Proceedings of the

4-7 july **2000** – Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910
(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

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Volume A, pages 61-79

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ABSTRACT

Abstract : Rabbit artificial insemination is now commonly practised in European countries. In order to maintain a "natural image" of rabbit meat, the author discusses the interest of biostimulation methods defined in opposition with "hormone use", to improve sexual receptivity of does at the moment of insemination and consequently their fertility and productivity. She resumes their study in rabbits. The efficiency of animal manipulation such as a change of cage, or doe gatherings, before insemination, is not clearly demonstrated; moreover these methods are time consuming and difficult to manage on large rabbit farms. A short (24 to 48h) dam-litter separation could be a real alternative to hormonal treatments (oestrus synchronisation), if the stimulation is applied just before insemination and if free suckling is applied before and after insemination. Nevertheless, new experiments are necessary to evaluate long term effects on total productivity and define precise conditions that allow the expression of effects of such a biostimulation under conditions compatible with animal welfare. A nutritional flushing following a restricted feeding period seems to improve reproduction performance. The results of studies using continuous or interrupted lighting programs illustrate the need to study photoperiodism more intensively in the rabbit. In opposition with other zootechnical species, no study has quantified the rabbit buck effects on oestrus induction. Since these methods have to be easy to apply, inexpensive and consistent with animal welfare, the author concludes that lighting programs, a short dam-litter separation and feeding programs are the most interesting ways to explore since they are perfectly adapted to cycled production.

INTRODUCTION

Artificial insemination (A.I.) is widely used on European rabbit farms. This method allows new systems of production such as "cycled production" : all the does of a same batch are inseminated on the same day whatever their sexual receptivity. THEAU-CLÉMENT and ROUSTAN (1992) evidenced a particularly strong antagonism between lactation and reproductive functions in non-receptive does : at the moment of insemination lactating non-receptive does have poor performance. This antagonistic effect represents a major problem since the intensive methods of production generally applied require does to be inseminated at the start of the nursing period (from 0 to 11 days *post partum*). It should be emphasised that with natural mating, the negative effect of the antagonism has often escaped until now, since non-receptive lactating does refuse to mate. A sexual receptive behaviour is correlated with more pre-ovulatory follicles on the rabbit ovary (KERMABON *et al*., 1994) and consequently with higher concentration of plasma oestradiol (REBOLLAR *et al*., 1992). So, to assure good

and regular production, it is necessary to have reliable techniques to induce and synchronise oestrus (leading to sexual receptivity behaviour) of lactating does.

Many studies have been made on hormone treatments (MAERTENS *et al.*, 1995b ; CASTELLINI, 1996). Pregnant Mare Serum Gonadotrophin (PMSG) is now largely used in rabbitries. Nevertheless the use of exogenous products (hormones, antibiotics,…) goes against the popular perception of the consumer. During the 6th World Rabbit Congress, CASTELLINI (1996) mentioned that, in the near future, the European Community policy might impose a restriction on the use of hormones (gonadotrophins) in relation to their residues in meat, animal welfare and the desire to preserve a "natural" image of meat. A similar trend concerns the use of antibiotics; in order to counteract antibiotic resistance, the European Community has already suppressed 4 of 8 antibiotics currently used in animal feeding. For these reasons, over these last years, important work has been done particularly by the International Rabbit Reproduction Group (I.R.R.G) to set up alternative methods which do not require the use of hormones, in order to increase sexual receptivity at the moment of insemination and consequently the productivity of rabbit does (BOITI, 1998). These are called "biostimulation" methods and comprehend a large spectrum of techniques. Up to date, several approaches have been tried such as animal manipulation, a short dam-litter separation, feeding programs, photoperiod and buck effects. These methods were sometimes drawn from results obtained in other zootechnical species. After a short section devoted to the physiological response to environmental *stimuli*, different biostimulation methods will be presented. The aim of this work is to analyse, in the light of zootechnical results, feasibility under farming conditions, compatibility with animal welfare, physiological interpretation..., the interest of these methods and the promising ways that can be explored [1].

PHYSIOLOGICAL RESPONSES TO ENVIRONMENTAL STIMULI

Reproduction is regulated by a complex hormonal system in which the hypothalamus and the pituitary gland play a leading role. The secretion of GnRH (Gonadotrophin Releasing Hormone) produced at the hypothalamus level is able to stimulate both the synthesis and release of two gonadotrophins : FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) at the anterior pituitary level. These proteic hormones act upon the ovaries : FSH is mainly responsible for follicular growth and LH controls the final follicular maturation and induces the ovulation of pre-ovulatory follicles.

In most species and the rabbit too, the ovarian steroid hormones (oestrogen and progesterone) seem to alternately exercise a positive and negative feedback, respectively for oestrogen and progesterone, on the secretion of GnRH, FSH and LH in the hypothalamo-pituitary complex. This whole system regulates the sexual activity of the does.

Moreover, complex mechanisms interfere with the hypothalamo-pituitary-ovarian axis with the participation of endogenous opioid peptides such as endorphins, catecholamines (such as DOPA, Norepinephrine..), corticotrophin releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and cortisol (GILMORE and COOK, 1981).

It has long been recognised that the environment plays an important role in the regulation of reproductive function and it now appears obvious, that environmental *stimuli*

[1] Some reactualised parts of this synthesis were taken from THEAU-CLÉMENT (1998)

must act through the nervous system and the hypothalamo-pituitary axis. Environmental *stimuli*, such as changing day-length or temperature and feeding, affecting animals by stress, auditory and/or olfactory *stimuli* can positively or negatively modify reproductive performance.

BIOSTIMULATION METHODS

1) ANIMAL MANIPULATION

1-1 Change of cage

In nulliparous rabbit does, LEFÈVRE and MORET (1978) and REBOLLAR *et al.* (1995) evidenced that a change of cage can improve fertility. In opposition, LUZI and CRIMELLA (1998) by transferring does (and their litter when lactating) to another cage, 2 days before insemination, did not confirm such an improvement on nulliparous does. However in non-nulliparous does, a change of cage 48h before insemination increased fertility (+ 14 %), compared to the control group, and gave +1.4 born alive/insemination in lactating pluriparous animals.

1-2 Doe gatherings

MIRABITO *et al.* (1994a), gathering 3 rabbit does immediately before insemination, did not obtain any improvement of performance, even in nulliparous does. DUPERRAY *et al.* (1999) studied the interest of doe gatherings (8/cage, 15 minutes before insemination) when a dam-litter separation was applied in both experimental and control groups. This stimulation increases the frequency of red and purple vulva suggesting the positive effect of the stimulation on rabbit doe receptivity and significantly increases fertility (+ 6.1%). Nevertheless, the positive effect on fertility is clear on nulliparous, multiparous lactating and non-lactating does but not on primiparous rabbit does. At birth, the size and the weight of the litter are not modified by the treatment. Productivity at birth is increased by + 0.6 born alive/insemination when doe gatherings are applied in addition to a dam-litter separation. Nevertheless, this biostimulation method has to be used only on healthy herds since the contact between animals could represent a source of contamination.

The efficiency of animal manipulation has not been clearly demonstrated to increase rabbit productivity, since conclusions made by different authors are often opposite. Moreover, these biostimulation methods are time consuming and difficult to manage on large rabbit farms (individual identification, frequent change of cage…).

2) DAM-LITTER SEPARATION

It is well known that shortly after weaning (2-3 days), a high percentage of does enter oestrus. Nevertheless, a regular post-weaning insemination (no competition between pregnancy and lactation) is likely to be cost-effective in view of production conditions today. During lactation, a short dam-litter separation, has potentially been shown to induce oestrus. In sows, a daily dam-litter separation from 6 to 12 hours at 2 to 5 weeks *post partum* induces oestrus in 65% of the dams, compared with only 50% in the control group (STEVENSON and

DAVIS, 1984). In rabbits, a short (24 to 48 hour) dam-litter separation has been studied, generally by closing the nest box. In order to precisely define an optimal method, different application conditions of this method were studied (see table 1) :

- Suckling system (free or controlled suckling)
- Separation duration (from 24 to 48 hours)
- A.I position related to the first suckling following dam-litter separation (from 2h before to 48h after controlled suckling)

The efficiency of this method will be successively approached at the level of productivity components (fertility and prolificacy, young viability and growth) and at the level of global productivity.

2-1 Fertility and prolificacy

Since rabbit farms generally use a 42-day rhythm, most of the studies use this condition. The results are presented in table 1. Nevertheless, results obtained with a 35-day reproduction rhythm will also be considered.

- ***Free suckling***

24h dam-litter separation, 3 days before insemination. CASTELLINI *et al.* (1998) compared two different techniques of mother-litter separation lasting 24 hours. The separations were performed 3 days before insemination by closing the nest box or by a change of cage (which implicates a mother-litter separation in addition to a modification of the does' micro-environmental conditions). These two methods of mother-litter separation do not significantly affect the reproduction performance of lactating does.

24h dam-litter separation, just before insemination (AI performed in the 15 min after suckling following the separation). This stimulation improves the sexual receptivity and fertility of 11-day lactating does (PAVOIS *et al*., 1994 ; THEAU-CLÉMENT and MERCIER, 1999, table 1). On the contrary, ALVARIÑO *et al.* (1998) did not obtain any improvement of fertility when the stimulation was applied on 10-day lactating does but on the contrary, fertility was improved by + 17% (47.4 *vs* 64.2 %) on 3-day lactating does. This short doe-litter separation has no effect on litter size.

36-48h dam-litter separation, just before insemination. This separation duration corresponds to at least one suckling omission. On 11-day lactating does, sexual receptivity is improved but fertility does not seem to be improved by a longer separation. On 4-day lactating does, ALVARIÑO *et al.,* 1998 confirmed the higher effect of biostimulation on fertility (36h : + 32%; 48h : + 34%) not clearly related to the separation duration. Only MAERTENS (1998) obtained a higher litter size when a 40h dam-litter separation was applied (8.2 *vs* 7.1 born alive).

48h dam-litter separation, insemination at the end of the separation, just before suckling. VIRAG *et al.* (1999) confirmed the improvement of fertility when a 48h dam-litter separation was applied (64.7 *vs* 44.9 % for the control group) without any effect on prolificacy.
Table 1. Reproductive performance of 11 day lactating does shortly separated from their litter, in comparison with a control group (without separation)

Table 1. Reproductive performance of 11 day lactating does shortly separated from their litter, in comparison with a control group (without separation).

Suckling system before and after D.L.S [(1)]	Separation duration	A.I position / 1st suckling following DLS[(1)]	*Authors*	Receptivity (%)	Fertility (%)	Born alive /litter
Free suckling	**24 h**	48 h after	*Castellini et al. (1998)*	NS	NS	NS
idem	**24 h**	15 minutes after	*Pavois et al. (1994)*	+ 26 %	+ 13 %	NS
			Theau-Clément et al. (1999)	+ 8 %	+ 13 %	NS
			Alvariño et al. (1998)	NS	NS	NS
idem	**36 h**	*idem*	*Pavois et al. (1994)*	+ 23 %	+ 11 %	NS
			Alvariño et al. (1998)		+ 11 %	NS
idem	**40 h**	*idem*	*Maertens (1998)*	+ 38 %	+ 11 %	+ 1.1
idem	**48 h**	*idem*	*Alvariño et al. (1998)*		+ 7 %	NS
idem	**48 h**	A.I just before suckling	*Virag et al. (1999)*		+ 20 %	NS
Controlled suckling	**48 h**	-2h, 0, + 2h	*Szendrö et al. (1999)*	NS	+ 7 %	NS

[(1)] DLS : Dam Litter Separation
NS : Non Significant (P > 0.05)

- ***Controlled suckling***

Receptivity and fertility are not improved by a 48h separation when controlled suckling is applied from 0 to 18 days *post partum* (SZENDRÖ *et al.,* 1999). BONANNO *et al.* (1999b) studied the effectiveness of delayed artificial insemination of non-receptive does, with or without oestrus synchronisation by a 48h dam-litter separation. Although sexual receptivity was improved in the stimulated group (+ 16%), the repercussion on fertility was not significant (+ 8%). Litter size at birth is not affected by the stimulation.

2-2 Young viability and growth

There is general agreement that neither the incidence of mastitis nor the young rabbit mortality is affected by a short dam-litter separation (MAERTENS, 1998 ; BONANNO *et al.* (1999a,b).

However, for a 24h separation, the weaning weight is depressed by nearly 5 % (TOMAS *et al.,* 1996; CASTELLINI *et al*., 1998; ALVARIÑO *et al*., 1998; THEAU-CLÉMENT and MERCIER, 1999). When weaning takes place between 28 and 32 days, a dam-litter separation greater than 36h significantly decreases growth of the young as the individual weaning weight decreases from -2 % (PAVOIS *et al.*, 1994; SZENDRÖ *et al.*, 1999) to - 10% (ALVARIÑO *et al.*, 1998; BONANNO *et al*., 1999b). Nevertheless, when weaning was delayed until 35 days, ALVARIÑO *et al*., (1999) did not evidence a significant decrease of individual weaning weight. When mother deprivation is applied on younger rabbits (4 days old), the decrease of weaning

weight at 28 days of age is greater than 10%. This suggests a differential sensitivity in relation to age, but the experiment did not allow to dissociate the effects of young age at the moment of stimulation from the weaning time.

Most of these studies point out that a 24-48 h mother deprivation leads to a lower weaning weight of young. When rabbits are weighted immediately after suckling, following the separation, THEAU-CLÉMENT and MERCIER (1999) and SZENDRÖ *et al.* (1999) evidenced a marked fall (24h separation : - 6% and 48h separation : - 13%).

MAERTENS (1998), applying a 40h dam-litter separation, registered a decrease in consumption between day 8 and day 11 *post partum* (282 *vs* 341 g/ day for the control group). Moreover, SZENDRÖ *et al*. (1999) evidenced that the day after the omission of suckling, the quantity of milk produced by the stimulated does increased by 22 % on the three subsequent days. Milk secretion lagged behind that of the does of the control group by 33%, 15% and 6% respectively. In addition, two days after the omission of suckling, the milk secreted was found to contain higher levels of dry matter (by 4.2%), fat (by 1.7%), protein (by 2.6%) and ash (by 0.5%) than previously. These values later returned to levels approaching the original values. Nevertheless, the milk production compensation was not large enough to counterbalance the negative effect of the separation on young growth till weaning. Moreover, SZENDRÖ *et al.* (1999) and BONANNO *et al.* (1999b) did not show compensatory growth during the fattening period ; young weight was depressed by -2% (70 days of age) and -3% (74 days of age) respectively.

Based on the above results, the decrease of weaning weight does not seem to be clearly related to the duration of the separation, but ALVARIÑO *et al.* (1999) evidenced under the same experimental conditions, that young weight measured (in comparison with the control group) after suckling the day of insemination, decreases in relation to the separation duration (-1.4%, -6.1%, -12.8%, respectively after 24 h, 36 and 48 h of separation).

2-3 Global productivity

These numerous studies indicate that a lot of factors can act upon the efficiency of a dam-litter separation: application conditions of biostimulation (suckling system before and after the stimulation, separation duration, interval between suckling following the separation and insemination) and the physiological status of does (lactation stage, sexual receptivity at the moment of insemination, parity....). Moreover, components of global productivity may be influenced differently. So, a short dam-litter separation can improve fertility but in parallel, slightly decreases young growth. In order to conclude more obviously the interest of a dam-litter separation, as a biostimulation method, two productivity indexes have been calculated, with available bibliographic data (studying at least 2 series of inseminations) :

- productivity at birth : the number of born alive/number of inseminations
- productivity at weaning : the total weight of rabbits obtained/ number of inseminations.

The relative difference between the productivity of the experimental and the control groups are presented. Since the dam-litter separation only concerns lactating does, the hypothesis is made that the separation does not affect temporarily non-lactating doe productivity.

- ***Conditions of application***

Separation duration : The separation duration does not seem to influence very much the relative difference of weaning productivity between the experimental and the control groups.

- 24h : + 19% (THEAU-CLÉMENT and MERCIER; 1999)
- 36h : + 14% (PAVOIS *et al.*, 1994)
- 40h : + 9% (MAERTENS, 1998)
- 48h : + 20% (VIRAG *et al.*, 1999)

Since all these studies were standardised as for the other components, using a 42 day reproductive rhythm and free suckling before and after the stimulation, the interest of a 24-48h mother-litter separation was evidenced. Unfortunately, some of these experiments were not applied through the entire reproductive lifetime of the does.

Insemination-suckling interval : When A.I is practised immediately after the first suckling following the separation, SZENDRÖ *et al.* (1999) showed that productivity at birth is significantly higher (+0.9 born alive/insemination). In opposition, when insemination is realised 2h before or 2h after suckling, the separation does not have any positive effect on reproductive performance of does.

Type of suckling before and after stimulation : Studies applying controlled suckling before and after stimulation did not lead to a clear increase of productivity at weaning (SZENDRÖ *et al.* 1999; TOMAS *et al.* 1996). Regular controlled suckling could limit or suppress the positive effect of a single dam-litter separation in increasing reproductive performance. Free suckling before and after the separation is recommended.

- ***Physiological status of does***

Lactation stage : A stimulation on 3-4 day lactating does (35 day reproduction rhythm) is more efficient to improve reproductive performance than on 9-10 day lactating does (42 day reproduction rhythm). Despite the marked young growth decrease, a 36 or 48h dam-litter separation applied on 3-4 day lactating does, can improve productivity at birth by 76 to 92% respectively (ALVARIÑO *et al.,* 1998). THEAU-CLÉMENT and ROUSTAN (1992) indicated that the antagonism between lactation and reproduction is specially marked during the first 3-5 days of nursing. Consequently, it can be considered that at this physiological status, production improvement possibilities are greater.

Sexual receptivity : Using free or controlled suckling, TOMAS *et al.* (1996) did not find any positive effect of a dam-litter separation using natural mating (i.e on receptive does) on productivity. On the contrary, as demonstrated by BONANNO *et al.* (1999b), a 48h separation on non-receptive does (lactation order<5) can improve productivity at weaning by +58,3%. These results suggest that a dam-litter separation is efficient in improving reproductive performance of non-receptive does.

Parity : Analysing the effect of biostimulation in relation with parity, MAERTENS *et al.* (1998) and VIRAG *et al.* (1999) evidenced that fertility is significantly improved only in primiparous does (+ 30% and + 43%, respectively). In the same way, a 48h separation improves fertility (+ 25%) of non-receptive does whose lactation number is lower than 5 (BONANNO *et al.*, 1999). It is interesting to underline that in this case, the authors did not notice any significant decrease of weaning weight. These results evidence a differentiated effect of biostimulation in

relation with parity. Primiparous does seem to be more sensitive to the positive effect of the stimulation.

Of course, others factors could interact with the effect of a dam-litter separation, such as genetic factors, nutrition, breeding conditions,....

2-4 Physiological aspects

At a physiological level, several hypotheses can be formulated to explain the positive response on reproductive performance of a dam-litter separation. When the separation duration varies from 24 to 48h, one or more sucklings are suppressed. In this case, prolactin secretion is temporarily reduced and delayed, and could suppress its antagonistic effect on reproductive performance (gonodotrophin release). In parallel, MCNEILLY (1998) suggests that β-endorphins produced in response to the suckling stimulus in the ewe, may inhibit the secretion of gonadotrophins. A temporal dam-litter separation could be able to break the inhibition and stimulate gonadotrophin secretion. A second hypothesis could be related to oxytocin release, necessary for milk ejection. Oxytocin also effects uterus contractions and thereby could contribute to the transport of spermatozoa up to the fertilisation site, if the insemination is performed shortly after suckling. A third hypothesis is that a doe-litter separation could act as a positive stress and influence the hormonal balance of the does. Of course, all these phenomena could interact.

2-5 Welfare aspects

At a welfare level, it is generally accepted that the European does (*Oryctolagus cuniculus*) only have limited contact with their young and visit them briefly (only about 3 to 4 minutes) just once a day to nurse, before dawn (HUDSON *et al.* 1996). As a consequence a 24h regular controlled suckling is similar to natural conditions (MYKYTOWYCZ, 1968 ; JILGE, 1995) and is regularly applied on farm conditions for 10 to 18 days *post partum*. Moreover, VERGA *et al.* (1986) evidenced that regular controlled suckling appears to produce less emotional young rabbits. Nevertheless, several opposite findings are found in the literature. Recently, SCHULTE and HOY (1997) evidenced that 57 % of rabbit does have between two to five suckling periods a day, generally from dusk to dawn. Applying a 16h dam-litter separation twice a week, SEITZ (1997) observed a modification of the nursing behaviour and a depressed weaning weight of the separated kids. On the contrary, none of the studies comparing regular controlled suckling *vs* free suckling, have shown a significant negative effect of controlled suckling on weaning weight (MORET, 1975; CORDIER, 1978; VERGA *et al.*, 1986; LE NORMAND *et al.* 1994; TOMAS *et al.* 1996). Consequentely, it may be suggested that controlled suckling applied once (with biostimulation) or twice a week (SEITZ, 1997) leads to a negative stress for young growth. It may be recommended that shorter dam-litter separation be used, therefore avoiding any disturbance of the normal behaviour of kids and their mother.

In conclusion, a single dam-litter separation at insemination can improve further productivity of lactating does. Now, a question is : could this method be a real alternative to hormonal treatments ? Only two experiments have compared the efficiency of a dam-litter separation with a PMSG treatment. When free suckling and a 42-day reproduction rhythm are used, a dam-litter separation could be a real alternative to hormonal treatments for inducing oestrus synchronisation and consequently improving productivity (MAERTENS, 1998 ; ALVARIÑO et al., 1998). The stimulation has to be done just before insemination and A.I has to be practised immediately after the first suckling following the separation. Nevertheless, the

positive effect of such a biostimulation on receptive does (natural mating) is not clear and when controlled suckling is applied before and after the stimulation. In order to definitively conclude on an optimal duration of the separation, new experiments are necessary to evaluate the long term effects on total productivity. In addition, the precise conditions that allow the expression of effects of such a biostimulation under conditions compatible with animal welfare are to be defined.

3) FEEDING PROGRAMS

PARIGI-BINI and XICCATO (1993) observed large energy losses (28%) by primiparous rabbit does during lactation due to large simultaneous requirements for lactation, body growth and pregnancy. This should partly explain the low receptivity and fertility generally observed in primiparous does. Because of increasing feed intake capacity with parity numbers, the negative effect of lactation should be less pronounced in multiparous does. FORTUN and LEBAS (1994) confirmed the detrimental effect of lactation in primiparous does, and showed that this effect decreases with a smaller number of suckling young rabbits and that a negative nutritional balance of does depresses foetal growth.

We will separately consider feeding programs applied continuously or during rearing in order to prepare the doe's reproductive carreer (long term feeding programs) and feeding programs applied just before the reproductive cycle (short feeding programs).

3-1 Long term feeding programs

When experimental diets (high-energy *vs* low-energy diet) are continuously provided to does over successive reproductive cycles, authors generally conclude a long-term positive effect of high plane nutrition on reproductive performance (HULOT *et al*., 1982; MAERTENS and DE GROOTE, 1988; CASTELLINI and BATTAGLINI, 1991; CERVERA *et al.*, 1993).

Another way to increase long term reproductive performance, may be appropriate feeding during the rearing period. On nulliparous and primiparous does, MANCHISI *et al.* (1988) comparing two feeding levels (*ad libitum vs* restricted, 50 days before the experiment) concluded that *ad libitum* feeding increases the percentage of ovulating does. HARTMANN and PETERSEN (1997) studied the effects of either restrictive or *ad libitum* feeding during the rearing period of does on reproductive performance (until the third insemination). The restricted does were heavier and from the second litter on, the restrictive feeding had a positive influence on the reproductive performance (second litter : + 1.4 young rabbit). On the contrary, in a study by EIBEN *et al.* (1999), raising young rabbits with a restricted 130g diet per day or with 9 hours' daily feeding time access, did not decrease nor significantly improve the performance of the does after 3 successive inseminations.

Moreover, it seems that a diet rich in fibre during the growing period (from 50 days old to day 10 of the first pregnancy) in comparison with a control group, increases the number of weaned rabbits per litter, measured over one year (NIZZA *et al*., 1997 : + 5%). On the contrary, XICCATO *et al.* (1999) did not increase the reproductive performance of nulliparous does receiving a high-fibre diet from weaning to their first littering.

3-2 Short-term feeding programs

The weight of ewes before mating reflects the mean nutritional status of the flock and has a clear influence on reproductive performance (ovulation rate, fertility and prolificacy; THERIEZ, 1984). Moreover, any increase in weight just before mating has a positive effect on reproductive performance. Conversely, a lower nutritional status before mating decreases the ovulation rate and embryo viability. Hence "flushing" is commonly practised in ovine production. This consists of increasing the doe's feeding (energy) level just before mating.

In short term feeding programs, flushing is applied just before mating or insemination. Some authors have used flushing without any previous restrictions, others have first applied a feeding restriction.

- ***Flushing without previous restriction***.

FORTUN-LAMOTHE (1998) studied the effect of a stimulation with pre-mating energy intake on reproductive performance at the subsequent mating or parturition of rabbit does during 4 successive cycles. The results suggest that increased pre-mating energy intake has a positive effect on conception rate. However, inadequate *pre-partum* energy intake (food restriction) has a detrimental effect on sexual receptivity and litter weight.
MAERTENS (1998) performed a 4 day flushing with an energetic diet before insemination on lactating does. However, the applied flushing failed to improve sexual receptivity, fertility and litter size, in comparison with the control group (-1.2 %, -12.2 % and -0.5 born alive, respectively). The author related these results to the low palatability of the experimental diet. The daily energy intake during the flushing period was lower than in the control group (-0.15 MJ ME/day). An energetic "flushing" using propylene glycol (2% in water, for 4 days before insemination) was studied by LUZI *et al.* (1999). The treatment increased fertility (64 *vs* 53% for the control group) but did not have any effect on litter size at birth and at weaning, nor on growth of young. Despite a higher mortality between birth and weaning (21 *vs* 14 %), the treatment increased productivity at weaning by + 15 %.

- ***Flushing with previous restriction*.**

VAN DEN BROECK and LAMPO (1977) demonstrated on nulliparous does, that flushing following a restricted feeding period improves reproduction performance. Similarly, GOSALVEZ *et al.* (1995) evidenced the positive effect of nutritional flushing (following restricted feeding for 2 weeks before the beginning of the experiment) on the percentage and the intensity of ovulation of 17 week-old females

3-3 Physiological aspects

In intensive systems, nutritional requirements are greatly increased by the contemporary presence of pregnancy and lactation. As previously affirmed, reproduction is affected by the energetic balance. At a physiological level, the main factors that link metabolism and reproduction (like insulin, IGFs, glucose, leptin and the neuropeptide Y), both act on the hypothalamo- pituitary axis (affecting gonadotrophin secretions) and directly on the ovaries (by altering gametogenesis; MONGET, 1997).

Flushing following a restricted feeding period seems to improve reproductive performance. Nevertheless, if the preparation of the future reproductrive career of the doe seems to be an interesting avenue to explore (restriction and/or a high-fibre diet during growth), its efficiency is controversial. So, new experiments should be done to study how to partially counterbalance the energy deficit of does during lactation and improve their reproductive performance. The durability of these effects has to be analysed. No scientific study on lactating does, has really defined a nutritional program well adapted to artificial insemination and for various production systems (depending on reproductive rhythm, genotypes...) and for various physiological statuses of the doe. If these feeding programs should be well adapted to cycled production, these methods also have to be easy to apply.

4) LIGHTING PROGRAMS

In our latitude, HAMMOND and MARSHALL (1925) and BOYD (1986) reported that wild rabbits (*Oryctolagus cuniculus*) have a well defined seasonal cycle of reproduction: most pregnancies occur between February and early August with a peak in May. It means that fertility is maximum with increasing daylength. WALTER *et al.* (1968) showed that a 16h constant photoperiod all year round reduces the reproduction problems normally associated with decreasing daylength periods. In the same way, UZCATEGUI and JOHNSTON (1992), in a study on Rex rabbits, concluded that 14 h of continuous light appears to be superior to both 10 and 12 hours for maximising doe reproduction and spermatogenesis of bucks. On the contrary, SCHÜDDEMAGE *et al.* (1999) evidenced in a one-year experiment that rabbit does housed under 8h artificial light per day produced 5% more live born pups than rabbits housed under 16h light per day.

4-1 Continuous lighting programs

By modifying the lighting program (8 h light/day until 8 days before insemination and 16 h light/day immediately after), THEAU-CLÉMENT *et al.* (1990) in comparison with a control group (constant 16 h light/day), found a significant improvement of sexual receptivity of the does (71.4 *vs* 54.3 %). However, the effect on fertility was not significant (61.4 *vs* 48.9 %). Because of lower viability and weaning weight (-5%) between birth and weaning, productivity at weaning was not improved by the experimental lighting program. MIRABITO *et al.* (1994b), using a similar lighting program (except that in the week after AI there was a progressive return to 16 h/ day) but with a longer reproductive rhythm (6 *vs* 5 weeks) obtained significantly higher receptivity and fertility on lactating does of the experimental group (+10%), but litters were lighter at weaning (-6%). In the same way, MAERTENS and LUZI (1995a) submitted rabbit does to a 16hL:8hD continuously lighting schedule or to a 10hL:14hD schedule. In order to synchronise the oestrus, the lighting period of the experimental group was suddenly increased till 16hL from 5 days before the insemination. No effect was seen on reproductive performance. However, in these studies, young viability or litter weight at weaning was significantly lower in the treated groups, suggesting that the lighting program can adversely affect the milking ability of females and/or the feeding behaviour of the young rabbits.

4-2 Interrupted lighting programs

UZCATEGUI and JOHNSTON (1992), in Rex rabbits, concluded that intermittent lighting schedules of 10, 12 and 14 h are equally as effective as 14 h of continuous light in promoting

doe reproduction. Feed consumption appears to be inversely related to the total hours of light. ARVEUX and TROISLOUCHES (1994), submitting does to different lighting programs (continuous:16h light/day or discontinuous: 2 periods of 8h light followed by 4h dark), increased fertility (82.6 *vs* 67.6 %, natural mating) without any reduction of young viability and litter weight at weaning. Moreover, productivity was increased by +13% weaned rabbits/doe/year and no abnormal behaviour was observed in the experimental group (nest abandonment, excitability, cannibalism, food waste...) and young seem to be well adapted to the situation.

4-3 Physiological aspects

Physiological mechanisms explaining how photoperiod can act on reproductive function are badly known in rabbits. Lighting programs are widely used in avian species. In sheep and goats, the photoperiodic information is transmitted via the retina to the pineal gland as nervous impulses. The knowledge of the different effects of photoperiod on neuroendocrine pathways and reproductive activity in these species has led to the successful application of light treatments to control seasonal reproductive activity (CHEMINEAU *et al.* 1992).

In conclusion, these results illustrate the need to study photoperiodism in the rabbit more intensively, since some recent results are in opposition with previous knowledge about the optimal artificial light program. Moreover, the preferential moment of nursing activity could interact with the light program. It is likely that these parameters could interact with feed consumption and milk production of the mother and consequently on young growth and animal welfare. Nevertheless, lighting programs are easy to apply and do not need large manpower costs. Only dark rooms (without windows) and a light programmer are required. They will be all the more efficient, since rabbit does will be in the same physiological condition. So, lighting programs are perfectly adapted to cycled production.

5) BUCK EFFECTS

In various physiological situations, the presence of the male influences the pattern of hormonal secretions and the behaviour in the females of many Ungulate species. In ewes, (MAULÉON and DAUZIER, 1965), cows (SIGNORET, 1980) and sows (ROWLINSON and BRYANT, 1974) the introduction of males into the herd reduces the duration of lactational anoestrus and advances ovulation relative to the oestrus onset (LINDSAY *et al.*, 1975; POINDRON *et al.*, 1980) by advancing the preovulatory surge of LH (MARTIN and SCARAMUZZI, 1983). In seasonally anoestrous ewes of several breeds, the introduction of males (if preconditioned by a period of isolation from rams ; teasing) induces and synchronises oestrous (OLDHAM *et al.*, 1978). In parallel, the introduction of bucks among anovulatory goats, after a period of complete segregation, induces synchronous ovulations in the following days (CHEMINEAU 1987).

In these species, olfactory cues constitute the sensory input emitted by the male at teasing. It has been found that exposure to wool collected in rams over certain skin regions which are rich in sebaceous glands will cause ovulation in seasonally anovular ewes isolated from rams (KNIGHT *et al*., 1980). Thus, in all cases, chemical compounds, acting as pheromones, appear to participate in the process of hypothalamic control of pituitary gonadotrophin secretions. This neuro-endocrine response can be triggered in a very short

delay : in ewes, the odour of the ram induces LH secretion after only a few minutes (POINDRON and LE NEINDRE, 1980). Other sensory cues (sight, sound and touch) are able to trigger the hormonal secretion by themselves (COHEN-TANNOUDJI and SIGNORET, 1987), but a direct and a sustained interaction with the ram always induces a greater answer compared with mere olfactory or visual stimulation. So, the different sensory information from the male acts in a cumulative way. In goats, when male stimulation is sufficient and anoestrus not too deep, the conception rate and litter size of females which were anovulatory before the introduction of males, are equivalent to those of previously cyclic females (CHEMINEAU 1987).

Accordingly, the "buck effect" has been used as an efficient method of controlling reproduction in these breeding species and appears to be a "biological" alternative to hormonal methods of stimulation, at least at some periods in the year. So far, we do not know whether similar mechanisms can be generalised to other taxa, such as the rabbit. Since the work of MYKYTOWYCZ's group, the paramount importance of olfactory exchanges between male and female rabbits has been clearly established. Animals of both sexes mark and overmark objects from the environment with secretions from their submandibular glands. In females, the rate of this chin marking activity is positively correlated with the oestrus state, and it has been hypothesised that they then emit specific signals that attract males and externalise information about their sexual stage (VODERMAYER, 1989; HUDSON AND DISTEL, 1990; MCNITT, 1992). The opposite, male-to-female odour exchanges have to our knowledge not been assessed, nor has the validity of the "buck effects" phenomenon been gauged in the rabbit. Accordingly, the role of the buck's odour in the induction of the doe's sexual receptivity is open to further experimental investigation.

CONCLUSION

Since the last World Rabbit Congress, a great number of scientific teams have begun to study several biostimulation methods. As a general rule, little information is available related to the relationship between the use of biostimulation and rabbit welfare. This deficiency could be partly explained by the difficulty to find methods able to quantify animal welfare.

The efficiency of animal manipulation such as a change of cage, or doe gatherings, before insemination, has not been clearly demonstrated; moreover these methods are time consuming and difficult to manage on large rabbit farms. A short dam-litter separation could be a real alternative to hormonal treatments (oestrus synchronisation), if the stimulation is applied just before insemination and if free suckling is applied before and after insemination. A nutritional flushing following a restricted feeding period seems to improve reproduction performance. The results of studies using continuous or interrupted lighting programs illustrate the need to study photoperiodism in the rabbit more intensively. On the contrary to other zootechnical species, no study has quantified the rabbit buck effects on oestrus induction.

If some of these methods succeed in increasing fertility, they sometimes decrease young growth (lighting programs, dam-litter separation...). Consequently, researchers have to pay particular attention to the global productivity of stimulated does and to the durability of these positive effects. Moreover, long-term biostimulation methods (such as feeding programs starting at the prepubertal age and more generally, the preparation of the further reproductive career of does) could be an interesting avenue to explore. These methods have to be easy to apply, inexpensive, consistent with animal welfare and well adapted with cycled production.

It obviously appears "more than ever" that strict collaborations between nutritionists, specialists of behaviour and animal welfare, physiologists, geneticians... are absolutely necessary to precisely define how on a healthy herd, biostimulations are able to improve and standardise rabbit doe reproductive performance.

ACKNOWLEDGEMENTS

The author would like to thank Laurence FORTUN-LAMOTHE, Wendy BRAND-WILLIAMS, J. M. BRUN, B. SCHAAL, and the colleagues of the International Rabbit Reproduction Group, for their help in preparing the manuscript.

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