing the experimental facilities and
236 reducing genetic drift (García and Baselga, 2002; Piles and Blasco, 2003), and it is a successful
237 strategy to re-establish a population to continue the breeding programme (Marco-Jiménez et
238 al., 2018). Our study suggests that selection for growth rate has no adverse effect on litter
239 size, foetal growth and reproductive performance, and we even observed a slight
240 improvement in the embryo implantation and litter size and bodyweight at birth after 18
241 generations of selection.

242 Livestock animals selected for high production efficiency (litter size, growth, feed
243 efficiency or carcass composition and meat quality) have impaired reproductive performance
244 (Rauw et al., 1998; Bunger et al., 2005) or health traits and robustness (Rauw et al., 1998;
245 Prunier et al., 2010; Phocas et al., 2014; Rauw and Gomez-Raya, 2015). These adverse effects
246 of selection are often difficult to reveal as a consequence of not being registered or because
247 the feeding or environmental conditions are being modified. For example, in swine a long-
248 term selection for a combined breeding goal (growth, feed efficiency, body composition and
249 litter size) has been accompanied by an improvement in litter size and weight, but
250 unfavourable effects of selection for several traits such as an increase in stillbirths and in
251 postnatal mortality, reduced longevity and productive life, a reduced milk production and
252 robustness (Silalahi et al., 2016 and 2017). It is known that rabbit selection for growth traits
253 has negative genetic correlations on ejaculate traits such as mass motility, volume, abnormal
254 sperm rate or head sperm morphometry (Brun et al., 2006; Lavara *et al.*, 2012 and 2013) and
255 the female contribution to fertility has been found (Piles and Tussel, 2012). Moreover, several
256 studies showed an impaired reproductive performance of paternal line R when it was
257 compared with maternal lines, with high embryonic, foetal, perinatal losses and during the
258 lactation period (Vicente et al., 2012 and 2013; Naturil-Alfonso et al., 2016). Nevertheless, the
259 present study showed that selection for growth rate does not adversely affect litter size and
260 reproductive performance. It is worth mentioning that, after 18 generations of the selection
261 process, females increased in implanted embryos, ending in improved litter size. Moreover,
262 similar prenatal losses were observed between both generations, in line with our previous
263 results in which implantation and gestational losses were around 20-30% and 50%,
264 respectively (Vicente et al., 2012). In maternal or crossed rabbit lines, embryonic and foetal
265 loss rates are 10 and 20%, respectively, and up to 15% for perinatal losses (Santacreu et al.,

266 1992; Fortun et al., 1993; García and Baselga, 2002; Santacreu et al., 2005; Mocé et al., 2005;
267 Vicente et al., 2012; Ragab et al., 2014). Some of the causes of the high implantation failures
268 and foetal losses of this paternal line could be linked to high levels of IGF-1 and leptin, lower
269 oestrogen and progesterone levels and lower mRNA expression levels of insulin-like growth
270 factor II receptor (IGF-IIR) at endometrial tissue found in the females (Vicente et al., 2012;
271 Llobat et al., 2012; Naturil-Alfonso et al., 2016).

272 Additionally, perinatal and lactation mortality rates were similar and high between
273 both generations. Perinatal and lactation losses found in both can be associated with impaired
274 maternal behaviour and were already reported for this line by Lavara et al. (2002). Moreover,
275 this could be related to the abnormal levels of oestradiol and progesterone during gestation
276 observed in this line (Vicente et al., 2013) and with a low litter size. This endocrine disruption
277 might trigger a cascade of events that would affect the construction of the nest, the
278 pheromonal cues, nursing behaviour and, finally, milk production. González-Mariscal et al.
279 (2016) reviewed maternal behaviour and sibling interactions in rabbits, describing the role of
280 changing concentrations of oestradiol, progesterone and prolactin throughout gestation to
281 prime the mother's brain to respond to the newborn and as regulators of nest-building, and
282 how the duration and periodicity of nursing will depend on the stimulation of the teats by the
283 kits (suckling young). This should be evaluated in a subsequent study in order to improve
284 reproductive efficiency, among other things, carry out adoptions at birth to prevent the
285 number of young rabbits from being less than 6 so that an adequate nursing behaviour
286 develops. Regarding negative outcomes of non-lactating does, it is probable that in spite of
287 feed control, these females tended to accumulate fat and had difficulty mobilizing during
288 gestation or lactation. R line does seem to prioritize maintaining their heavier body size rather
289 than litter development, a difference from other lines (Arnau-Bonachera et al., 2018 a and b),

290 which may be further aggravated if does do not become pregnant and continue to gain weight
291 (Rommers et al. 1999) and, consequently, negatively affects their long-term reproductive
292 function (Castellini et al., 2006).

293 This study also enabled us to explore whether selection for growth rate affects
294 placentae and foetal growth. Our findings revealed that the foetal sac and foetal placenta area
295 at day 12 of gestation and foetal placenta area at day 19 of gestation was higher in the R38
296 generation. Nevertheless, no differences between generations were found at day 26 of
297 gestation, indicating that the possible effects of both selection and the gestation-lactation
298 overlap were compensated at the end of gestation but not in the weight of liveborn kits. This
299 could be because during the last week of gestation the fastest increase in bodyweight takes
300 place (Vicente et al., 1995 and 2013; Argente et al., 2003). So, we have observed evidence
301 that growth selection influenced foetal structures and final weight of foetuses. The changes
302 in foetal structures take place at a critical period between day 12 and 19 of gestation, in which
303 organogenesis is defined, which could be important in the final growth of gestation and during
304 postnatal life (Vuguin, 2007; Sartori et al., 2020). During foetal development, extrauterine
305 signalling provides a link between environmental changes and physiology of the foetus as the
306 impetus to prepare the organism for the postnatal environment, guided mainly by epigenetic
307 changes (Gluckman et al., 2005; Sarkies, 2020). If these adaptive responses are directed to a
308 nutritionally deficient postnatal environment, they could potentially affect muscle, adipose
309 and reproductive tissue development (Ford et al., 2007; Ford and Long, 2012). Skeletal muscle
310 or reproductive tissue have a lower priority in nutrient partitioning compared to the brain and
311 heart in response to challenges to the foetus during development, and are particularly
312 vulnerable to nutrient deficiency (Caton et al., 2019; Crouse et al., 2019). Females from the
313 paternal rabbit line used in this study, regardless of the repercussions due to the selection

314 process, shows some negative phenotypic characteristics at endocrine level and a different
315 nutritional partition that could be triggered during prenatal development and in the first steps
316 of postnatal development (suckling). So, a more in-depth study of these stages is necessary.

317 In conclusion, selection for growth rate does not adversely affect components of litter
318 size, foetal growth and reproductive performance in rabbit does. Nevertheless, this study
319 reinforces some significant reproductive problems, such as high prenatal and perinatal
320 mortality in this paternal line, that were already present in generation 18. Therefore, further
321 studies must elucidate how the founders and not the selection process could play a
322 fundamental role in the adverse reproduction outcomes.

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327

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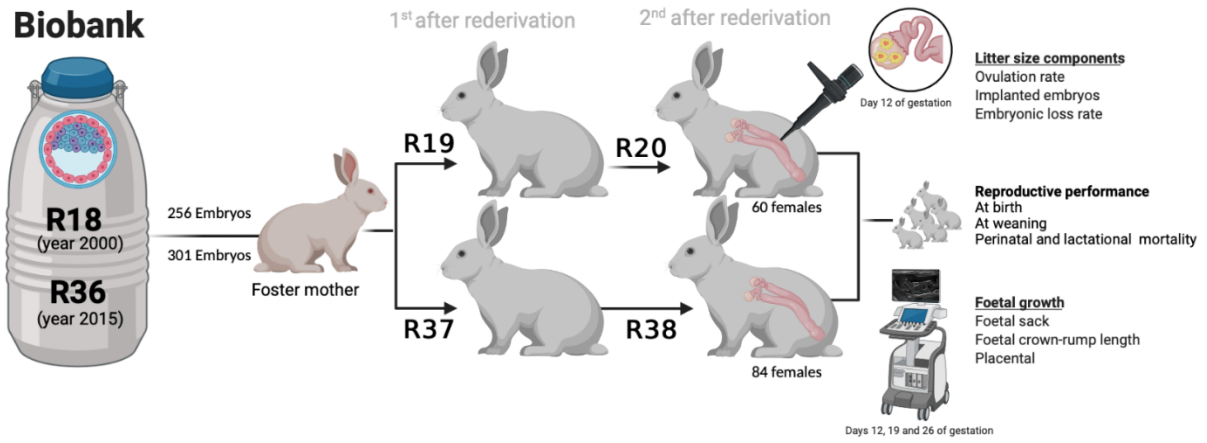
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531 **Graphical abstract.**

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536 **Table 1.** Effect of selection for growth rate on total litter size, liveborn, litter size at weaning, perinatal and lactation mortality rates and
537 delivery rate from two rederived population separated by 18 generations.

	Type	n	Litter size			Mortality rate (%)		Delivery rate (%)
			Total	Liveborn	At weaning	Perinatal	Lactation	
Generation	R20	179	6.5±0.32 ^b	5.1±0.36	3.1±0.34	23.2±3.33	46.1±4.11	0.57±0.029
	R38	278	7.1±0.29 ^a	5.8±0.33	3.7±0.31	21.9±3.03	42.5±3.68	0.59±0.024
Lactation status	Nulliparous	142	5.9±0.37 ^b	4.1±0.35 ^b	2.3±0.39 ^b	29.8±3.86	56.7±4.80 ^b	0.76±0.032 ^a
	Primiparous Lactating	75	7.1±0.46 ^a	5.7±0.52 ^a	3.2±0.49 ^{ab}	23.3±4.76	46.4±5.91 ^{ab}	0.59±0.044 ^b
	Multiparous lactating	143	7.1±0.36 ^a	6.1±0.41 ^a	4.2±0.38 ^a	17.3±3.72	33.8±4.60 ^a	0.58±0.032 ^b
	Non-lactating	97	7.1±0.37 ^a	5.9±0.42 ^a	3.9±0.39 ^a	19.9±3.86	40.3±4.72 ^a	0.37±0.031 ^{ca}

538 n: Number of inseminations. Data are expressed as least squared mean ± standard error of means. ^{a,b} Values with different superscripts in
539 column differ significantly ($p < 0.05$).

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543 **Table 2.** Effect of selection and lactation status on litter size components from two rederived population separated by 18 generations.

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Type	Ovulation rate ¹	Implanted embryo		Loss rate (%)		Litter size	
		Implanted embryo	² Implanted embryos	¹ Embryonic	Foetal		
Generation	R20	12.1±0.45	5.1±0.79 ^b	8.1±0.73	37.0±5.19	42.8±7.31	5.0±0.80
	(n)	(44)	(44)	(27)	(27)	(27)	(26)
Generation	R38	12.7±0.39	7.2±0.71 ^a	9.4±0.60	28.5±4.26	33.4±6.00	6.7±0.65
	(n)	(59)	(59)	(45)	(45)	(45)	(44)
Lactation status	Non-lactating	12.8±0.47	6.5±0.86	8.3±0.71	36.7±5.11	40.0±7.19	5.7±0.79
	(n)	(36)	(36)	(28)	(28)	(28)	(27)
Lactation status	Lactating	12.0±0.37	5.8±0.64	9.1±0.61	28.9±4.36	36.2±6.14	6.1±0.66
	(n)	(67)	(67)	(44)	(44)	(44)	(43)

557 Data are expressed as least squared mean ± standard error of means. n: number of laparoscopies.

558 ¹ It was determined as the number of corpora lutea.559 ² Implanted embryos in pregnant does.560 ^{a,b} Values in the same column and factor with different superscripts are statistically different (P < 0.05).

561

562 **Table 3.** Effect of selection and lactation status on foetal sac and placentae area and foetal size at 12, 19 and 26 days of gestation from two
 563 rederived population separated by 18 generations.

	Group	Day of gestation	n	Foetal sac area (cm ²)	Maternal placenta area (mm ²)	Foetal placenta area (mm ²)	Crown-rump length of foetus (mm)
Generation	R21	12	82	2.07±0.071 ^b	44.9±2.46	42.0±2.29	11.2±0.35
	R39		95	2.44±0.070 ^a	50.7±2.44	46.7±2.28	11.5±0.34
	R21	19	77	5.81±0.178	111.8±6.30	116.0±6.31 ^b	35.8±0.68 ^b
	R39		94	6.27±0.176	118.2±6.13	136.7±6.14 ^a	38.0±0.68 ^a
	R21	26	66	10.01±0.530	197.8±12.21	247.6±15.67	73.1±1.85
	R39		62	10.24±0.560	193.1±13.57	244.5±17.59	72.0±1.92
Lactation status	Non-lactating	12	70	2.36±0.072 ^a	48.4±2.49	46.8± 2.32	11.3±0.35
	Lactating		107	2.15±0.058 ^b	47.2±2.02	42.0±1.88	11.4±0.29
	Non-lactating	19	69	6.49±0.181 ^a	116.2±6.23	139.0±6.27 ^a	36.9±0.71
	Lactating		104	5.59±0.147 ^b	113.8±5.13 ^b	113.7±5.14 ^b	36.9±0.57
	Non-lactating	26	54	10.15±0.695	211.7±17.01	233.2±22.04	71.6±2.42
	Lactating		74	10.10±0.602	179.1±13.90	258.9±17.91	73.6±2.08

a,b Values in the same column and factor with different superscripts are statistically different (P < 0.05).

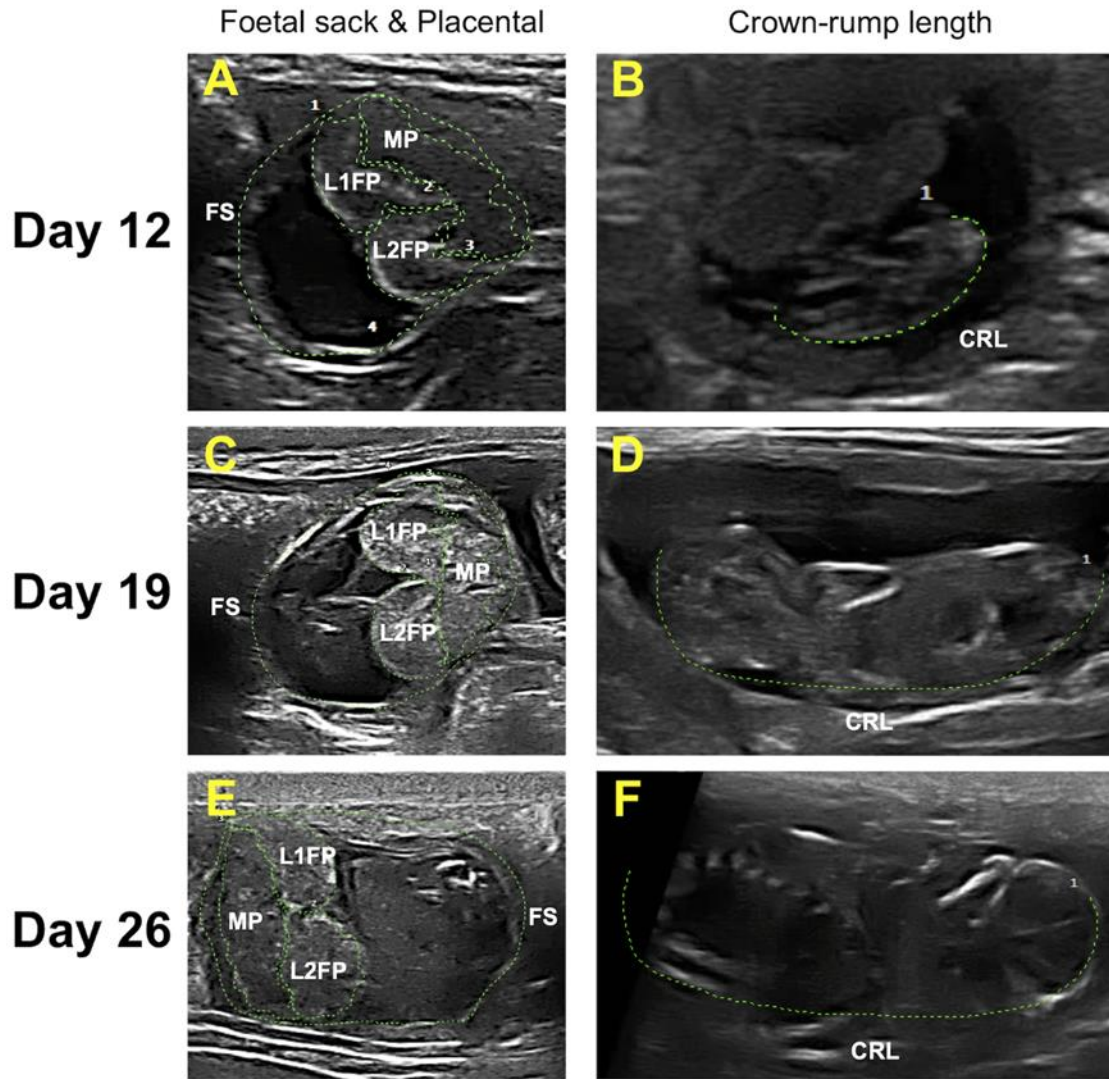
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568 Figure. 1. Ultrasonography measurements of the foetal sac (FS), crown-rump length (CRL) of
 569 foetus and the placental measurements of the two-lobed foetal (L1FP and L2FP) and maternal
 570 (MP) at 12, 19 and 26 day of gestation.



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