

INTERNATIONAL MASTER ON ANIMAL BREEDING  
AND REPRODUCTION BIOTECHNOLOGY

**Genetic selection for ovulation rate and litter  
size in rabbits: estimation of genetic  
parameters, direct and correlated responses**

Thesis of Master

Valencia, 7<sup>th</sup> June 2012

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♥♥♥ A Mes Très Chers Parents ♥♥♥

*Tous les mots du monde ne sauraient exprimer l'immense amour que je vous porte, ni la profonde gratitude que je vous témoigne pour tous les efforts et les sacrifices que vous n'avez jamais cessé de consentir pour mon instruction et mon bien-être.*

*C'est à travers de vos encouragements et que j'ai opté pour cette noble profession, et c'est à travers de vos critiques que je me suis réalisée.*

*J'espère avoir répondu à aux espoirs que vous avez fondé en moi.*

*Je vous rends hommage par ce modeste travail en guise de ma reconnaissance éternelle et de mon infini amour.*

*Vous résumez si bien le mot parents qu'il serait superflu d'y ajouter quelque chose.*

*Que **Dieu** tout puissant vous garde et vous procure santé, bonheur et longue vie pour que vous demeuriez le flambeau illuminant le chemin de vos enfants.*

**W**e know very little, and yet it is astonishing that we know so much, and still more astonishing that so little knowledge can give us so much power.

- Albert Einstein-

## ACKNOWLEDGMENTS

In the name of **Allah**, the Most Gracious and the Most Merciful

I'm very much indebted to Dra. María Antonia Santacreu and Dra María Lorena Mocé for being an outstanding advisors and excellent professors. Their constant encouragement, support, and invaluable suggestions made this work successful. I'm so grateful for their patience with all the problems which arise during this work. They have been everything that one could want in an advisor.

I am deeply indebted to my committee members Dr. Manuel Baselga and Dr. Duni Gabiña. Thank you for their time and effort in reviewing this work.

I would like to thank to Dr. Agustín Blasco for being excellent professor, always available for scientific discussions and for being patient all the time repeating for me the same things for a good understanding of their fields.

I would like to express my gratitude to my colleagues in the Department of Animal Science, especially in the fourth floor (Mohamed, Pilar, Paty, Ayman, Carlos and Ahmad) for creating and maintaining a cheerful atmosphere in our group. Special thanks go to Cristina and Vero for being not only excellent colleagues but also very good friends, for their understanding and help in all the occasions.

I would like to express my thanks to my Palestinian lovely friend Rima, who always give help in any time, encouragement and hear to my problems. My sincere thanks to my Egyptian friends: Abeer, Hanan, Hadeer, Afaf, Tarek, for supporting, encouragement me and for the good time we shared in our apartment.

I'm most grateful to the Mediterranean Agronomic Institute of Zaragoza (IAMZ), for supporting me with a full grant to study two years for having the international master degree, which helped me to exchange powerful scientific ideas with persons from different countries and upgrade my knowledge.

Last but not least, my deepest gratitude goes to my beloved parents and also my lovely sister for their endless love, prayers and encouragement.

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# **Chapter 1**

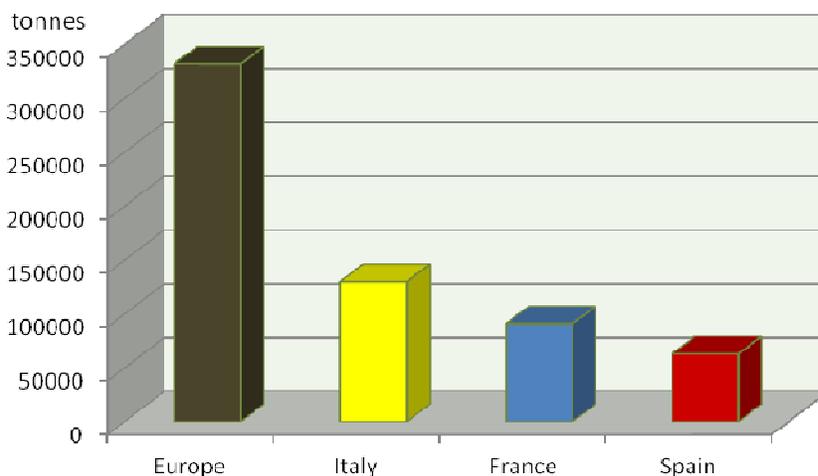
## **General Introduction**

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## I. General introduction to rabbit production

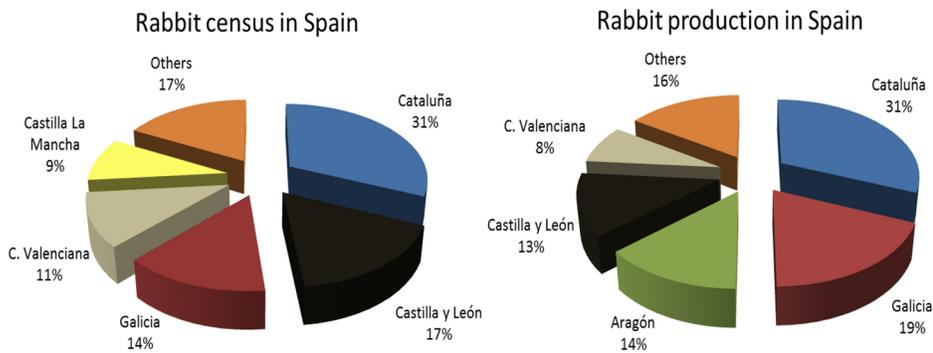
Global meat production is around 285 million tonnes (FAO, Food and Agriculture Organization, 2011) and rabbit carcass meat production is near 1.2 million tonnes (0.4% of the global meat production). The majority is produced in Europe (50%) while Asia (predominantly China) produces 41%. In 2010, the main European producers were located in Italy (39%), France (27%) and Spain (19%) (Figure 1.1). These three European countries produce rabbits mainly under intensive farming conditions, although there still does exist a significant level of home production (REGA, Registro General de Explotaciones Ganaderas, 2010).



**Figure 1.1:** Rabbit carcass meat production in Europe in 2010. Developed from:

[http://www.marm.es/app/vocwai/documentos/Adjuntos\\_AreaPublica/INDICADORES%20ECON%C3%93MICOS%20SECTOR%20CUN%C3%8DCOLA%202010.pdf](http://www.marm.es/app/vocwai/documentos/Adjuntos_AreaPublica/INDICADORES%20ECON%C3%93MICOS%20SECTOR%20CUN%C3%8DCOLA%202010.pdf)

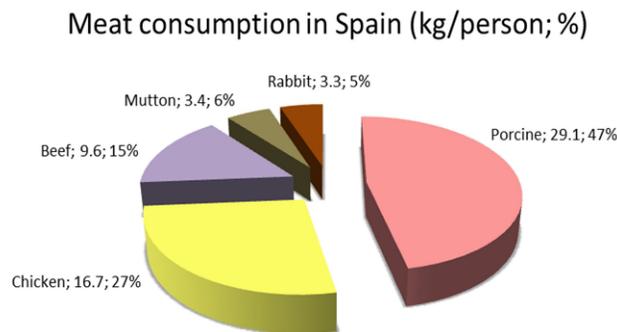
In Spain, Cataluña stands out as the main producer (30%). Data from 2010 show that a high proportion of the rabbits censused in Spain (near 80%) is concentrated in only 5 communities: Cataluña (1.840.394), Castilla y León (988.866), Galicia (823.898), Comunidad Valenciana (653.663) and Castilla La Mancha (537.321), while rabbit carcasse meat is mainly produced in Cataluña (19.778), Galicia (11.836), Aragón (8.549), Castilla y León (8105) and Comunidad Valenciana (5067) (Figures 1.2 and 1.3; REGA, Registro General de Explotaciones Ganaderas).



**Figures 1.2 and 1.3.** Rabbit census and rabbit carcass meat production in Spain in 2010. Developed from:

[http://www.marm.es/app/vocwai/documentos/Adjuntos\\_AreaPublica/INDICADORES%20ECON%C3%93MICOS%20SECTOR%20CUN%C3%8DCOLA%202010.pdf](http://www.marm.es/app/vocwai/documentos/Adjuntos_AreaPublica/INDICADORES%20ECON%C3%93MICOS%20SECTOR%20CUN%C3%8DCOLA%202010.pdf)

Rabbit consumption is stable or slightly decreasing in the European countries. In Spain, only 5% of the meat consumed is rabbit (Figure 1.4, INRA SAGA). Efforts are underway to increase the consumer appeal of rabbit meat by introducing “quick cook” meals and a variety of cuts of meat, rather than rely on the traditional whole carcass presentation (including heads) (Eady, 2008).



**Figure 1.4.** Meat consumption in Spain in 2001 (kg/person; %). Developed from:

<http://www.avicampus.fr/PDF/PDFlapin/selectionlapin1.pdf>

In rabbit production, a high proportion of the total costs correspond to feeding (60%; Pascual et al., 2011). Feeding costs can be reduced through an improvement in the feed conversion. Feed conversion is difficult and costly to measure but the beneficial correlation with growth rate means selection for growth rate will also result in improvement in feed conversion (Gosey, 2003). Fixed costs (30%; Pascual et al., 2011) can be reduced by increasing female productivity, because costs can be distributed

among a higher number of animals produced. Therefore, breeding programs in rabbits include growth rate and reproductive efficiency as the most important aspects.

The genetic improvement programs in rabbits are organized in a pyramidal structure with three levels: nucleus populations on the top of the pyramid, multipliers and commercial farms on the bottom. In most nucleus populations, selection is performed on three specialized rabbit lines, two maternal lines, selected to increase litter size and a paternal line, selected for growth rate. In the multipliers, animals from both maternal lines are mated to obtain hybrids. Hybrid females present heterosis in reproductive traits, which leads to higher litter sizes in maternal lines. Therefore, three way crosses are usually carried out. Finally, in the commercial farms, hybrid females are mated to males from the paternal line, and the final product is sent to slaughterhouse.

## **II. Litter size and its components: ovulation rate and prenatal survival**

Litter size at birth in polytocous species is mainly determined by two parameters: number of ova shed by estrus and proportion of these ova represented by normal fetuses at birth, also called prenatal survival. Fertilization rate is usually high in rabbits (Adams, 1960a; Torrès et al., 1984; Santacreu et al., 1990; Theau-Clement et al., 2009), pigs (Bazer et al., 1988; Soede et al., 1995; Geisert and Schmitt, 2002) and mice (Joakimsen and Baker, 1977; Wilmut et al., 1986) and is therefore not considered as a cause of variation of litter size.

### **1. Ovulation rate**

#### **1.1 Estimation of ovulation rate**

In rabbits, ovulation rate is usually estimated as the number of corpora lutea in both ovaries, counted *in vivo* by laparoscopy around d 12 of gestation or *post mortem* after dissection of the ovary. Both measurements of ovulation rate have shown to have a high regression coefficient (0.91; Santacreu et al., 1990) reflecting the high precision of the technique in the measurement of ovulation rate.

#### **1.2 The mechanism of ovulation**

Ovulation rate is the total number of ova shed by the ovaries at ovulation. In rabbits, ovulation is induced by the coitus stimulus. The coitus leads to a nervous stimulus that induces release of the gonadotropin-releasing hormone (GnRH) in the hypothalamous. This hormone activates the synthesis and secretion of the follicle-stimulating hormone

(FSH) and the luteinizing hormone (LH) at the anterior pituitary gland. The FSH is known to be very important for the terminal growth of the follicles, while an acute rise in LH triggers ovulation (Rosell, 2000). Follicular development can determine the degree of the oocyte maturation (oocyte quality) and the ovulatory timing. Both factors can affect posterior embryonic and fetal development.

To our knowledge, there is only one study reporting the relationship between oocyte quality and ovulation rate (Koenig et al., 1986). In this study in pigs, a higher proportion of immature oocytes was found in females selected for ovulation rate and in superovulated females compared to unselected females and to naturally ovulated ones, respectively. No study has been found relating the ovulatory timing and ovulation rate.

### **1.3 Timing of ovulation**

Approximately 8h *post-coitum* (*p.c.*), the ovulatory follicles begin to release the oocytes. Most of the follicles ovulate simultaneously in a short period of time, with a small proportion of them ovulating later (Fujimoto et al., 1974 in rabbits). Ovulation is completed in rabbit 14 h *p.c.* (Fujimoto et al., 1974). Late ovulating oocytes may be fertilized later, leading to lesser developed embryos. The more developed embryos advance the uterine secretions (Torres et al., 1984 in rabbits; Pope, 1988 and Xie et al., 1990 in pigs; Wilmut et al., 1986 and Al-Shorepy et al., 1992 in mice). The lesser developed embryos may not tolerate the degree of asynchrony associated with the advanced uterine environment, and they may die during the embryonic or the fetal period (Peiró et al., 2007 in rabbits; Wilde et al., 1988 and Pope et al., 1990 in pigs). Synchrony between the developing embryos and the secretions of the uterus has been recognized as a critical factor to maintain a successful pregnancy (reviewed by Pope, 1988 and Barnes, 2000).

### **1.4 Oocyte quality**

Oocyte quality, or developmental competence, is acquired during folliculogenesis as the oocyte grows and during the period of oocyte maturation (Krisher, 2004). Both nuclear and cytoplasmic maturation have to be completed successfully to be a competent oocyte. Oocyte quality affects the establishment and maintenance of pregnancy modifying early embryonic survival and fetal development (Krisher, 2004).

Multiple methods have been proposed to assess oocyte quality. The best method is to evaluate the fertilization ability of classified oocytes and their developmental

competence along gestation following embryo transfer. However, this is usually not possible because of economic and technical factors. The study of oocyte morphology is relatively quick and simple; however, it is unreliable if it is not accompanied by other methods (Balaban and Urman, 2006). Other methods to assess oocyte quality that have been proposed are: measurement of ATP, an important energy source for maintaining protein synthesis and other cellular functions (reviewed by Krisher, 2004); measurement of glutathione (GSH), the main compound that protects the cell against the oxidative stress (reviewed by Luberda, 2005; Rausell and Tarín, 2005); quantification of mitochondrial DNA; quantification of oocyte mRNA and proteins (reviewed by Krisher, 2004). Besides, some authors have studied the expression of genes in the granulosa cells or in the oocyte itself, looking for specific molecular markers of oocyte quality, or have performed polar body biopsy to screen oocytes with chromosomal abnormalities deriving from errors in the two meiotic divisions (reviewed by Revelli et al., 2009).

## **2. Prenatal survival**

Prenatal survival is an important character in animal production, a high prenatal survival leads to increased litter size at birth and consequently to greater economic benefits (Santacreu, 2006).

### **2.1 Components of prenatal survival: embryonic and fetal survival**

Prenatal survival is by definition the proportion of ova shed giving birth to young. It comprises two periods: the embryonic and the fetal period. In rabbits, it has been accepted to call embryonic period to the period before implantation (d 7) and fetal period to the period from implantation until birth (d 30) (Mocé et al., 2010).

### **2.2 Estimation of embryonic and fetal survival**

Embryonic survival is calculated as the proportion of implanted embryos from the number of corpora lutea and fetal survival is calculated as the proportion of kits born from the number of implanted embryos. Besides, prenatal survival is the product of both embryonic and fetal survival; it is calculated as the proportion of kits born from the number of corpora lutea. The estimation of survival during gestation requires the counting of implanted embryos or fetuses. In rabbits the laparoscopic method permits the estimation of embryonic and fetal survival in the same female without affecting litter size (Santacreu et al., 1990). However, in pigs, it is not possible to estimate ovulation rate, embryo survival and foetal survival in the same females without

compromising litter size (Neal and Johnson, 1986) because in this species implantation sites cannot be determined by observation of the external surface of the uterus. This makes the rabbit a particularly useful model for examining the relationships between litter size and its components in the same females.

### **2.3 Timing and extent of prenatal mortality**

Prenatal mortality is the major limiting factor of litter size in rabbits like in pigs and mice. In rabbits, prenatal mortality is around 30% (Adams, 1960a, b; García and Baselga, 2002), 10- 14% corresponding to the embryonic period, and 20-22% to the fetal period. Prenatal mortality in mice is lower, around 20%. This percentage is almost equally distributed between the pre and the post-implantation period (reviewed by Wilmot et al., 1986). In pigs, a prenatal loss of 40 to 60% has been reported (reviewed by Foxcroft et al., 2006); the largest proportion of it occurs before d 30-35 of gestation of the 114- day gestation period.

### **2.4 Factors associated with prenatal mortality**

Physiologically, prenatal survival is a complex trait which depends on a series of events ranging from gamete maturation to the birth of viable offspring (Blasco et al., 1993). Prenatal survival depends on the genotype of the dam, the embryo and their interaction. It seems that the maternal genotype plays the most important role whereas the embryo genotype has a minor effect (reviewed by Bradford, 1969 and Blasco et al., 1993). The part of prenatal survival due to the female is called uterine capacity. Uterine capacity has been defined as the maximal number of fetuses a female can carry to term when the number of potentially viable embryos is not limiting (Bennett and Leymaster, 1989). Thus, uterine capacity depends on both embryo survival and fetal survival.

Different causes have been suggested to explain the prenatal losses related to the maternal genotype in early stages of gestation: an increased number of immature oocytes when ovulation rate is high (Torres, 1982 in rabbit, Koenig et al., 1986 in pigs) and an inadequate secretion of certain proteins and hormones necessary for the development of the embryo (Beier, 2000; Daniel, 2000 in rabbit; Bagchi et al., 2001 in humans and rats; Vallet et al., 1998 in pigs). In the later stages of gestation, it has been suggested that the main cause of mortality is competition among embryos for the availability of space and nutrients when number of embryos in the uterus is large.

(Adams, 1960b; Hafez, 1969). The most characteristics studied are the length, weight and degree of vascularization of the uterus (Argente et al., 2003).

### III. Genetic improvement for litter size

#### 1. Conventional selection for litter size

The most common criteria used in selection programs of rabbit maternal lines are litter size at birth or at weaning (for a review by Khalil and Al-Saef, 2008).

Litter size has a low heritability (reviewed by Mocé and Santacreu, 2010 in rabbits; Rotschild and Bidanel, 1998 in pigs). Direct selection on litter size in closed populations have led to a response much lower than expected, around 0.1 young per generation (Table 1.1, for a review in rabbits; Ollivier and Bolet, 1981; Bolet et al., 1989; Holl and Robison, 2003 in pigs). However, in mice, direct selection for litter size has obtained a higher response than in pigs and rabbits, 0.15 to 0.20 young per generation (Bradford, 1968, 1969; Falconer, 1971; Bakker et al., 1978; Gion et al., 1990).

**Table 1.1:** Direct responses in number of kits born alive (NBA) or number of kits weaned (NW) and correlated responses in ovulation rate (OR) and prenatal survival (PS) estimated per generation in rabbits, with their standard errors (in parenthesis).

					Responses		
	Line	G	Cr	Method	NBA/NW	OR	PS
Gómez et al.,	Prat	3	NW	BLUP/REML	0.09 /year	-	-
Rochambeau et al., 1998	1077	18	NW	BLUP/REML	0.08	0.06*	-
				Control	0.08	-	-
	2066	18	NBA	BLUP/REML	0.13	-	-
García and Baselga, 2002a	V	0-21	NW	BLUP/REML	0.09	-	-
		15-21		Control	0.09	0.18	0.06%
García and Baselga, 2002b	A	1-26	NW	BLUP/REML	0.18	-	-
		17-26		Control	0.09	0.01	0.41%

G: Generations; Cr: Criterium of selection

\* Response estimated by Brun et al. (1992) after 13 generations of selection.

Modified from Laborda (2011).

The puzzling results obtained from selection experiments for litter size in close populations led to the search for alternative methods of selection: experiments of

selection for ovulation rate, uterine capacity and prenatal survival for improving indirectly litter size.

## **2. Selection for the components of litter size**

An approach to increasing litter size is to select for its components, ovulation rate and prenatal survival. The efficiency for improving litter size through its components is highly dependent on their genetic parameters. There is also little information of the heritabilities of these traits and their correlations (Tables 1.2a, b). Ovulation rate has higher heritability than litter size, but this heritability is lower than in pigs and mice (reviewed by Blasco et al., 1993b). Genetic correlation between ovulation rate and litter size is positive and low. Prenatal survival has a low heritability similar to litter size, and a high correlation with litter size.

Few selection experiments for components of litter size have been reported in rabbits: two divergent selection experiments for uterine capacity and one selection experiment for ovulation rate.

### **2.1 Selection for ovulation rate**

In rabbits, and another species like pigs and mice, the increase in litter size is basically associated to an increase in ovulation rate (Bolet et al., 1989 in pigs; Brun et al., 1992; García and Baselga, 2002a in rabbits; Bakker et al., 1978; Gion et al., 1990 in mice). This phenomenon, in addition to the fact that ovulation rate presents a higher heritability than litter size (Blasco et al., 1993b), and that both traits were correlated, led to propose selection for ovulation rate as an indirect way to improve litter size. Moreover, ovulation rate sets the upper limit for litter size and it could be easily counted by laparoscopy, laparotomy or after slaughter.

The first experiments of selection for ovulation rate were proposed in mice by Bradford (1969) and Land and Falconer (1969) and in pigs by Zimmerman and Cunningham (1975).

There are six selection experiments for ovulation rate in prolific species, three in pigs (Cunningham et al., 1979; Leymaster and Christenson, 2000; Rosendo et al., 2007), two in mice (Bradford, 1969 and Land and Falconer, 1969) and only one selection experiment for ovulation rate has been carried out in rabbits (Laborda et al., 2011, 2012). The estimated responses to selection in these experiments are summarized in Table 1.3. In these experiments, ovulation rate responded to selection but no correlated

**Table 1.2a:** Estimated heritabilities of ovulation rate (OR), litter size (LS), and prenatal survival (PS) and phenotypic and genetic correlations between these traits and litter size (LS) at the day of gestation indicated (DG) in mice, pigs and rabbits.

	Species	DG	Heritability			Phenotypic correlation			Genetic correlation		
			OR	LS	PS	OR, LS	OR, PS	PS, LS	OR, LS	OR, PS, LS	PS, LS
Land and Falconer, 1969	Mice	-	0.31	-	-	-	-	-	-	-	-
Bradford, 1969	Mice	-	0.10	-	-	-	-	-	-	-	-
Clutter et al., 1990 <sup>a</sup>	Mice	17	0.33	0.18	0.15	0.45	-0.04	0.86	0.81	0.06	0.60
Long et al., 1991	Mice	Birth	0.18 (0.07)	0.33 (0.13)	-	-	-	-	0.62 (0.24)	-	-
Young et al., 1977	Pigs	30	0.21 (0.20)	0.39 (0.17)	-	-	-	-	-	-0.26	-
Young et al., 1978	Pigs	Birth	0.59 (0.12)	0.72 (0.22)	-	0.06	-	-	-0.01 (0.46)	-	-
Cunningham et al., 1979	Pigs	Birth	0.42 (0.06)	-	-	-	-	-	0.07	-	-
Bolet et al., 1989	Pigs	Birth	0.21 (0.12)	0.03 (0.08)	-	-	-	-	0.85	-	-
Bidanel et al. 1992	Pigs	30	0.11 (0.02)	-	0.03 (0.03)	0.41 (0.04)	-0.13 (0.04)	0.87 (0.01)	0.98 (0.33)	-0.13	0.99
Haley and Lee, 1992	Pigs	Birth	0.30 (0.10)	0.09 (0.06)	0.00	0.21 (0.05)	-0.28 (0.05)	0.87 (0.01)	0.98 (1.00)	*	*

**Table 1.2b:** Estimated heritabilities of ovulation rate (OR), litter size (LS), and prenatal survival (PS) and phenotypic and genetic correlations between these traits and litter size (LS) at the day of gestation indicated (DG) in mice, pigs and rabbits (continuation of Table 1.2a).

	Species	DG	Heritability			Phenotypic correlation			Genetic correlation		
			OR	LS	PS	OR, LS	OR, PS	PS, LS	OR, LS	OR, PS	PS, LS
Bidanel et al., 1996	Pigs	30	0.27 (0.02)	-	0.08 (0.03)	-	-0.12 (0.04)	-	-	-0.11 (0.15)	-
Johnson et al., 1999	Pigs	50	0.24	0.16	0.14	0.03	-0.47	0.48	0.24	-0.86	0.36
Ruiz-Flores and Johnson, 2001 <sup>b</sup>	Pigs	Birth	0.42 (0.06)	0.18 (0.08)	0.12 (0.09)	0.16	0.59	-0.69	0.52	0.83	-0.04
Rosendo et al., 2007 <sup>c</sup>	Pigs	Birth	0.34	-	0.14	0.06	-0.18	0.82	0.41	-0.26	0.66
Blasco et al., 1993a	Rabbits	Birth	0.21 (0.11)	0.27 (0.21)	0.23 (0.10)	0.25 (0.06)	-0.30 (0.05)	0.84 (0.02)	0.36 (0.31)	-0.14 (0.35)	0.87 (0.08)
Bolet et al., 1994	Rabbits	-	0.24 (0.04)	0.11 (0.03)	-	-	-	-	-	-	-

\* Not estimated because the estimate of the heritability of PS was zero.

<sup>a</sup> Standard errors range from 0.05 to 0.06 for the heritabilities and from 0.06 to 0.66 for the genetic correlations. Litter size was estimated as the number of fetuses at d 17 of gestation. <sup>b</sup> They measure prenatal loss instead of prenatal survival.

<sup>c</sup> Standard errors range from 0.01 to 0.03 for the heritabilities and from 0.03 to 0.13 for the correlation.

Modified from Laborda (2011).

response on litter size at birth was obtained. The lacking correlated response in litter size was associated with an increase in prenatal mortality. There is little information about the timing of prenatal mortality in the experiments of selection for ovulation rate, probably due to the difficulties in measuring the number of fetuses in live animals in pigs and mice.

In all cases, fetal survival has decreased with selection for ovulation rate in these three species.

**Table 1.3:** Direct response in ovulation rate (OR) and correlated responses in total number born (TNB) and prenatal survival (PS) with their standard errors (SE) estimated per generation in the experiments of selection for OR in mice and pigs.

	Species	G	Response in OR (SE)	Response in TNB (SE)	Response in PS (SE)
Land and Falconer, 1969	Mice	12	0.40 <sup>b</sup>	no clear changes <sup>b</sup>	-
Bradford, 1969	Mice	11	0.26 (0.11) <sup>a</sup>	0.07 (0.05) <sup>a</sup>	-
			0.12 <sup>b</sup>	0.02 <sup>b</sup>	-0.7% <sup>b</sup>
Cunningham et al., 1979	Pigs	9 <sup>1</sup>	0.38 (0.08) <sup>a,1</sup>	0.15 (0.13) <sup>a</sup>	-
			0.49 (0.10) <sup>b,1</sup>	0.06 (0.07) <sup>b</sup>	-1.6% (0.5%) <sub>b,2</sub>
Leymaster and Christenson, 2000	Pigs	10	0.29 <sup>b</sup>	0.06 <sup>b</sup>	-
Rosendo et al., 2007	Pigs	6	0.49 (0.10) <sup>c</sup>	0.08 (0.11) <sup>c</sup>	-1.0% (0.9%) <sup>c</sup>
			0.51 (0.10) <sup>b</sup>	0.06 (0.11) <sup>b</sup>	-1.6% (0.9%) <sup>b</sup>

G: number of generations; Parity: parity number for litter size; <sup>a</sup> Regression of line means on generation number; <sup>b</sup> Response estimated with a control population; <sup>c</sup> REML estimate

<sup>1</sup> Johnson et al., 1984, responses estimated at generation 10.

<sup>2</sup> Geisert et al., 1978: response per generation in survival at d 30 and at d 70, 0.5% and 1.1%, respectively.

Modified from Laborda (2011).

## 2.2 Selection for prenatal survival

There are two experiments of selection for prenatal survival in polytocous species, one in pigs (Rosendo et al., 2007) and the other one in mice (Bradford, 1969). In pigs, the selection criterion was the average prenatal survival over the first two parities corrected for ovulation rate (prenatal survival + 0.018 x ovulation rate). In mice, selection was

based on [(number of normal fetuses at d 16 / ovulation rate) x number of normal fetuses at d 16]. The number of normal fetuses at d 16 was used as an estimator of litter size at birth. The objective in both experiments was to select for prenatal survival avoiding selection against ovulation rate. Responses to selection in pigs and mice are presented in Table 1.4. Selection for prenatal survival increased litter size both in pigs and mice compared to a control line, and a correlated response in ovulation rate was observed in mice. In mice, the increases in ovulation rate and litter size in the line selected for prenatal survival nearly equalled those of two contemporarily lines directly selected for ovulation rate and for litter size, respectively. In pigs, it is not possible to determine if the estimated response was higher than response to direct selection for litter size due to the high standard error of the estimate and to the absence of a contemporary line selected for litter size. Summarizing, selection for prenatal survival increased litter size, but it was not more effective than direct selection for litter size.

**Table 1.4:** Responses in prenatal survival (PS), ovulation rate (OR) and litter size (LS) estimated per generation in pigs and mice selected for prenatal survival, with their standard errors (in parenthesis).

Species		Pigs		Mice	
Generations		6		11	
Method		Control population <sup>1</sup>	REML <sup>1</sup>	Control population <sup>2</sup>	Regression <sup>2*</sup>
RESPONSE	PS (%)	1.0 (0.9)	0.8 (0.9)	0.8	0.4 (0.4)
	OR (ova)	0.04 (0.11)	0.11 (0.11)	0.15	0.23 (0.09)
	LS (kits)	0.21 (0.11)	0.24 (0.11)	0.20	0.25 (0.06)

<sup>1</sup> Rosendo et al., 2007; <sup>2</sup> Bradford, 1969

\* Regression of generation mean on generation number.

Modified from Laborda (2011).

### 2.3 Selection for uterine capacity

Selection for increased uterine capacity has been proposed as an indirect way of improving litter size (Bennett and Leymaster, 1989, 1990). In rabbits, Blasco et al. (1994) proposed using unilateral ovariectomy to measure uterine capacity.

There is little information on genetic parameters of uterine capacity. Heritability of uterine capacity was low (0.05, and 0.11 in rabbits reported by Bolet et al. (1994) and Blasco et al. (2005) respectively; 0.08 in mice (Kirby and Nielsen, 1993).

In pigs, there is only one experiment of selection for uterine capacity (Leymaster and Christenson, 2000) and their results have not been fully published yet. There are three more experiments of selection for uterine capacity: two experiments of divergent selection in rabbits (first experiment: Bolet et al., 1994; Santacreu et al., 1994; second experiment: Blasco et al., 2005; Mocé et al., 2005; Santacreu et al., 2005), and one experiment in mice (Clutter et al., 1990; Gion et al., 1990; Kirby and Nielsen, 1993). The estimated responses to selection in these experiments are summarized in Table 1.5.

In rabbits, in the first experiment (Bolet et al., 1994), selection was performed on number of dead fetuses from implantation to birth. After 4 generations of selection it was observed that the number of dead fetuses did not change and no significant response was obtained in litter size and its components. The second experiment consisted in selection on litter size in unilateral ovariectomized females, which includes both embryo and fetal survival (Blasco et al., 2005; Mocé et al., 2005; Santacreu et al., 2005). After 10 generations of selection for uterine capacity, correlated response to selection in litter size was not symmetric and a response was detected in the low line. A divergence of 2.35 kits was found between the high and low lines, mainly because of a higher correlated response in the low line.

In mice, Gion et al. (1990) and Kirby and Nielsen (1993) found a favorable correlated response in litter size when selecting for high uterine capacity, but selection for uterine capacity was not more effective than direct selection for litter size.

In conclusion, direct responses to increase uterine capacity and correlated responses in litter size were low or close to zero in rabbits and mice.

#### **2.4 Index selection for components of litter size: Ovulation rate and prenatal survival**

Cunningham et al. (1979) suggested that litter size could be regarded as a natural index of ovulation rate and embryonic survival. Johnson et al. (1984) used this idea to develop a model in which litter size is determined by the product of ovulation rate and embryonic survival and an index was constructed to optimize weights on component traits. Selection on the optimum index was predicted to increase ovulation rate

restricting the decrease in embryonic survival and to increase litter size more than direct selection.

**Table 1.5:** Responses to selection to increase uterine capacity (UC) and correlated responses in ovulation rate (OR), litter size (LS) and prenatal survival (PS) estimated per generation.

Species		Rabbits (1 <sup>st</sup> exp.)		Rabbits (2 <sup>nd</sup> exp.)	Pigs	Mice
Generations		10		4	11	13 <sup>6</sup> , 21 <sup>7</sup>
Method		Control Population <sup>1,2</sup>	Genetic Trends <sup>3</sup>	Genetic Trends <sup>4</sup>	Control population <sup>5</sup>	Control population
RESPONSE	UC (kits)	-0.01 <sup>1</sup>	0.08	-0.15	0.11	0.10 (0.02)
	OR (ova)	-0.03 <sup>2</sup>	0.03	-0.3	0.00	0.03 <sup>6</sup>
	LS (kits)	0.05 <sup>2</sup>	-	-	0.08	0.00 <sup>7</sup>
	PS (%)	0.5 <sup>2</sup>	0.4	0	-	0.3 <sup>6</sup>

<sup>1</sup> Mocé et al., 2005; <sup>2</sup> Santacreu et al., 2005; <sup>3</sup> Blasco et al., 2005; <sup>4</sup> data calculated from results presented in Santacreu et al., 1994, assuming a symmetric response; <sup>5</sup> Leymaster and Christenson, 2000; <sup>6</sup> Gion et al., 1990; <sup>7</sup> Kirby and Nielsen, 1993;

\*Standard errors (SE) in parenthesis.

Modified from Laborda (2011).

To our knowledge, there are only two experiments of selection for an index of ovulation rate and prenatal survival: one in pigs (Johnson et al., 1984; Neal et al., 1989; Bennett and Leymaster, 1989, 1990; Casey et al., 1995; Johnson et al., 1999), and the other one in mice (Clutter et al., 1990; Gion et al., 1990; Kirby and Nielsen, 1993; Ribeiro et al., 1997a, b).

Selection was efficient in increasing litter size when compared to the control line (Table 1.6) but this response was similar to the observed response to direct selection for litter size in other experiments. In pigs, the index was recalculated during the experiment to optimize response to selection. In mice, response to selection was estimated by comparison with a control line and with a line selected for litter size (Gion et al., 1990). As in pigs, litter size increased with selection compared with the control line (Table 1.6), but it increased at a similar rate to the line selected for litter size. The increase in litter size in the mice line selected for the index was due to a higher ovulation rate and prenatal survival in the selected line than in the control line. The index was used along

the selection experiment without reweighting their components. The authors suggested that selection for the index could have been more effective than selection for litter size if optimally weighted components have been used.

Summarizing, in these experiments, response was lower than expected. The reason for the lower than expected responses is probably the construction of the index using genetic correlations estimated with low precision (Falconer and Mackay, 2001), which is usually a problem in this kind of experiments. Besides, another limit of the index selection is the determination of the optimum economic weights and their reweighting along the selection process.

**Table 1.6:** Responses to selection in ovulation rate (OR), litter size (LS) and prenatal survival (PS) estimated per generation in pigs and mice selected for an index of ovulation rate and prenatal survival, with their standard errors (in parenthesis).

Species		Pigs		Mice
Generations		10 <sup>1</sup> , 11 <sup>2</sup>		13 <sup>3</sup>
Method		Control population <sup>1</sup>	REML <sup>2</sup>	Control population <sup>3</sup>
RESPONSE	OR (ova)	0.78 (0.04)	0.67 (0.12)	0.15 <sup>3</sup>
	LS (kits)	0.11 (0.05)	0.21 (0.04)	0.17 (0.01) <sup>3</sup>
	PS (%)	-0.9 (0.1)	-0.01 (0.01)	0.15 <sup>3</sup>

<sup>1</sup>Casey et al., 1996; <sup>2</sup>Johnson et al., 1999; <sup>3</sup>Gion et al., 1990.

Modified from Laborda (2011).

### 2.5 Two-stage selection for ovulation rate and litter size

An alternative to selection for an index could be two-stage selection, which would be less affected by the precision of the genetic correlations. One experiment of two-stage selection in a line of pigs (Line IOL) was performed by Ruiz-Flores and Johnson (2001). It was based on theoretical work of Bennett and Leymaster (1989; 1990a, b) and experimental results of Johnson et al. (1984). In the first stage, females born in litters with highest number of fully formed pigs were selected. In stage two, these females underwent laparotomy to count their ovulation rate at second estrus, and they were selected on their ovulation rate. Line IOL was previously selected for an index selection of ovulation rate and embryo survival during 8 generations. The estimated heritabilities of ovulation rate and litter size were higher than those in rabbits (0.42 and 0.18 for

ovulation rate and litter size respectively). Also, the genetic correlation between ovulation rate and litter size was highly moderate (0.52) but it was reported without standard error. Response to 8 generations of selection, estimated as the regression of line means on generation number was greater than expected for the number total born ( $0.33 \pm 0.06$  pigs per generation) and for ovulation rate ( $0.26 \pm 0.07$  ova per generation). Thus, litter size increased 130% compared with ovulation rate. These responses in litter size are higher than the responses observed in other experiments of direct selection for litter size in pigs. Besides, two-stage selection led to an increase in prenatal survival (7.87%). Two-stage selection for ovulation rate and number of fully formed pigs was effective because litter size in gilts with increased ovulation rate was a good measure of uterine capacity.

In rabbits, the first experiment of two-stage selection for ovulation rate and litter size is currently being carried out in the Universitat Politècnica de València (UPV). Results from seven generations of two-stage selection for ovulation rate and litter size are going to be presented in this work.

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## **Chapter 2**

### **Objectives**

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## **Objectives**

The objectives of this Thesis are:

1. To study the phenotypic and genetic parameters of ovulation rate, litter size, embryonic, fetal and prenatal survival rates in a rabbit population selected for six generations for ovulation rate and then for seven generations for both ovulation rate and litter size.
2. To estimate genetic responses to selection for ovulation, litter size, implanted embryos and survival rates in the same rabbit line.

## **Chapter 3**

### **Genetic selection for litter size and ovulation rate in rabbits: estimation of genetic parameters, direct and correlated responses**

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### **Abstract**

The aim of this work was to estimate direct and correlated responses in survival rates in an experiment of selection for ovulation rate and litter size in rabbits (OR\_LS line). The experiment consisted of 2 periods of selection. In period 1, selection was performed for ovulation rate during 6 generations. In period 2, line underwent a two-stage selection for ovulation rate and litter size during 7 generations. Selection in period 1 was based on the phenotypic value of ovulation rate estimated at d 12 of gestation by laparoscopy. Two-stage selection was based on the phenotypic value of ovulation rate and the average litter size over the first two parities. Total selection pressure was about 30%. The line had approximately 17 males and 75 females per generation. Traits recorded were: ovulation rate (OR) estimated as the number of corpora lutea in both ovaries; number of implanted embryos (IE), estimated as the number of implantation sites; litter size (LS), estimated as total number of rabbits born recorded at each parity; embryo survival (ES) estimated as IE/OR, fetal survival (FS) estimated as LS/IE, and prenatal survival (PS) estimated as LS/OR. Data were analyzed using Bayesian methodology. The estimated heritabilities of LS, OR, IE, ES, FS and PS were 0.07, 0.21, 0.10, 0.07, 0.12 and 0.16 respectively. The estimated repeatabilities of LS, OR, IE and ES were 0.16, 0.27, 0.20 and 0.14 respectively. In the first period of selection, OR increased 1.36 ova in 6 generations, but no correlated response was observed in LS due to a decrease on fetal survival. Correlated responses for implanted embryos, embryo, fetal and prenatal survival in the first selection period were 1.11, 0.00, -0.04 and -0.01 respectively. After 7 generations of two-stage selection for ovulation rate and litter size, OR increased 1.0 ova and correlated response on LS was 0.9 kits. Correlated responses for implanted embryos, embryo, fetal, and prenatal survival in the second selection period were 1.14, 0.02, 0.02, and 0.07 respectively. Two-stage selection for ovulation rate and litter size could be a promising procedure to improve litter size in rabbits.

**Key words:** Rabbit, two-stage selection, litter size, ovulation rate, survival rates.

## INTRODUCTION

Litter size is the trait responsible for most of the variation in overall reproductive performance in polytocous species, especially rabbits and pigs. Heritability of litter size in rabbits and other prolific species is low. Unlike mice, response to direct selection for litter size in closed populations has been low in most experiments of rabbits and pigs (reviewed by Mocé and Santacreu, 2010 in rabbits, Rothschild and Bidanel, 1998 in pigs).

In early experiments, several authors predicted greater response in litter size from selecting for indexes of its component traits than from direct selection for litter size in pigs (Johnson et al., 1984; Bennett and Leymaster, 1989, 1990) and mice (Clutter et al., 1990; Ribeiro et al., 1997a, b). These same authors concluded that an optimized selection index can produce a better balance of genetic changes in components of litter size than did direct selection for either ovulation rate or litter size. Cunningham et al. (1979) suggested that litter size could be regarded as a natural index of ovulation rate and embryonic survival. However, response on litter size after selection based on indexes of ovulation rate and prenatal survival was similar to the observed response after direct selection for litter size (Gion et al., 1990 in mice; Casey et al., 1994 in pigs).

The only experiment in which the response in litter size was higher than in the experiments of direct selection for litter size consisted in a two-stage selection experiment designed by Ruiz-Flores and Johnson (2001) in pigs; selection was performed for ovulation rate and number of fully formed pigs at birth in a line previously selected for an index to increase ovulation rate and embryonic survival. Response obtained in that experiment for eight generations of selection was higher ( $0.33 \pm 0.06$  pigs per generation) than the responses observed in other experiments of selection for litter size in rabbits and pigs (around 0.1 young per generation in both species). Selection on litter size was also successful in an experiment with pigs previously selected for ovulation rate (Lamberson et al., 1991; Johnson, 1992). Response obtained in this experiment for eight generations was 1.06 pigs (0.13 pigs per generation).

The first experiment of two-stage selection for ovulation rate and litter size in rabbits is currently being carried out at the Universitat Politècnica de València (UPV). The objective of this experiment is to test whether selection on both ovulation rate and litter

size is successful for increasing litter size in a line previously selected for ovulation rate. Responses in ovulation rate, litter size, implanted embryos and survival rates were estimated for six generations of direct selection for ovulation rate, and for seven generations of two-stage selection for ovulation rate and litter size.

## **I. MATERIALS AND METHODS**

All experimental procedures involving animals were approved by the Universitat Politècnica de València Research Ethics Committee.

### **1. Animals and experimental design**

Animals involved in this study came from the line OR\_LS whose origin was a synthetic line (V) founded by mating crossbred males and females of two commercial hybrids that were commonly used as maternal lines. Line V was first selected for litter size at weaning for 12 generations (García and Baselga, 2002), then for high uterine capacity (CU+) for 10 generations (Blasco et al., 2005), and then selection was relaxed for 6 generations. From there, founders were chosen at random to create the line OR\_LS. This line underwent 13 generations of selection and 2 periods of selection can be distinguished:

1. Selection for ovulation rate: from generation 0 to 6, females were selected only for ovulation rate at second gestation estimated by laparoscopy. Selection for ovulation rate was continued until generation 10 (Line OR) and results were already presented in previous articles by Laborda et al. (2011, 2012a, b).
2. Two-stage selection for ovulation rate and litter size: from generation 7 to 13, a two-stage selection experiment was carried out. In stage one, females were selected having high ovulation rate at second gestation. In stage two, selection was for the higher average litter size over the first two parities of females from stage one. There were no generations of relaxed selection between both periods of selection. Thus, lines OR\_LS and OR have the first 6 generations in common and share 4 contemporaneous generations.

In both periods and stages of selection, pressure of selection in females was about 30%. Males were selected within sire families from litters of best dams to avoid increase of inbreeding. Two males, a breeder and an alternate were selected from each sire family. The base population consisted of 85 females and 19 males. Does were mated for the

first time at 18-20 weeks of age and 11-12 days after each parturition thereafter. Females which did not accept males were mated again one week afterwards. Pregnancy was checked approximately 13 days after mating by simple palpation of the abdomen. Animals were bred at the experimental farm of the Universitat Politècnica de València. Cages were “flat-deck”, with extractable nest box with isolated plastic floor. Does were kept under a constant photoperiod of 16-h light: 8-h dark with controlled ventilation and fed a commercial diet.

## **2. Traits**

Ovulation rate (OR), estimated as the number of corpora lutea in both ovaries, and the number of implanted embryos (IE), estimated as the number of implantation sites, were measured by laparoscopy at d 12 of second gestation. Surgical technique is described by Argente et al. (1997) and it was shown by Santacreu et al. (1990) to be an accurate technique for measuring ovulation rate without affecting litter size. Litter size (LS) was measured as the total number of kits born per litter; it was measured in a maximum of 5 parities in each female. Embryo survival (ES) was estimated as IE/OR, fetal survival (FS) was estimated as LS/IE and prenatal survival (PS) was estimated as LS/OR. Females from all generations had a second measurement of OR, females from the 1<sup>st</sup> to the 5<sup>th</sup> generation and females from 12<sup>th</sup> and 13<sup>th</sup> generations had a second post mortem measurement of IE, and ES. Data from 969 laparoscopies and 4370 parities were analysed. Number of records for each trait will be presented ahead in Table 1. The number of animals in the pedigree was 1289.

## **3. Statistical Analysis**

Data from 13 generations of selection were used in the analysis. Bayesian inference was used.

Bivariate and trivariate repeatability animal models were fitted in order to estimate the genetic parameters and genetic responses. Genetic parameters and correlations between OR and LS were estimated using bivariate model. Trivariate analyses were used to estimate genetic parameters between traits different from OR and LS. Each three-trait analysis included ovulation rate, litter size and one of the four remaining traits. The model assumed for OR, LS, IE and ES was:

$$y_{ijklm} = P_i + YS_j + L_k + a_l + p_l + e_{ijklm}$$

where  $P_i$  is the effect of parity (5 levels for LS, 4 levels for the other traits, since there was no records for them in the first gestation),  $YS_j$  is the effect of year-season (one year season every three months: 38 levels for LS, 37 levels for OR and IE and 34 levels for ES),  $L_k$  is the effect of lactation state of the doe (2 levels: 1 for lactating and 2 for not lactating does when mated),  $a_l$  is the additive value of the animal,  $p_l$  is the permanent environmental and non-additive effects of the doe and  $e_{ijklm}$  is the residual of the model. The model for FS and PS did neither have the parity effect nor the permanent environmental effect, because records came only from the second parity, and the year-season effect had 30 levels.

For the bivariate repeatability model, the traits were assumed to be conditionally normally distributed as follows:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} | \mathbf{b}_1, \mathbf{b}_2, \mathbf{a}_1, \mathbf{a}_2, \mathbf{p}_1, \mathbf{p}_2, \mathbf{R} \sim \mathbf{N} \left( \mathbf{X} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \mathbf{Z} \begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} + \mathbf{W} \begin{bmatrix} \mathbf{p}_1 \\ \mathbf{p}_2 \end{bmatrix}, \mathbf{R} \right)$$

where  $\mathbf{b}_1$  and  $\mathbf{b}_2$  were random vectors including the effects of YS, L and P;  $\mathbf{a}_1$  and  $\mathbf{a}_2$  were vectors of individual additive genetic effects;  $\mathbf{p}_1$  and  $\mathbf{p}_2$  were vectors of permanent environmental effects.  $\mathbf{X}$ ,  $\mathbf{Z}$  and  $\mathbf{W}$  were known incidence matrices;  $\mathbf{R}$  was the residual (co)variance matrix. Between individuals, only the additive random effects were assumed correlated. Between traits, the additive, the permanent environmental and the residual effects were assumed correlated. The residual (co)variance matrix can be written as  $\mathbf{R}_0 \otimes \mathbf{I}_n$ , with  $\mathbf{R}_0$  being the  $2 \times 2$  residual (co)variance matrix between the traits analyzed and  $\mathbf{I}_n$  an identity matrix of appropriate order. Bounded uniform priors were used to represent vague previous knowledge of distributions of  $\mathbf{b}_1$  and  $\mathbf{b}_2$ . Prior knowledge concerning additive and permanent effects was represented by assuming that they were normally distributed, conditionally on the associated (co)variance components, as follows:

$$\begin{bmatrix} \mathbf{a}_1 \\ \mathbf{a}_2 \end{bmatrix} | \mathbf{G} \sim \mathbf{N}(\mathbf{0}, \mathbf{G}) \quad \begin{bmatrix} \mathbf{p}_1 \\ \mathbf{p}_2 \end{bmatrix} | \mathbf{P} \sim \mathbf{N}(\mathbf{0}, \mathbf{P})$$

where  $\mathbf{0}$  was a vector of zeroes,  $\mathbf{G}$  was the genetic (co)variance matrix and  $\mathbf{P}$  was the (co)variance matrix of the non additive genetic plus permanent environmental effects of

the doe. Matrices G and P could be written as  $G_0 \otimes A$  and  $P_0 \otimes I_s$ , respectively, where  $G_0$  and  $P_0$  were the  $2 \times 2$  genetic and permanent (co)variance matrices, A was the known additive genetic relationship matrix and  $I_s$  the identity matrix of the same order as the number of levels of permanent effects. Bounded uniform priors were used for the components of the (co)variance matrices  $R_0$  and  $G_0$  and  $P_0$ . For trivariate repeatability analyses the order of R, G and P matrices was  $3 \times 3$ .

Marginal posterior distributions of all unknowns were estimated by using the Gibbs sampling algorithm. Data augmentation was carried filling the data vector to have the same design matrices for all traits. Augmented data were not used for inferences, but permitted to simplify computing by sampling from a predictive distribution of missing data (Sorensen and Gianola, 2002). The program TM developed by Legarra et al. (2008) was used for all Gibbs sampling procedures. After some exploratory analyses, chains of 1,000,000 samples were used, with a burning period of 200,000. One sample each 100 for the bivariate analysis and one sample each 500 for the trivariate one was saved to avoid high correlations between consecutive samples. Convergence was tested using the Z criterion of Geweke.

## II. RESULTS AND DISCUSSION

Descriptive statistics for all traits are presented in Table 3.1. Realized cumulative selection differentials for OR and LS, means and standard deviations (SD) for all traits in generations 0 to 13 are presented in Table 3.2. Values are in agreement with the ones published by other authors in maternal rabbit lines (Brun et al., 1992; Garcia and Baselga, 2002; Piles et al., 2006; Theau-Clement et al., 2009). For the period of direct selection for ovulation rate, cumulative differential of selection for OR was 17.2 ova. For the two-stage selection, corresponding to the period from generation 7 to generation 13, cumulative differentials of selection for OR and LS were 10.16 ova and 15.82 kits respectively.

**Table 3.1.** Descriptive statistics for litter size (LS), ovulation rate (OR), implanted embryos (IE), embryo survival (ES), fetal survival (FS), and prenatal survival (PS).

<b>Trait</b>	<b>N</b>	<b>Mean</b>	<b>s.d</b>	<b>Min</b>	<b>Max</b>	<b>CV (%)</b>
LS	3563	9.0	3.13	1.0	18	34.6
OR	1703	15.8	2.56	8.0	25	16.1
IE	1315	12.2	3.8	1.0	23	31.1
ES	1311	0.76	0.22	0.06	1.0	29.0
FS	884	0.75	0.17	0.07	1.0	22.6
PS	889	0.59	0.20	0.05	1.0	33.9

N: number of data; s.d: standard deviation; CV: coefficient of variation.

### 1. Genetic and phenotypic parameters

For all the traits analyzed, Monte Carlo standard errors were small and are not offered in the tables. The Geweke test did not detect lack of convergence in any case, except for fetal survival. Mean and median are equal for all the traits showing that, in all cases, the marginal posterior distributions were symmetric. Median has some advantages; it is not sensitive to outlying points and some transformations, and the risk of the estimator is minimal when the loss function is the absolute value of the difference between the true and the estimated value. Features of the estimated marginal posterior distributions of heritability and repeatability for the traits studied are summarized in Table 3.3. In general, estimates of heritabilities were low with the exception of OR, which was moderate (0.21), with a probability of 95% of being at least 0.14 (value  $k$ ; Table 3.3). Both LS and IE had low heritability estimates (0.07 and 0.10, respectively). For estimates heritabilities of survival rates, ES showed the lowest value (0.07). Heritability estimate of PS was 0.16 with a probability 99% of being at least 0.10 and of the same order of magnitude as that of FS (0.12). Estimates of the genetic parameters of fetal survival are scarce in the literature. Our result was similar with the one estimated with high error standard reported by Blasco et al. (1993) but slightly lower than the estimate published in rabbits by Laborda et al. (2012).

In general, our heritability estimates for the majority of traits were in agreement with other studies in rabbits (Blasco et al., 1993, 1996; Argente et al., 2000; Garreau et al., 2004; Laborda et al., 2011, 2012), in pigs (Johnson et al., 1999; Ruiz-Flores and Johnson, 2001; Rosendo et al., 2007) and in mice (Clutter et al., 1990). In the case of

**Table 3.2.** Means and SD (in parentheses) for ovulation rate (OR), litter size (LS), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS) in generations 0 to 13.

	Generation													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
<b>N</b>	85	75	92	80	65	59	102	67	82	74	62	49	76	76
<b>S<sup>*,a</sup></b>	2.71	2.43	3.65	2.91	3.85	1.65	1.65	1.51	1.15	1.55	1.01	1.53	1.76	-
<b>S<sup>*,b</sup></b>	0.44	0.68	0.65	0.37	0.09	0.23	2.13	2.34	2.41	1.86	2.64	1.94	2.50	-
<b>OR<sup>a</sup></b>	14.9 (2.2)	15.5 (2.7)	15.8 (2.6)	16.4 (2.4)	15.8 (2.7)	15.5 (2.4)	16.3 (2.3)	15.9 (2.5)	15.7 (2.4)	15.5 (2.7)	16.4 (2.4)	16.1 (2.5)	16.5 (2.9)	16.1 (2.6)
<b>LS<sup>b</sup></b>	8.1 (3.0)	8.5 (2.6)	9.1 (2.8)	9.1 (3.0)	8.6 (2.9)	8.7 (3.1)	9.3 (2.9)	9.1 (3.3)	8.8 (3.5)	9.4 (3.0)	9.4 (3.5)	9.6 (3.1)	9.1 (3.1)	9.6 (3.2)
<b>IE<sup>c</sup></b>	12.5 (3.1)	12.6 (3.6)	12.5 (3.6)	12.1 (3.6)	11.1 (4.1)	11.5 (4.3)	13.1 (3.4)	12.1 (4.1)	12.5 (4.0)	12.9 (3.3)	12.6 (4.5)	11.5 (3.7)	12.4 (3.7)	11.5 (4.4)
<b>ES</b>	0.82 (0.18)	0.81 (0.20)	0.79 (0.20)	0.75 (0.21)	0.71 (0.24)	0.74 (0.26)	0.79 (0.18)	0.75 (0.23)	0.76 (0.21)	0.82 (0.18)	0.76 (0.24)	0.73 (0.24)	0.76 (0.20)	0.71 (0.26)
<b>FS</b>	0.72 (0.19)	0.73 (0.20)	0.78 (0.17)	0.68 (0.22)	0.75 (0.16)	0.69 (0.18)	0.74 (0.14)	0.75 (0.19)	0.75 (0.18)	0.79 (0.15)	0.80 (0.15)	0.79 (0.14)	0.74 (0.16)	0.79 (0.17)
<b>PS</b>	0.59 (0.19)	0.57 (0.18)	0.62 (0.20)	0.51 (0.22)	0.58 (0.19)	0.55 (0.21)	0.58 (0.17)	0.58 (0.19)	0.57 (0.19)	0.66 (0.18)	0.61 (0.22)	0.58 (0.19)	0.57 (0.19)	0.63 (0.24)

N: number of females at each generation. S<sup>\*</sup>: Selection differential applied to animals at generations 0 and consecutively to the other generations for ovulation rate [superscript a] and litter size [superscript b]. <sup>a</sup> Unit = ova. <sup>b</sup> Unit = kits. <sup>c</sup> Unit = embryos.

OR, recent studies in pigs reported higher heritability estimates than in rabbits (Ruiz-Flores and Johnson, 2001; Rosendo et al., 2007).

The repeatability estimate of LS was 0.16 with HPD<sub>95%</sub> [0.13, 0.20] (Table 3.3). Repeatability estimate for LS agrees with estimates reported for a maternal line by Khalil (1993), but are lower than the ones reported by Lukefahr and Hamilton (1997) ( $r = 0.23$ ) and Rastogi et al. (2000) ( $r = 0.30$ ). Ovulation rate and IE had a moderate repeatabilities estimates (0.27 and 0.20 for OR and IE respectively). The repeatability estimate of ES was 0.14 with HPD<sub>95%</sub> [0.08, 0.21] (Table 3.3). No repeatability or  $p^2$  estimates for the traits IE and ES have been reported in the literature. These repeatability estimates lead to an estimated ratio of the permanent environmental variance to the phenotypic variance ( $p^2$ ) of 0.09, 0.06, 0.07 and 0.07 for LS, OR, IE and ES, respectively. Our estimates of  $p^2$  are inside the range reported for litter size in rabbits (reviewed by Garreau et al., 2004).

**Table 3.3.** Features of the marginal posterior distributions of the heritability ( $h^2$ ) and the repeatability ( $r$ ) of litter size (LS), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES) and the heritability of fetal survival (FS) and prenatal survival (PS).

Traits	$h^2$	HPD <sub>95%</sub> ( $h^2$ )	$P_{0.10}$	$k$	$r$	HPD <sub>95%</sub> ( $r$ )
LS	0.07	0.02, 0.12	0.16	0.03	0.16	0.13, 0.20
OR	0.21	0.13, 0.29	1.00	0.14	0.27	0.21, 0.35
IE	0.10	0.05, 0.17	0.60	0.06	0.20	0.14, 0.26
ES	0.07	0.02, 0.12	0.19	0.03	0.14	0.08, 0.21
FS	0.12	0.06, 0.21	0.69	0.07	-	-
PS	0.16	0.10, 0.20	0.99	0.11	-	-

HPD<sub>95%</sub>: high posterior density interval at 95%.  $P_{0.10}$ : probability of the heritability being higher than 0.10.  $k$ : limit for the interval [ $k, +\infty$ ) of the heritability having a probability of 95%.

Features of the estimated marginal posterior distributions of the genetic correlations are summarized in Table 3.4. The estimate of the genetic correlation between LS and OR was positive ( $P = 0.92$ ; Table 3.4), but imprecise (HPD<sub>95%</sub>, Table 3.4). Estimated genetic correlations between LS and the remaining traits were positive (value  $P$ ; Table 3.4) being moderate with ES and FS and high with PS with a probability of 95% of being at least 0.78 (value  $k$ ; Table 3.4). Estimated genetic correlations of OR with FS

and PS were negative ( $P = 1.00$ ; Table 3.4), and nothing can be said about the sign of the estimated genetic correlation between OR and ES because it was imprecise.

Genetic correlation between OR and LS was in agreement with values reported by other authors (Ruiz-Flores and Johnson, 2001; Rosendo et al., 2007 in pigs; Blasco et al., 1993a and Laborda et al., 2011a). Estimated values of the genetic correlation between OR and LS founded in literature was generally positive, but they were reported without or with high standard errors. Higher genetic correlations between LS and IE were obtained in other experiments in rabbits and pigs, possibly because the number of fetuses was measured at a later point of gestation (Blasco et al., 1993a in rabbits; Johnson et al., 1999 in pigs). The positive genetic correlations between LS and survival rates agree with estimates in the literature (Blasco et al., 1993; Argente et al., 1997; Laborda et al., 2012 in rabbits; Rosendo et al., 2007 in pigs). Besides, the genetic correlation between OR and IE was in accordance with the ones obtained in rabbits (0.58 by Laborda et al., 2012), pigs (0.44 by Johnson et al., 1999) and mice (0.81 by Clutter et al., 1990).

Genetic correlations between all traits were estimated with low precision, especially for the genetic correlations between LS with both OR and ES, and OR with ES which have a very large interval of confidence. To obtain estimated genetic parameters with high precision, a large set of data would be needed. Nevertheless, the nature of this kind of experiments, which need techniques such as laparoscopy or slaughter the female to measure ovulation rate prevents from collecting a large number of data making the estimation of precise genetic correlations difficult. Although in this study, genetic parameters were estimated with limited database and low precision, they are within the range of the values reported in the literature.

Features of the estimated marginal posterior distributions of the phenotypic correlations are summarized in Table 3.5. Phenotypic correlation between LS and OR was positive ( $P = 1.00$ , Table 3.5) but low. Estimated phenotypic correlation between LS and IE was high and positive ( $P = 1.00$ , Table 3.5). The posterior mean of phenotypic correlation

**Table 3.4.** Features of the marginal posterior distributions of the genetic correlation between the traits analyzed: litter size (LS), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS).

Traits	mean	median	HPD <sub>95%</sub>	P	k
LS, OR	0.30	0.30	-0.12, 0.71	0.92 <sup>a</sup>	-0.05 <sup>a</sup>
LS, IE	0.66	0.68	0.34, 0.99	1.00 <sup>a</sup>	0.34 <sup>a</sup>
LS, ES	0.54	0.59	-0.04, 0.95	0.94 <sup>a</sup>	-0.04 <sup>a</sup>
LS, FS	0.63	0.63	0.34, 0.96	1.00 <sup>a</sup>	0.34 <sup>a</sup>
LS, SP	0.85	0.86	0.77, 0.91	1.00 <sup>a</sup>	0.78 <sup>a</sup>
OR, IE	0.70	0.72	0.44, 0.93	1.00 <sup>a</sup>	0.46 <sup>a</sup>
OR, ES	-0.09	-0.09	-0.53, 0.34	0.67 <sup>b</sup>	0.29 <sup>b</sup>
OR, FS	-0.53	-0.50	-0.82, -0.27	1.00 <sup>b</sup>	-0.30 <sup>b</sup>
OR, PS	-0.35	-0.35	-0.62, -0.09	0.99 <sup>b</sup>	-0.12 <sup>b</sup>

HPD<sub>95%</sub>: high posterior density interval at 95%; P: probability of the genetic correlation being greater than zero (superscript a), or less than zero (superscript b); k: limit for the interval <sup>a</sup> [k, +∞), <sup>b</sup> (-∞, k], having a probability of 95%.

**Table 3.5.** Features of the marginal posterior distributions of the phenotypic correlation between the traits analyzed: litter size (LS), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS)

Traits	mean	median	HPD <sub>95%</sub>	P	k
LS, OR	0.19	0.20	0.13, 0.26	1.00 <sup>a</sup>	0.14 <sup>a</sup>
LS, IE	0.72	0.72	0.70, 0.75	1.00 <sup>a</sup>	0.70 <sup>a</sup>
LS, ES	0.67	0.68	0.64, 0.71	1.00 <sup>a</sup>	0.65 <sup>a</sup>
LS, FS	0.49	0.49	0.45, 0.54	1.00 <sup>a</sup>	0.45 <sup>a</sup>
LS, PS	0.88	0.88	0.87, 0.89	1.00 <sup>a</sup>	0.87 <sup>a</sup>
OR, IE	0.40	0.40	0.35, 0.45	1.00 <sup>a</sup>	0.36 <sup>a</sup>
OR, ES	-0.09	-0.09	-0.15, -0.04	1.00 <sup>b</sup>	-0.05 <sup>b</sup>
OR, FS	-0.23	-0.23	-0.30, -0.17	1.00 <sup>b</sup>	-0.18 <sup>b</sup>
OR, PS	-0.25	-0.25	-0.31, -0.20	1.00 <sup>b</sup>	-0.20 <sup>b</sup>

HPD<sub>95%</sub>: high posterior density interval at 95%; P: probability of the genetic correlation being greater than zero (superscript a), or less than zero (superscript b); k: limit for the interval <sup>a</sup> [k, +∞), <sup>b</sup> (-∞, k], having a probability of 95%.

between OR and IE (Table 3.5) had similar magnitude and sign than the ones obtained in pigs and mice. Estimated phenotypic correlations between OR and survival rates were negative ( $P = 1.00$ , Table 3.5); however they were of low magnitude, especially the phenotypic correlation between OR and ES. Phenotypic correlations between LS and survival rates were positive ( $P = 1.00$ , Table 3.5), being moderate with ES and FS and high with PS.

Positive correlations between LS and survival rates and negative correlations between OR and both FS and PS were in agreement with the estimates founded in the literature (Blasco et al., 1993a; Blasco et al., 1993b for a review in rabbits; Johnson et al., 1999; Rosendo et al., 2007 in pigs).

## **2. Response to selection**

In each period of selection, total responses to selection for all traits were estimated by the difference of line means between first and last generations. The estimated responses to selection for OR, LS and IE are shown in Figure 3.1. The correlated responses in ES, FS, and PS are shown in Figure 3.2. We can distinguish two periods of genetic responses.

### **2.1 Selection for ovulation rate**

After six generations of selection, OR increased in 1.36 ova, almost 1.5% per generation (0.22 ova/generation, Figure 3.1). In this experiment, selection for OR did not practically modify LS; correlated response in LS was 0.30 kits in 6 generations (0.05 kit/generation, Figure 3.1). Thus, only 22 % of the average increase in ovulation rate was realized as more kits at birth. Implanted embryos increased 1.11 embryos in 6 generations (0.18 embryos/ generation, Figure 3.1). Prenatal survival apparently showed a little decrease (0.013 in 6 generations, Figure 3.2). We did not observe any response in ES, but FS decreased consistently (0.038 in 6 generations, around 0.9 % per generation, Figure 2). Thus, this decrease in fetal survival seems to be responsible for the lack of correlated response observed in litter size. Our results are in agreement with estimated responses published by Laborda et al. (2011, 2012a, b) using data of 10 generations of selection for OR (line OR).

Our estimated response of OR was similar to the ones reported in pigs by Leymaster and Christenson (2000) and in mice by Bradford (1969), but lower than those obtained in other studies (Cunningham et al., 1979; Rosendo et al., 2007 in pigs; Land and

Falconer, 1969 in mice). In these studies, the correlated response on litter size was close to zero, except the one observed by Cunningham et al. (1979), but it was estimated with a very high standard error ( $0.15 \pm 0.13$  pigs/ generation). In all cases, an increase in prenatal mortality was observed. As in this experiment, Freking et al. (2007) in pigs and Bradford (1969) in mice observed that post-implantation losses were the main cause for the uncorrelated response in litter size. Possible physiological causes for the lacking correlated response in litter size were already discussed with details by Laborda et al. (2011, 2012a, b).

In conclusion, the results show that selection for ovulation rate could increase fetal mortality, whereas embryo mortality does not seem to have been modified. This fetal mortality has been the main cause for the lacking observed correlated response in litter size. Further studies are needed to explain the mechanisms that have increased fetal mortality in rabbits selected for high ovulation rate.

## **2.2 Two-stage selection for ovulation rate and litter size**

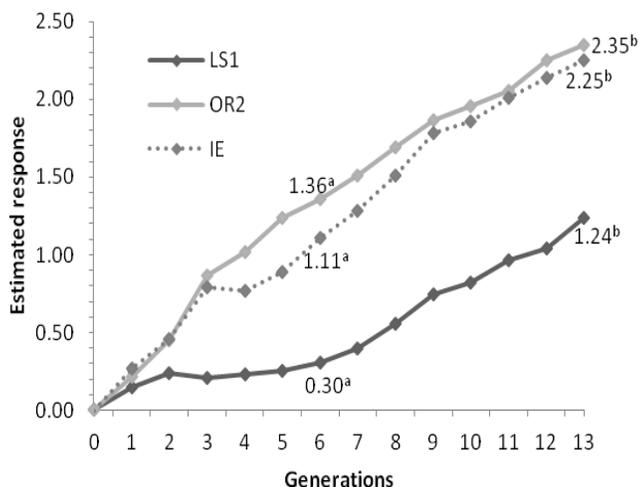
In the second period, seven generations of selection for ovulation rate and litter size have been performed. Ovulation rate continued increasing throughout the two-stage selection but with a lower rate than during the first period of selection, due to a decrease on the selection differential applied (Table 3.1). In fact, from generation 6, the proportion of females with extremely high ovulation rate increased 4.1% per generation in line OR against 0.9% in line OR\_LS. Response in OR was estimated to be 1 ova, almost 0.9 % per generation (0.14 ova /generation, Figure 3.1). Direct response for LS was approximately 0.9 kits (0.13 kit /generation, Figure 3.1). Thus, around 93 % of the average increase in ovulation rate was realized as more kits at birth. The correlated response in IE was 1.14 embryos (Figure 3.1). Both embryonic and fetal survivals have been shown to contribute with the same amount in the increase observed in prenatal survival. A small positive change in ES and FS was observed (approximately 0.020 in 7 generations, Figure 3.2). Prenatal survival increased 0.077 in 7 generations, around 2 % per generation (Figure 3.2). The direct response in litter size was similar to the response estimated by Lamberson et al. (1991) after direct selection for LS during 8 generations in a line previously selected to increase ovulation rate. In pigs, after 8 generations of two-stage selection Ruiz-Flores and Johnson (2001) obtained greater direct responses in number of fully formed pigs and ovulation rate ( $0.33 \pm 0.06$  pig/generation and  $0.26 \pm$

0.07 ova/generation respectively). Their estimate of correlated response in prenatal survival was similar to the one observed in this study (0.078 in 7 generations).

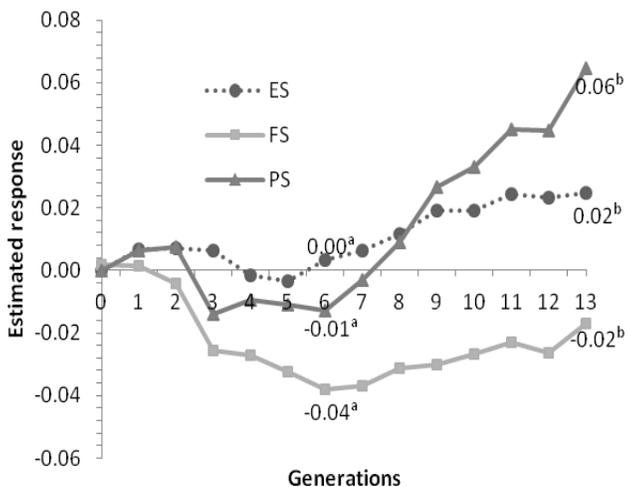
In the second period of selection, total number of kits born could be a good measurement of uterine capacity, since ovulation rate was high enough due to the direct selection applied during the first period. In populations selected for increasing ovulation rate, the total number of kits born is expected to represent uterine capacity more closely than in unselected populations (Lamberson et al., 1991, Johnson et al., 1999; Ruiz-Flores et al., 2001). Thus, selection in the second period was performed to ameliorate uterine capacity and then indirectly prenatal survival.

Observed changes in prenatal survival either happened during pre and post-implantation periods of gestation. In early stages of gestation, an improvement in the quality of oocytes (Torres, 1982 in rabbits; Koenig et al., 1986 in pigs) and lower variability of embryo development (Pope et al., 1988; Xi et al., 1990 in pigs) could explain the increase in embryo survival. To assess oocyte quality in both lines OR and OR\_LS, one study was designed by Laborda et al. (2012c) to measure concentrations of ATP and glutathione (GSH), the main compound that protects the cell against the oxidative stress. Their results showed a difference in the concentration of GSH of 0.7 pmol /oocyte between the line OR and the line OR\_LS that could indicate a higher number of mature oocytes in the line OR\_LS. No difference between lines was found for ATP concentration. Both oocyte quality and variability of embryo development can affect embryonic and fetal survival. In later stages of gestation, an increase in prenatal survival could be associated with more uterine space and resources (Adams, 1960; Hafez, 1969), and more blood supply to the fetuses (Hafez, 1965; Duncan, 1969; Argente et al., 2003 in rabbits).

Results from period one of selection show that, after six generations of selection for ovulation rate, ovulation rate responded to selection, but no correlated response on litter size was observed. Results from period two of selection show that, two-stage selection for ovulation rate and litter size would be effective in improving ovulation rate and litter size. Moreover, this increase has been due to reducing both pre and post-implantation mortalities.



**Figure 3.1:** Genetic trends for ovulation rate (OR), litter size (LS), and number of implanted embryos (IE) of line OR\_LS. This line was selected for OR from generation 0 to 6, and for OR and LS from generation 7 to 13. Superscript a: mean of the estimated breeding value of the character at generation 6. Superscript b: mean of the estimated breeding value of the character at generation 13.



**Figure 3.2:** Genetic trends for embryo survival (ES), fetal survival (FS) and prenatal survival (PS) of line OR\_LS, this line was selected for OR from generation 0 to 6, and for OR and LS from generation 7 to 13. Superscript a: mean of the estimated breeding value of the character at generation 6. Superscript b: mean of the estimated breeding value of the character at generation 13.

### **Implications**

Summarizing, the results obtained show that two-stage selection for ovulation rate and litter size could be more effective to increase both litter size and prenatal survival than either direct selection for litter size or ovulation rate. To support this hypothesis, results from this study will be compared with a control line which has been vitrified in generation six when the second period of selection was initiated.

### **ACKNOWLEDGEMENTS**

This study was supported by the Comisión Interministerial de Ciencia y Tecnología CICYT- AGL2005-07624-C03-01 CICYT- AGL2008-05514-C02-01 and by funds from Generalitat Valenciana research programme (Prometeo 2009/125).

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## **Chapter 4**

### **General Discussion**

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### **1. Selection for ovulation rate**

Selection for ovulation rate was proposed as an indirect way for increasing litter size in mice (Bradford, 1969; Land and Falconer, 1969), pigs (Cunningham et al., 1979; Leymaster and Christenson, 2000; Rosendo et al., 2007) and rabbits (Laborda et al., 2011, 2012). In rabbits, selection for ovulation rate was performed for 10 generations and results have been already presented and discussed with details by Laborda (Doctoral thesis, 2011). In this study, only data from the first 6 generations which correspond to the first period of selection has been analyzed and results agree with those of Laborda (2011). Ovulation rate increased but there was no correlated response in litter size.

In all experiments of selection for ovulation rate in pigs and mice, the correlated response in litter size was close to zero. A higher proportion of immature oocytes and less developed embryos, a greater variability in embryonic development and a higher embryonic or fetal competence for resources (reviewed by Santacreu, 2006 in rabbits; Geisert and Schmitt, 2002 in pigs) were suggested to explain the increase in prenatal survival. These different factors affecting embryonic and fetal mortalities have been summarized and discussed with details by Laborda (Doctoral thesis, 2011).

Selection for ovulation rate seems not to be a good alternative to improve litter size more efficiently than direct selection for litter size.

### **2. Two- stage selection for ovulation rate and litter size**

Selection for ovulation rate and other component of litter size (uterine capacity or prenatal survival) was proposed as an indirect mean of increasing litter size with the expectation that it could be more efficient than direct selection for only one of the components of litter size. Several authors predicted greater responses from index selection for optimally weighted components of litter size. However, in two experiments of index selection, responses were lower than expected (Casey et al., 1996 in pigs; Gion et al., 1990 in mice). The reason for the lower than expected responses is probably the construction of the index using genetic correlations estimated with low precision (Falconer and Mackay, 2001). The nature of this kind of experiments, which need laparoscopies, laparotomies or slaughtering the female to measure ovulation rate, prevents from collecting a large number of data, making the estimation of precise genetic correlations difficult.

Two-stage selection was proposed as an alternative to the index selection, which would be less affected by the precision of the genetic parameters and the models used for data analysis. There is an experiment of two-stage selection in pigs, performed by Ruiz-Flores and Johnson (2001) in which, response in litter size was approximately twice the response observed in experiments for litter size in pigs. These results were the basis for the two-stage selection performed during the second period of our experiment. Through the second period of selection, mean ovulation rate was high due to the direct selection applied during the first and second periods, and a subsequent number of potentially viable embryos exceeding uterine capacity was expected. Therefore, litter size could be a good measurement of uterine capacity. Thus, selection in the second period was performed to ameliorate indirectly uterine capacity (i.e prenatal survival).

After seven generations of two-stage selection, a change in litter size was observed (0.13 kit/ generation) as a result of the increase of both ovulation rate and prenatal survival (0.14 ova and 0.01 per generation for OR and PS respectively). Thus, two-stage selection resulted in approximately 30% greater response in litter size than direct selection for litter size. It could be concluded from this experiment that the applied two-stage selection procedure resulted in substantial changes in both ovulation rate and litter size at birth, with a subsequent reduction in prenatal mortality.

Similar conclusion was obtained by Ruiz-Flores and Johnson (2001) in the two-stage pig experiment, though there was a greater direct response in litter size ( $0.33 \pm 0.06$  pig/generation). This higher response in litter size could be due to a higher response in ovulation rate ( $0.26 \pm 0.07$  ova per generation) since their estimate of correlated response in prenatal survival was the same as the one estimated in our study (0.01 per generation). Generally, estimates of heritability for ovulation rate were higher in pigs than in rabbits, but causes of this high estimates in pigs were not clear.

Direct and correlated responses from this study were estimated by genetic trends in both periods of selection, as the difference of line means between first and last generations divided per generation number. This common method to estimate genetic response has one limit that it strongly depends on genetic parameters and the model used in the analysis. An alternative would be the use of a control population (Rochambeau et al., 1989; Baselga, 2004) (i.e the control population must be raised contemporaneously and under the same environment as the selected population). The control line has the

advantage of providing independent information of the model used in the data analysis. However, the main problems of using a control line are: the genetic drift that acts through generations and the undesired selection (usually for small size and closed populations), and the need of economic and experimental facilities. The use of cryopreserved control population can avoid disadvantages of maintaining control population without selection. Thus, in this experiment of selection, embryos from donor females belonging to 6<sup>th</sup> generation of line OR\_LS (just when the two-stage selection period started) were vitrified and stored in liquid N<sub>2</sub> to produce the control population. Two-stage selection will continue until generation sixteen, i.e. the 10<sup>th</sup> generation of two-stage selection, and responses will be estimated by comparison with the cryopreserved control population.

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## **Chapter 5**

### **Conclusions**

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1. The estimated heritabilities for all traits were low, with the exception of ovulation rate which had a moderate heritability (0.21).
2. The estimated genetic correlation between ovulation rate and litter size was positive but low. It was estimated with low precision, having a probability of 95% of being in the interval from -0.12 to 0.71.
3. The estimated genetic correlations between litter size and the survival rates were positive, being moderate with embryo survival and fetal survival and high with prenatal survival.
4. The estimated genetic correlations of ovulation rate with fetal survival and prenatal survival were negative. Nothing can be said about the sign of the estimated genetic correlation between ovulation rate and embryo survival.

**During the first period of selection:**

5. Estimated response for ovulation rate was 0.22 per generation, but litter size did not respond to selection, due to an increasing in fetal mortality.

**During the two-stage selection:**

6. Estimated response for litter size was 0.13 per generation, as a result of the increase in ovulation rate and prenatal survival. Estimated responses were 0.14 ova and 0.01 per generation for ovulation rate and prenatal survival, respectively.
7. The increase in prenatal survival occurred during both pre and post-implantation periods.