

## Criteria and methodology used to characterize reproductive abilities of pure and crossbred rabbits in comparative studies

Bolet G., García-Ximénez F., Vicente J.S.

in

Rouvier R. (ed.), Baselga M. (ed.).  
Rabbit production and genetics in the Mediterranean area

Zaragoza : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 17

1991

pages 95-104

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=92605166>

To cite this article / Pour citer cet article

Bolet G., García-Ximénez F., Vicente J.S. **Criteria and methodology used to characterize reproductive abilities of pure and crossbred rabbits in comparative studies.** In : Rouvier R. (ed.), Baselga M. (ed.). *Rabbit production and genetics in the Mediterranean area*. Zaragoza : CIHEAM, 1991. p. 95-104 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 17)



<http://www.ciheam.org/>  
<http://om.ciheam.org/>

# Criteria and methodology used to characterize reproductive abilities of pure- and crossbred rabbits in comparative studies

G. BOLET\*  
F. GARCIA-XIMENEZ\*\*  
J.S. VICENTE\*\*

\* INRA, STATION D'AMÉLIORATION GÉNÉTIQUE DES ANIMAUX, B.P.27, 31326 CASTANET-TOLOSAN, FRANCE

\*\* UNIVERSIDAD POLITÉCNICA DE VALENCIA, DPTO DE CIENCIA ANIMAL, CAMINO DE VERA 11, 46020 VALENCIA, SPAIN

**SUMMARY** - In a comparative study involving purebreeding and crossbreeding of rabbits a mere estimation of their zootechnical value is not sufficient. We therefore propose a procedure based on two approaches:

- use of DICKERSON's model to resolve genetic values into genetic effects (additive and heterosis effects, direct, maternal and grand-maternal effects) in crossbreeding and in some embryo transfer experiments. With a view to understanding the genetic and physiological determinism of litter size in rabbits, this model can be used to analyse some characters measured during pregnancy. These characters and the way of measuring them are described.
- use of experimental techniques at different stages of pregnancy to dissociate maternal, paternal and embryonic genotypes. Some of them are already available for genetic studies while others have to be improved. The most useful and attractive techniques are reviewed, e.g. laparoscopy, embryo freezing and transfer, artificial insemination, in vitro fertilisation.

**Key words:** Rabbit, reproduction, crossbreeding, breed comparison, genetic effects, ovulation, embryo.

**RESUME** - "Critères et méthodologie utilisés pour la caractérisation des qualités reproductives de lapins croisés et de race pure lors d'études comparatives". Dans une comparaison de races pures et de croisement, une simple estimation des valeurs zootechniques n'est pas suffisante. Nous proposons une démarche basée sur deux approches.

- Le modèle de décomposition des effets génétiques de DICKERSON (effets additifs et d'hétérosis, directs, maternels ou grand-maternels) peut être utilisé pour analyser des expériences de croisement et certains types d'expériences de transfert d'embryons. Pour comprendre le déterminisme génétique et physiologique de la taille de portée chez le lapin, il peut être utilisé pour analyser un certain nombre de mesures réalisées au cours de la gestation. Nous décrivons ces caractères et la façon de les mesurer.
- Un certain nombre de techniques expérimentales permettent d'intervenir à différentes étapes de la gestation et dissocier les génotypes du père, de la mère et de l'embryon. Certaines sont dès maintenant utilisables pour des études génétiques, d'autres doivent auparavant être améliorées. Nous passons en revue les plus courantes et les plus prometteuses (laparoscopie, congélation et transfert d'embryons, insémination artificielle, fécondation in vitro....).

**Mots-clés:** Lapin, reproduction, croisement, comparaison de races, effets génétiques, ovulation, embryon.

## Introduction

Before choosing a strategy for using genetic resources, the following points have to be defined:

- breeds or genotypes involved,
- methods for renewal of pure populations (if necessary including a genetic improvement programme),
- methods for using these breeds.

If several genotypes are available, these three steps will be defined on the basis of a certain number of parameters:

- the zootechnical value of the genotypes in pure- and crossbreeding,
- the genetic components of this value in pure- and crossbreeding.

In a comparative study of breeds, these two groups of parameters should therefore be measured. The purpose of the present paper is to describe some

methods and tools available for such measurements, especially those applied to reproductive performances. The factors of variation which affect these parameters (e.g. herd management method, reproductive rhythm) will not be treated here and therefore we are not going to mention the criteria describing the reproductive lifespan of doe rabbits, the choice of which depends on these factors of variation.

We shall limit ourselves to the genetic and physiological parameters of litters.

## Definition of parameters

### ZOOTECNICAL VALUE

This parameter is based on comparisons of purebred and crossbred genotypes; the conditions of this comparison are not treated in this publication, but will be mentioned elsewhere. Two points should however be emphasized, i.e. the sampling conditions of each population according to which compared animals are representative of the genotype to which they belong and the genotype/environment interactions rendering the choice of the environment(s) of the comparison very important.

### GENETIC COMPONENTS

A simple comparison in terms of mean breed value does not supply much information and does not permit to define an optimum strategy for the utilisation of crossbreds. The genetic value of a breed can be divided into a certain number of elements using for instance DICKERSON's model (1969). The direct genetic effects are the effects of an individual's genes on its performance; they are mainly additive (gI) and heterosis (hI) effects. The maternal genetic effects are the effects of the dam's genes which contribute to a trait affecting indirectly the performance of an individual; they can be direct (gM) and heterosis (hM) effects. The grand-maternal, paternal effects can also be defined. Concerning prolificacy, MATHERON and MAULEON (1979) suggested to define litter size as an offspring and not a dam trait. This allows us to take into account the influence of the sire of the litter and to better explain the maternal effects (e.g. ovulation rate and milk production are then defined as maternal effects). These authors showed that litter size depends on the joint action of different groups of genes from the young, their mother and grand-mother. Any strategy of discontinuous crossbreeding involves a phase of selection and renewal of pure breed populations. The major alternatives are: a pure breed selection independently of crossbreeding, a selection of pure breeds for heterosis or the complementarity

(SELLIER, 1982). In the case of the rabbit, BRUN (1985) discussed the interest of selection for crossbreeding. The choice of a strategy of selection and subsequent crossbreeding (SELLIER, 1976) will depend on the value of the above mentioned parameters and also on the genetic parameters by which the selection response in pure- and crossbreeding can be predicted (BRUN, 1985). These parameters are estimated from covariances between related individuals (FOULLEY and LEFORT, 1978) accounting for the additive, dominance and maternal environment effects.

## Measurement of crossbreeding genetic parameters

### STUDIED TRAITS

The analysis of litter size according to DICKERSON's model allows us to estimate crossbreeding parameters, but also to provide biological explanations of genetic variations of prolificacy. It leads to assessing the type of maternal effects by distinguishing for instance between ovulation rate, whose direct consequence is the theoretical maximum crowding of the uterine horns, and the effect of the uterine environment during gestation. Accordingly, an estimation of crossbreeding parameters for litter size will rapidly lead to determining these parameters more accurately using the analysis of the biological components of litter size. The major components are the number of ova laid by the females (currently named ovulation rate), their fertilization rate, and embryo survival and a distinction is made between the main phases of preimplantation, implantation, placentation and the foetal phase. These different points will be discussed in chapter III.

### ESTIMATION METHODS

#### Experimental crossbreeding

Crossbreeding parameters can be estimated from the information supplied by a certain number of crossings. Thus, by combining diallel, back and terminal crossings, all crossbreeding parameters can be estimated theoretically in the model set a priori, either on litter size at birth or at weaning (BRUN and ROUVIER, 1984; BRUN, 1990) or on the biological components during pregnancy, considering ovulation rate as a female trait and the number of embryos at the different stages as a litter trait (BOLET *et al.*, 1990b).

#### Interventions on pregnancy

Also experimental means are available to dissociate the genotype of the embryo from that of the dam. Thus,



with embryo transfer it is possible to freely choose and exploit the genotype of the donor, embryo (according to sire genotype) and recipient. These experiments can be analysed using DICKERSON's model and they theoretically make possible the estimation of parameters by reducing the number of different crossings and by combining pure- and crossbreds in donors and recipients (BOLET and THEAU-CLEMENT, 1985) (Table 1).

**Table 1. Decomposition of breeding types according to Dickerson's parameters in a crossbreeding experiment involving transfer of embryos.**

Genotype of										
Male	Female		$\mu$		gI		gM		hI	hM
	D	R		A	B	A	B			
A	A		1	1		1				
A	A	B	1	1			1			
A	A	A x B	1	1		1/2	1/2			1
A	B		1	1/2	1/2		1	1		
A	B	A	1	1/2	1/2	1		1		
A	B	A x B	1	1/2	1/2	1/2	1/2	1	1	

D : Donor doe.

R : Recipient doe.

A, B, A x B : Breeds and crossbreedings.

gI, gM : Direct and maternal genetic effects.

hI, hM : Direct and maternal heterosis effects.

**Table 2. Theoretical components of observed covariances between related individuals in the case of transfer of embryos between donor and recipient dams.**

Covariance between :	Var gI	Var dI	Cov gI gM	Var gM	Var dM + C	Var E
Dam/Daughter D = R	1/2	0	5/4	1/2	0	0
Sire/Daughter	1/2	0	1/4	0	0	0
Recipient/Daughter D ≠ R	0	0	3/4	1/2	0	0
Sibs D = R	1/2	1/4	1	1	1	0
Sibs D ≠ R						
Same R	1/2	1/4	0	1	1	0
Sibs 1 and 2 D1 = R1 D2m ≠ R2	1/2	1/4	1/2	0	0	0
Unrelated 1 & 2 D1 = R2 D2 = R1	0	0	1	0	0	0

D = Donor doe.

R = Recipient doe.

Var gI = Additive direct genetic variance.

Var dI = Dominance direct genetic variance.

Cov gI gM = Direct-maternal additive genetic covariances.

Var gM = Additive maternal genetic variance.

Var dM = Dominance maternal genetic variance.

Var C = Common environmental variance.

Var E = Residual environmental variance.

Using embryo transfer it is also possible to estimate within-breed genetic parameters by choosing the parental relationships between donors and recipients. This method was developed by RISKAL *et al.* (1985) in crossfostering (equivalent to transfers after birth) and subsequently proposed for embryo transfers (BOLET and THEAU-CLEMENT, 1985) (Table 2).

However, there are 2 limits in the transfer of embryos, on the one hand the difficulty in performing enough transfers to draw exhaustive conclusions and, on the other hand, the consideration of the transfer effect on embryo viability. Authors who have used this technique for genetic purposes in the rabbit, but also in other species, have been confronted with this problem (see for instance TORRES *et al.*, 1987a; BOLET and THEAU-CLEMENT, 1988).

With the aim of dissociating at the maximum the genotype of the embryo from that of the dam, other experimental techniques can be associated to embryo transfer. Thus, KOBAYASHI *et al.* (1983) used donor ovarian cultures, in vitro fertilization of recovered ova, embryo culture and then their transfer. This association of techniques (described in chapter IV) is interesting theoretically, but limited by the results obtained at each step; thus these authors used the ovaries of 52 donors and only obtained 9 births of recipients

## Description of traits

### OVULATION RATE

Sensu stricto, the ovulation rate corresponds to the number of oocytes released during ovulation (as a matter of fact this term is incorrect as it is not a rate and may be confused with the percentage of ovulating females, but it is currently used). It determines the maximum number of young produced by a female during each gestation. The most reliable estimation of ovulation rate therefore consists in counting the number of oocytes actually released by isolating them in the oviducts just at the end of ovulation (not taking into account those lost in the peritoneal cavity). However, if admitting that only one oocyte is released from each follicle (which might be wrong in some cases according to ADAMS, 1982), any system which allows to distinguish without error between ovulated and non ovulated follicles would represent a method for estimating the ovulation rate. After ovulation, a scar can be seen on the apical part of these follicles corresponding to the rupture of the peripheral layers. For determination of ovulation rate in late gestation or in pseudo-gestation, the usual method consists in counting the corpora lutea formed in the ovulated

follicles, characterized by their appearance and the presence of a protuberance at the ovulation point. There may however be slightly protuberating corpora lutea which are difficult to detect or corpora lutea within the follicles which have not succeeded in fully breaking their superficial layers (KATZ, 1988). If the observation is made with a magnifying glass after slaughter, the individual enucleation of the corpora lutea increases the counting reliability.

## EMBRYONIC DEVELOPMENT AND LOSS

Embryonic development is divided into various phases which are interesting to observe using one of the techniques described below. Our purpose is not to describe these different phases of embryonic and foetal development in the rabbit (see for instance THIBAUT, 1967; TORRES, 1982). However, let us briefly recall that these phases involve preimplantation, where the embryo changes from the 1-cell to the morula stage and then to the blastocyst stage, implantation, placentation and foetal phase. The parameters to be observed are numerous and different according to stages: size, number of cells, metabolic activity during preimplantation (see TORRES *et al.*, 1987b; GARCIA *et al.*, 1983a; BOLET and THEAU-CLEMENT, 1988; FISHER, 1989), the appearance, development of appendages, weight of the different components at the post-implantation and foetal stages (TORRES, 1974, 1982; GARCIA *et al.*, 1983b). This examination can be used to estimate the number of live embryos during the different phases and to characterize embryonic mortality.

Taken as a whole, embryonic mortality is the difference between the number of young born alive and ovulation rate. However, some points should be emphasized:

### Fertilization failure

Some or all of the oocytes released into the oviduct may not be fertilized. It is important to distinguish between fertilization failure and total mortality as the true range of the former may be underestimated (BOLET *et al.*, 1990a). It is difficult to establish whether an egg recovered at the 1-cell stage has been fertilized or not. The examination has to be done with a magnifying glass and may consist in determining the presence of 2 polar bodies and the 2 pronuclei (if necessary by staining) (TATEMOTO *et al.*, 1989) or considering morphological criteria (presence of cortical granules). This examination becomes more and more uncertain as the interval from the moment of fertilization increases. In the absence of gestation (diagnosed by abdominal palpation around day 11 - 12), examination of the ovaries allows to determine whether the female has ovulated or not (search for visible

corpora lutea throughout pseudo-gestation), but does not allow to distinguish between fertilization failure or total mortality. And yet when estimating the value of a genotype, it is important to be able to distinguish between fertilization failure, total losses and partial losses. The relative importance of these 3 parameters may be determining for the mode of utilisation of that genotype in a crossbreeding schedule as the genetic effects of the sire, the dam and the embryo are different in the 3 cases.

### Preimplantation losses

The accuracy of estimation of preimplantation losses mainly depends on the quality of embryo recovery and on their examination with a magnifying glass (see chapter III).

### Postimplantation losses

When the estimation of embryo losses is made during stages corresponding to placentation or later on, the number of live embryos or foetuses at the different stages can be estimated by some residues in the uterus, e.g. implantation marks on the endometrial mucosa, placentas, momified or dead foetuses (TORRES, 1982; GARCIA *et al.*, 1983a; TORRES *et al.*, 1987a).

Estimation of the "uterine capacity" represents an important factor for these examinations. Several criteria may be taken into account:

- the minimum distance between 2 embryos required for the development of each placenta and a normal display of the foetal phase.

- the existence of a negative relationship between the number of young and their mean weight as well as a positive relationship between their number and total litter weight resulting in the fact that the uterine capacity of a female is all the larger, the higher the total weight and the lower the mean weight above the minimum required for progeny survival (GARCIA *et al.*, 1983b; ESTANY *et al.*, 1986).

Other parameters can be measured (e.g. anatomical, endocrine, blood flow), but their relationship with the uterine capacity is not evident.

## Examination techniques

### FLUSHING OF OVIDUCTS AND UTERINE HORNS

This technique consists in injecting a perfusion medium into the oviduct or uterine horn (or both) so as to wash down and collect oocytes or embryos. The

medium may be very simple (saline, phosphate buffer) or more complex (culture medium such as Ham's F10, B2), enriched or not (BSA or homologous serum) according to the fate of recovered oocytes or embryos. Flushings can be performed from late ovulation (about 10 h after mating) until the phase immediately prior to implantation (about 6 days after mating). This is followed by the utilisation of other counting techniques (see below).

Flushings can be made after slaughter or in vivo. The recovery rate ranges around 100% in the first case (BOLET and THEAU-CLEMENT, 1988) while the in vivo recovery rate is lower (ADAMS, 1982) so that the latter technique cannot be used for obtaining a reliable estimation of ovulation rate.

Until day 2 post coitum, oviducts may be perfused only. However, the normal transit of embryos might be modified by some ovulation induction treatments (ADAMS, 1982; BOURDAGE and HALBERT, 1988). One of the limits of this technique is that for it to be reliable, the females have to be slaughtered and if necessary the recovered embryos transferred for subsequent development which may affect them.

### Post-mortem flushing of the oviduct

The genital tract is withdrawn after slaughter and the oviduct separated from the uterine horn below the uterotubal junction; the flushing catheter is introduced via the ampulla. Generally, the amount flushed is 2 cc at a moderate pressure.

### Flushing of the uterine horn

The 2 horns are separated by cutting them in the vaginal area of the cervix. The catheter is fitted not too deep into the horn and 5 to 10 cc of medium is injected. It is possible to compare the number of recovered embryos with the number of corpora lutea on the ovary and to repeat the flushing, which may increase the embryo recovery rate.

### LAPAROTOMY

This operation consists in making an incision in the abdominal wall to reach the genitals of the female and directly observe or manipulate the ovaries and uterine horns. This technique makes possible in vivo flushing, the observation of embryos from day 6 of gestation (TORRES, 1974) and embryo transfer (see below).

As gestation goes on after laparotomy, it is possible to obtain information on litter size components between day 6 and 16. But this operation must not be repeated several times in the same female. Moreover,

risks of infections and lesions are greater in suckling females.

### LAPAROSCOPY (OR CELIOSCOPY)

This technique has the same applications as laparotomy, but it is less aggressive, can be repeated several times on the same rabbit and does not give rise to particular problems in suckling females.

It was described for the first time in rabbits by FUJIMOTO *et al.* (1974) and used for in vivo observation of ovulation. GARCIA-XIMENEZ *et al.* developed a technique by means of which it is possible to count the corpora lutea at ovulation and the embryos from day 10 of gestation (MOLINA, 1987; SANTACREU *et al.*, 1990a). THEAU-CLEMENT and BOLET (1987) developed a slightly different method for counting the corpora lutea.

### CAESAREAN OPERATION

Using this technique it is possible to count the young in the 2 uterine horns and possibly identify each animal according to its location in the horn. The young rabbits are then adopted by a nursing doe, as the mother is generally slaughtered after the operation. The intervention should be made 28-29 days after mating, before normal birth term in order to increase the survival chances of the young. The different steps of this operation, i.e. extraction of the genital tract, withdrawal of the young followed by the adoption, should be as rapid as possible.

## Techniques used to intervene on gestation

### INDUCTION OF OVULATION

The doe rabbit which does not exhibit any apparent oestral cycle, generally ovulates as stimulated by mating. This has the experimental advantage of knowing the ovulation time, 10 h after mating. Ovulation can be induced artificially and dissociated from the emission of fertilizing sperm by acting on 3 levels of the ovulatory reflex:

- utilisation of a sterile male, the closest situation to normal mating reconstituting the whole reflex arch which induces pseudogestation with the same ovulation rate. These males can be obtained by vasectomy, (ligature and section of the deferent ducts), followed by an examination of the semen to verify the result of the operation.
- utilisation of natural or synthetic GnRH (Gonadotropin Releasing Hormone) or of an

analogue which allows to simulate the pituitary stimulation by GnRH secreted by the hypothalamus.

- utilisation of LH or hCG allows to act directly on the ovary by simulating endogenous LH release (which may however coexist). The disadvantage of hCG is a decrease in the response after several treatments.

Whether GnRH (THEAU-CLEMENT *et al.*, 1990) or hCG (HULOT and POUJARDIEU, 1976; HULOT *et al.*, 1988) is used, the ovulation frequency is high, but variable according to the physiological stage of the doe rabbit and the ovulation rate is not significantly different from that recorded with natural mating.

### SUPEROVULATION

In the doe rabbit the most currently used hormones are FSH and PMSG.

In most cases, FSH treatments give high mean responses. To induce ovulation the hormone is generally administered at low doses applied twice a day for 3-4 days, followed by the induction of ovulation (Table 3).

**Table 3. Superovulation treatments with FSH.**

Authors	Total dose	Freq.	Period	Via	OI	OR	NOE
KENNELLY and FOOTE (1965)	2 mg	2	3	i.m.	0,33 mg LH	—	42
TROTNOW <i>et al.</i> (1981)	1,6 mg	1	4	subc.	100 UI hCG	—	32
FISCHER (1987)	1,8 mg	2	3	subc.	75 UI hCG	—	22
DELBOS-WINTER <i>et al.</i> (1988)	2 mg	1	4	subc.	75 UI hCG	33	—

OI : Way of induction of ovulation

OR : Ovulation rate

NOE : Number of ova or embryos obtained

PMSG is usually administered in a single dose; this mode of use and its low cost represent the main advantages; in contrast, its dose-response relationship is variable and via its LH effect it may induce an anticipated ovulation. The mode of use, dose, interval between treatment and induced ovulation as well as the efficiency vary according to authors (Table 4).

**Table 4. Superovulation treatments with PMSG.**

Authors	Dose	Via	Hours before OI	OI	OR	NOE
HATTON <i>et al.</i> (1985)	75 UI	i.m.	66-68	50 UI hCG	—	25
KANAYAMA <i>et al.</i> (1985)	50 UI	i.v.	72	75 UI hCG	—	15
KEEFER <i>et al.</i> (1985)	150 UI	—	77	—	—	28
GARCIA-XIMENEZ and VICENTE (1989)	50 UI	subc.	48	50 UI hCG	20	18
MAYAN <i>et al.</i> (1989)	100 UI	subc.	72	50 UI hCG	24	17
REBOLLAR <i>et al.</i> (1989)	50 UI	i.m.	72	40 µg GnR	16	—

OI : Way of induction of ovulation

OR : Ovulation rate

NOE : Number of ova or embryos obtained



## IN VITRO FERTILIZATION

It was in the rabbit that this technique was applied for the first time (THIBAUT and DAUZIER, 1961). It allows to perfectly dissociate the embryo from the maternal environment and is thus of great theoretical interest. The technique has been improved (KEEFER *et al.*, 1985), but, however aleatory, its efficiency remains.

## ARTIFICIAL INSEMINATION

This technique is a valuable tool for controlling mating plans especially in pure- and crossbred comparisons, and even more if it involves an international dimension. After a period where it has been less used, it is now gaining interest among farmers; hence, studies concerning its control and factors of variation are being undertaken (THEAU-CLEMENT and VRILLON, 1989). Concerning semen freezing, a method was developed by ANDRIEU and COUROT in 1976. However, it still gives rise to a variety of problems and its efficiency is quite variable.

## HEMIOVARECTOMY

In the rabbit like in other polytocous species, this operation causes a functional compensation of the remaining ovary whose ovulation rate increases by two (FLEMING *et al.*, 1984). Accordingly, all ova and embryos are located in a single horn (it is noteworthy that in the rabbit the 2 horns do not communicate so that it is not necessary to make a hemi-hysterectomy after this operation). Thus, this technique is of great interest for studies on embryo viability and especially on uterine capacity. It can be performed before or during the reproductive life; its disadvantage is that its effect is irreversible.

## EMBRYO TRANSFER

In a previous chapter we showed the theoretical interest of embryo transfer for comparisons of pure- and crossbreds. In the rabbit, this technique is facilitated because synchronization (or control of dissynchronization) between donor and recipient rabbits can easily be obtained by inducing a pseudogestation in the recipient at a suitable time (see chapter "Flushing of oviducts and uterine horns"). In contrast, transfer by natural routes gives poor results and the most efficient method is to place the eggs in the oviduct or in the uterus according to the stage at which the transfer is made.

The transfer yield, expressed in percentage of live embryos or young per transferred egg, varies according to the transfer stage, site and synchronization between donors and recipients (THEAU-CLEMENT, 1986;

HEYMAN, 1988; TECHAKUMPHU *et al.*, 1987a; FISCHER, 1989). The least favourable stage seems to be the 2 to 4-cell stage; the yield is very high at the morula or blastocyst stage (day 3 and 4 p.C.). It may also vary with the genotype of the embryos (BOLET and THEAU-CLEMENT, 1988).

The most current method consists in slaughtering the donor and flushing the uterine horns for recovery of the embryos and performing a laparotomy in the recipient. It is however possible to recover the embryos by flushing the uterine horns after a superovulation treatment followed by an injection of prostaglandins (TSUTSUMI *et al.*, 1980; GARNIER *et al.*, 1986). SANTACREU *et al.* (1990b) developed a technique of embryo transfer using a method of laparoscopy derived from the already mentioned counting technique.

Concerning the different techniques described (e.g. laparotomy, laparoscopy, vasectomy, ovariectomy), we have not given details about the surgical conditions. It should however be emphasized that a perfect asepsis should be applied and that the rabbits should be treated with antibiotics to reduce the risks of infection. The most delicate point is the anaesthesia. It may either be via the gaseous route (fluothan) or by injection of various substances. The administration of a ketamine and promethazine mixture at variable doses according to authors gives good results (PEETERS *et al.*, 1988).

## EMBRYO FREEZING

Embryo freezing and creation of embryo banks may provide valuable information for the programmes of evaluation of pure- and crossbreds. The techniques of rabbit embryo freezing were first described by BANK and MAURER (1974). Later on, several teams (e.g. TSUNODA and SUGIE, 1977; RENARD *et al.*, 1982; KOJIMA *et al.*, 1985, 1987) have adapted the freezing programmes developed in the mouse by WHITTINGHAM *et al.* (1972) to the rabbit.

The viability of thawed embryos after transfer is rather low: 15 to 45 % at the 2 to 16-cell stages (BANK and MAURER, 1974; RENARD *et al.*, 1984; RAO *et al.*, 1984). The morula stage seems to be less sensitive to the freezing thawing programme and gives a survival rate after transfer of 41 - 56 % according to authors (TSUNODA *et al.*, 1982; KOJIMA *et al.*, 1985; RENARD *et al.*, 1982; TECHAKUMPHU and HEYMAN, 1987). In contrast, KOJIMA *et al.* (1987) obtained survival rates of 80 to 90 % until the blastocyst II stage using in vitro cultures.

The synchronisation of pseudopregnant recipient rabbits and the site of embryo deposition also affect the survival rate. The authors generally use a dissynchronisation between donors and recipients in order to obtain a resumption of embryo development



(TSUNODA *et al.*, 1982; KOJIMA *et al.*, 1985, 1987). However, TECHAKUMPHU and HEYMAN (1987) obtained 55 % of live fetuses when performing transfers of morula-stage embryos into the oviduct of synchronous pseudopregnant recipients. On the other hand, it seems that the transfer after culture/freezing should be asynchronous (TECHAKUMPHU *et al.*, 1987b). At present, all these techniques are being further developed (KOBAYASHI *et al.*, 1990) and after improvement of the freezing yield they will most likely represent a useful experimental tool.

## Conclusion

The methods described in this paper are those used in our laboratories in Toulouse and Valencia for genetic studies of the reproductive abilities of our rabbit strains. Our experimental approach consists in using a genetic methodology together with simple laboratory methods for better assessing the genetic and physiological determinism of litter size. In our opinion, use of these techniques in keeping with the experimental facilities of each research team may contribute to improving the programmes for comparison of rabbit breeds in pure and crossbreeding.

We wish to thank Kirsten Rérat, (INRA, Unité Centrale de Documentation, 78352 Jouy-en-Josas Cedex) for the translation into English.

## References

- ADAMS, C.E. (1982): Mammalian egg transfer. C.R.C. Press, Inc., Boca Raton, Florida.
- ANDRIEU, R., COUROT, M. (1976): Congélation du sperme de lapin en vue de l'utilisation en insémination artificielle. 1st World Rabbit Congress, DIJON, 31 March-2 April 1976, comm. n° 67.
- BANK, H., MAURER, R.R. (1974): Survival of frozen rabbit embryos. *Exp. Cell. Res.*, 89, 188-196.
- BOLET, G., BRUN, J.M., HULOT, F., POUJARDIEU, B., DE ROCHAMBEAU, H., ROUVIER, R. (1990a): Stratégies pour améliorer la taille de portée chez le lapin : résultats et perspectives. 41st annual meeting of the EAAP, TOULOUSE, 9-12 July 1990.
- BOLET, G., BRUN, J.M., HULOT, F., THEAU-CLEMENT, M. (1990b): Variabilité génétique et effet de la sélection dans le croisement de trois souches de lapins. 2 - Composantes biologiques de la taille de portée. 5èmes Journées de la Recherche Cunicole en France, PARIS, 12-13 December 1990.
- BOLET, G., THEAU-CLEMENT, M. (1985): Applications actuelles et futures de nouvelles biotechnologies aux recherches en génétique quantitative sur la reproduction chez le lapin. Commission spécialisée Lapins de l'INRA, 6-7 November 1985, TOULOUSE (doc. polycopié)
- BOLET, G., THEAU-CLEMENT, M. (1988): Timing of embryonic development in two rabbit strains. 11th International Congress on Animal Reproduction and Artificial Insemination. DUBLIN, 26-30 June 1988, 3, 391.
- BOURDAGE, R.J., HALBERT, S.A. (1988): Distribution of embryos and 500  $\mu$ M microspheres in the rabbit oviduct. Controls for acute motion analysis during transport. *Biol. Reprod.*, 38, 282-291.
- BRUN, J.M. (1985): Interactions géniteur x population des partenaires. III. Synthèse bibliographique. *Génét. Sél. Evol.*, 17, 561-578.
- BRUN, J.M. (1990): Variabilité génétique et effet de la sélection dans le croisement de trois souches de lapins. 1- Caractères des portées. 5èmes Journées de la Recherche cunicole en France, PARIS, 12-13 December 1990.
- BRUN, J.M., ROUVIER, R. (1984): Effets génétiques sur les caractères des portées issues de trois souches de lapins utilisés en croisement. *Génét. Sél. Evol.*, 16, 367-384.
- DELBOS-WINTER, R., FISCHER, B., BEIER, H.M., (1987): Einflüsse der FSH-/hCG-stimulierten polyovulation auf uterussekretion und implantation beim kaninchem. *Fertil.*, 3, 87-93.
- DICKERSON, G.E. (1969): Experimental approaches in utilizing breed resources. *Anim. Breed. Abstr.*, 37, 191-202.
- ESTANY, J., BALASCH, S., PLA, M., MOLINA, I., GARCIA, F. (1986): Un modelo de desarrollo placentario y fetal en el conejo I.T.E.A., 64, 21-35.
- FISCHER, B. (1987): Development retardation in cultured preimplantation rabbit embryos. *J. Reprod. Fertil.*, 79, 115-123.
- FISCHER, B. (1989): Effects of asynchrony on rabbit blastocyst development. *J. Reprod. Fertil.*, 86, 479-491.
- FLEMING, M.W., RHODES, R.C., DAILLEY, R.A. (1984): Compensatory responses after unilateral ovariectomy in rabbits. *Biol. Reprod.*, 30, 82-86.
- FOULLEY, J.L., LEFORT, G. (1978): Méthodes d'estimation des effets directs et maternels en sélection animale. *Ann. Génét. Sél. Anim.*, 10, 475-496.
- FUJIMOTO, S., RAWSON, J.M., DUKELOW, W.R. (1974): Hormonal influences on the time of ovulation in the rabbit as determined by laparoscopy. *J. Reprod. Fertil.*, 38, 97-103.
- GARCIA, F., BASELGA, M., PLA, M., (1983a): Mortalidades embrionaria y fetal en las distintas etapas de la gestación en el conejo de carne. *An. INIA/Ser. Ganadera*, 18, 11-27.
- GARCIA, F., BASELGA, M., PLA, M. (1983b): Determinación del peso de los fetos a los 19 y 28 días de gestación en conejos. *An. INIA/Ser. Ganadera*, 18, 29-36.
- GARCIA-XIMENEZ, F., VICENTE, J.S. (1990): Effect of PMSG treatment-mating interval in the superovulatory response of primiparous rabbit. *J. Appl. Res. Rabbit* (sous presse).

- GARNIER, V., RENARD, J.P., MENEZO, Y. (1986): Collecte vaginale d'embryons chez la lapine après le traitement à la prostaglandine. 4èmes Journées de la Recherche Cunicole en France, PARIS, 10-11 Décembre 1986, 1, 16.
- HATTON, J.B., KELUS, A.S., LEIBO, S.P. (1985): Transfer of fresh and frozen-thawed rabbit embryos to produce live young. *Experientia*, 41, 755-756.
- HEYMAN, Y. (1988): Moment de la transplantation et succès de la gestation chez les mammifères. *Reprod. Nutr. Dévelop.*, 28, (6B), 1773-1780.
- HULOT, F., MARIANA, J.C., CATTIAU, G. (1988): HCG-Induced ovulation in two rabbit breeds: effects of dose, season and sexual behaviour. *Livest. Prod. Sci.*, 20, 257-267.
- HULOT, F., POUJARDIEU, B. (1976): Induction artificielle de l'ovulation et fertilité chez la lapine allaitante ou non. *Ann. Biol. Anim. Bioch. Biophys.*, 16, 635-643.
- KANAYAMA, K., ENDO, T., KOBAYASHI, G., SAKUMA, Y. (1985): Studies on artificial insemination into the oviduct through the tubal fimbria in rabbits. *Jap. J. Fertil. Steril.*, 30, 65-70.
- KATZ, E. (1988): The luteinized unruptured follicle and other ovulatory dysfunctions. *Fertil. Steril.*, 50, 839-850.
- KEEFER, C.L., BENNETT, K.A., BRACKETT, B.G. (1985): In vitro fertilization in the rabbit after delayed ovum recovery. *Biol. Reprod.*, 33, 388-392.
- KENNELLY, I.I., FOOTE, R.H. (1965): Superovulatory response of pre- and post pubertal rabbits to commercially available gonadotrophins. *J. Reprod. Fert.*, 9, 177-188.
- KOBAYASHI, K., NAGASHIMA, H., YAMAKAWA, H., KATO, Y., OGAWA, J. (1990): The survival of whole and bisected rabbit morulae after cryopreservation by the vitrification method. *Theriogenology*, 33, 777-788.
- KOBAYASHI, Y., SANTULLI, R., WRIGHT, K.H., WALLACH, E.E. (1983): In vitro fertilization of rabbit ova ovulated in vitro during ovarian perfusion. *J. Reprod. Fert.*, 68, 41-44.
- KOJIMA, T., SOMA, T., OGURI, N. (1985): Effect of rapid addition and dilution of dimethyl sulfoxide on viability of frozen-thawed rabbit morulae. *Cryobiology*, 22, 409-416.
- KOJIMA, T., SOMA, T., OGURI, N. (1987): Effect of rapid addition and dilution of dimethyl sulfoxide and 37°C equilibration on viability of rabbit morulae thawed rapidly. *Cryobiology*, 24, 247-255.
- MATHERON, G., MAULEON, P. (1979): Mise en évidence de l'action conjointe des effets directs, maternels et grand-maternels sur la taille de portée. *Bull. Tech. Dép. Génét. Anim.*, 29-30, 232-263.
- MAYAN, M.D., VICENTE, J.S., SANTACREU, M.A., VIUDES-DE-CASTRO, M.P. (1989): Superovulación con PMSG en conejas primíparas para la obtención de embriones. ITEA, III Jornadas de Producción Animal, 9, 231-233.
- MOLINA, I. (1987): Determinación de los efectos directos y maternos sobre la tasa de ovulación y la viabilidad embrionaria y fetal en el conejo, mediante aplicación de las técnicas de transferencia de embriones y laparoscopia. Doctoral thesis, Valencia University.
- PEETERS, M.E., GIL, D., TESKE, E., EYZENBACH, V., BROM, W.E., LUMEIJ, J.T., DE VRIES, H.W. (1988): Four methods for general anaesthesia in the rabbit: a comparative study. *Lab. animals*, 22, 355-360.
- RAO, V.H., MONGHA, M.R., ANSARI, M.R., BHATTACHARYYA, N.K. (1984): Survival of rabbit embryos after rapid freezing and thawing. *Experientia*, 40, 821-822.
- REBOLLAR, P.G., RODRIGUEZ, J.M., DIAZ, M., UBILLA, E. (1989): Efecto de la estimulación con PMSG sobre la respuesta ovárica y resultados de la inseminación artificial en conejas de baja receptividad sexual. ITEA, III Jornadas de Producción Animal, 9, 262-264.
- RENARD, J.P., GARNIER, V., PARVEX, R. (1982): Production et congélation de l'embryon de lapin. 3èmes Journées de la Recherche Cunicole, Paris, 8-9 Décembre 1982, 1, 18.
- RENARD, J.P., BUI-XUAN-NGUYEN, GARNIER, V. (1984): Two-step freezing of two cell rabbit embryos after partial dehydration at room temperature. *J. Reprod. Fert.*, 71, 573-580.
- RISKA, B., RUTLEDGE, J.J., ATCHLEY, W.R. (1985): Genetic analysis of crossfostering data with sire and dam records. *J. Hered.*, 76, 247-250.
- SANTACREU, M.A., VICENTE, J.S., GARCIA-XIMENEZ, F. (1990b): Transfert de morulae dans l'utérus de la lapine par coelioscopie. 5èmes Journées de la recherche Cunicole en France, PARIS, 12-13 December 1990.
- SANTACREU, M.A., VIUDES-DE-CASTRO, M.P., BLASCO, A. (1990a): Evaluation par coelioscopie des corps jaunes et des embryons et influence de cette technique sur la taille de portée. *Reprod., Nutr. Dévelop.*, 30, 583-588.
- SELLIER, P. (1976): The basis of crossbreeding in pigs. A review. *Livest. Prod. Sci.*, 3, 203-226.
- SELLIER, P. (1982): Selecting populations for use in crossbreeding. 2nd World Congress on Genetics applied to Livestock production. Madrid, 4-8 October 1982, 6, 15-49. Editorial Garsi, Madrid.
- TATEMOTO, H., MAEDA, T., TERADA, T., TSUTSUMI, Y. (1989): Observations on the polar body in rabbit eggs by staining with fluorescent dye and acetolacmoid. *Jap. J. Anim. Reprod.*, 35, 140-146.
- TECHAKUMPHU, M., HEYMAN, Y. (1987): Survival of frozen-thawed rabbit morulae after synchronous or asynchronous transfer. *Anim. Reprod. Sci.*, 12, 305-312.
- TECHAKUMPHU, M., WINTENBERGER-TORRES, S., SEVELLEC, C. (1987a): Survival of rabbit embryos after synchronous or asynchronous transfer. *Anim. Reprod. Sci.*, 12, 297-304.
- TECHAKUMPHU, M., WINTENBERGER-TORRES, S., SEVELLEC, C., MENEZO, Y. (1987b): Survival of rabbit

embryos after culture or culture/freezing. *Anim. Reprod. Sci.*, 13, 221-228.

THEAU-CLEMENT, M., BOLET, G. (1987): Mesure du taux d'ovulation par coelioscopie chez la lapine. *Reprod. Nutr. Dévelop.*, 27, 701-705.

THEAU-CLEMENT, M., BOLET, G., ROUSTAN, A., MERCIER, P. (1990): Comparaison de différents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise à la reproduction. 5èmes Journées de la Recherche Cunicole en France, PARIS, 12-13 December 1990.

THEAU-CLEMENT, M. (1986): Comparaison de 3 stades de transfert d'embryons de lapin. 4èmes Journées de la Recherche Cunicole en France, PARIS, 10-11 Décembre 1986, 1, 18.

THEAU-CLEMENT, M., VRILLON, J.L. (1989): Le point sur l'insémination artificielle. Bibliographie : quelques résultats. *Cuniculture*, 87, 141-149.

THIBAUT, C. (1967): Analyse comparée de la fécondation et de ses anomalies chez la brebis, la vache et la lapine. *Ann. Biol. Anim. Bioch. Biophys.*, 7, 5-23.

THIBAUT, C., DAUZIER, L. (1961): Analyse des conditions de la fécondation in vitro de l'oeuf de la lapine. *Ann. Biol. Anim. Bioch. Biophys.*, 1, 277-294.

TORRES, S. (1974): Relation entre la taille des blastocystes de lapine à l'implantation et la survie embryonnaire. *Ann. Biol. Anim. Bioch. Biophys.*, 14, 41-52.

TORRES, S. (1982): Etude de la mortalité embryonnaire chez la lapine. 3èmes Journées de la Recherche Cunicole en France, PARIS, 8-9 Dec. 1982, 1, 15.

TORRES, S., HULOT, F., MEUNIER, M., SEVELLEC, C. (1987a): Comparative study of preimplantation development and embryonic loss in two rabbit strains. *Reprod. Nutr. Dévelop.*, 27, 707-714.

TORRES, S., HULOT, F., SEVELLEC, C. (1987b): Early stages of embryonic development in two rabbit genotypes. *Reprod. Nutr. Dévelop.*, 27, 715-719.

TROTNOW, S., AL-HASANI, S., SADTLER, C. (1981): Experience with in vitro fertilization of follicular rabbit oocytes and embryo transfer. *Gynecology*, 231, 41-50.

TSUNODA, Y., SUGIE, T. (1977): Survival of rabbit eggs preserved in plastic straws in liquid nitrogen. *J. Reprod. Fertil.*, 49, 173-174.

TSUNODA, Y., SOMA, T., SUGIE, T. (1982): Effect of post-ovulatory age of recipient on survival of frozen-thawed rabbit morulae. *J. Reprod. Fertil.*, 65, 483-487.

TSUTSUMI, Y., TERAMI, Y., TAKEDA, T., SUZUKI, H., MATSUI, S. (1980): In vivo egg recovery from the vaginae and the pattern of egg distribution in superovulated rabbits. *Jap. J. Anim. Reprod.*, 26, 6-14.

WITTINGHAM, D.G., LEIBO, S.P., MAZUR, P. (1972): Survival of mouse embryos frozen to -186°C and - 268°C. *Science*, 178, 411-414.