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BIOSTIMULATIONS APPLIED TO RABBIT REPRODUCTION : THEORY AND PRACTICE*

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ABSTRACT : Rabbit artificial insemination is now commonly practised in European countries. In order to maintain a "natural image" of rabbit meat and to be consistent with animal welfare, authors discuss the interest of biostimulation methods defined in opposition with "gonadotrophin treatment" to improve sexual receptivity of does at the moment of insemination. They first comment how at a physiological level biostimulations can improve reproduction performance. In a second part, they draw up the balance-sheet of their use in zootechnical species and rabbits. They concluded that these methods have to be easy to apply, inexpensive, consistent with animal welfare and have to improve also general productivity. Lighting programs, controlled lactation through temporary mother-litter separation and feeding programs seem the most interesting ways to explore.

RESUME : Biostimulations appliquées à la reproduction du lapin : théorie et pratique.
L'insémination artificielle de la lapine est maintenant couramment utilisée dans les pays européens. Afin de maintenir une "image naturelle" de la viande de lapin et d'être compatible avec le bien-être animal, les auteurs discutent l'intérêt de méthodes de biostimulation définies en opposition avec les méthodes hormonales, pour améliorer la réceptivité sexuelle des lapines au moment de l'insémination. Les auteurs montrent comment au niveau physiologique les biostimulations peuvent améliorer les performances de reproduction. Dans une deuxième partie, ils font le point de leur utilisation chez les espèces zootechniques et chez le lapin. Ils concluent que ces méthodes encore peu utilisées chez le lapin, doivent être faciles d'application, peu onéreuses et doivent aussi améliorer la productivité globale. Les traitements lumineux, une séparation momentanée de la mère et sa portée et la définition de programmes alimentaires adaptés aux différents systèmes pratiqués sont des voies qu'il conviendrait d'explorer.

INTRODUCTION

Rabbit Artificial Insemination (AI) is now commonly practised in European countries, allowing new systems of production known as "cycled production". A group of animals is inseminated every 5 or 6 weeks and the number of batches varies from 1 to 6. So, to assure good and regular production, it becomes more and more important to control factors acting upon the productivity.

If rabbit does can be inseminated immediately after kindling, their reproductive performance may vary with their parity, lactating status and sexual receptivity at the moment of insemination. THEAU-CLÉMENT and ROUSTAN (1992) showed that the lowest reproduction performance is shown by lactating non receptive does. This antagonistic effect of lactation on the reproductive function 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). This antagonism is particularly pronounced after 3–5 days of lactation. It should also be emphasized that in the case of natural mating, the negative effect has often escaped notice until now, since this antagonism is hidden when non receptive lactating females refuse to mate. So, to improve performance it is necessary to have reliable techniques to induce and synchronize oestrus of lactating does.

Many studies have been made on hormone treatments (MAERTENS *et al.*, 1995) but only a few on bio-stimulations. However, already twenty years ago, LEFEVRE and MORET (1978) showed the importance of an increasing daylength and cage changing on the onset of oestrus in nulliparous does. In lactating does, Pregnant Mare Serum Gonadotrophin (PMSG) is now largely used in rabbitries. Some authors have reported its efficiency to increase sexual receptivity, fertility and consequently productivity, but also have demonstrated an immune response due to successive injections. Others, showed a clear positive effect only during the first 4 cycles of insemination. So new studies are necessary to define more clearly the optimal conditions for the use of PMSG. During the 6th World Rabbit Congress, CASTELLINI (1996) mentioned that, in the near future, the European Community policy will impose a restriction on the use of hormones (gonadotrophins) in relation to their residues in meat,

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animal welfare and the desire to preserve a "natural" image of meat. So, the International Rabbit Reproduction Group (I.R.R.G.) decided to study some "Bio-stimulation" methods which do not require the use of hormones, in order to increase sexual receptivity and consequently the productivity of the rabbit does. In the first part, we will study how at the physiological level, bio-stimulation can improve the reproductive performance. In the second part, we will draw up the balance-sheet of their use in zootechnical species and rabbits.

INTERNAL RESPONSES TO ENVIRONMENTAL STIMULI

Reproduction is regulated by a complex hormonal system in which the hypothalamus and the pituitary gland play a leading role (THIBAULT and LEVASSEUR, 1991). The pituitary is controled by the hypothalamus. To understand fully the manner in which the pituitary function is regulated, it is necessary to be aware of the close anatomical relationship between this gland and the hypothalamus. The hypothalamus is a small gland located at the base of the brain. The pituitary, immediately under the hypothalamus, is composed of an anterior and a posterior lobe. The two lobes differ from each other embryologically, morphologically and functionally. Two factors may be considered:

1) Some hypothalamic secretory neurones receive stimulations coming from the higher nervous system and react by the delivery of neuro-hormones at the axon endings.

2) From a capillary plexus in the region of the ventral hypothalamus, long portal vessels pass down the pituitary.

So the hypothalamus, being the intersection between the nervous system and the endocrine apparatus, appears to be the "conductor" of the hormonal system.

Hypothalamo-pituitary hormones

GnRH (Gonadotropin Releasing Hormone) is a decapeptide hormone secreted at the hypothalamic level and able to stimulate both the synthesis and release of the two gonadotrophins FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) at the anterior pituitary level (KNOBIL and NEILL, 1988). These proteic hormones act upon the ovary: FSH is mainly responsible for follicular growth and LH controls the final follicular maturation and induces the ovulation of pre-ovulatory follicles. Thus the hypothalamo-pituitary axis is a functional unit.

Of course, the hypothalamo-pituitary axis synthesizes many hormones. We will just mention prolactin and oxytocin secreted by the anterior and the posterior pituitary, respectively. Prolactin acts on the mammary cells to cause them to produce milk, but also seems to antagonize gonadotrophins secretion (CASTELLINI, 1996). Oxytocin contributes to uterus contractions and milk ejection from the mammary gland (Fig.1).

Ovarian hormones

In most species and rabbit too, the ovarian steroïd hormones (oestrogen and progesterone) seem to exercise alternately a positive and a negative feedback, respectively for oestrogen and progesterone, on the secretion of GnRH, FSH and LH in the hypothalamo-pituitary complex. All this system regulates the sexual activity of the does (INRAP, 1988).

Moreover, complex mechanisms interfere with the hypothalamo-pituitary-ovarian axis with the participation of endogenous opioïd peptides such as endorphins, catecholamines (such as DOPA, Norepinephrine..), corticotrophin releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and cortisol (BAULIEU, 1990).

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

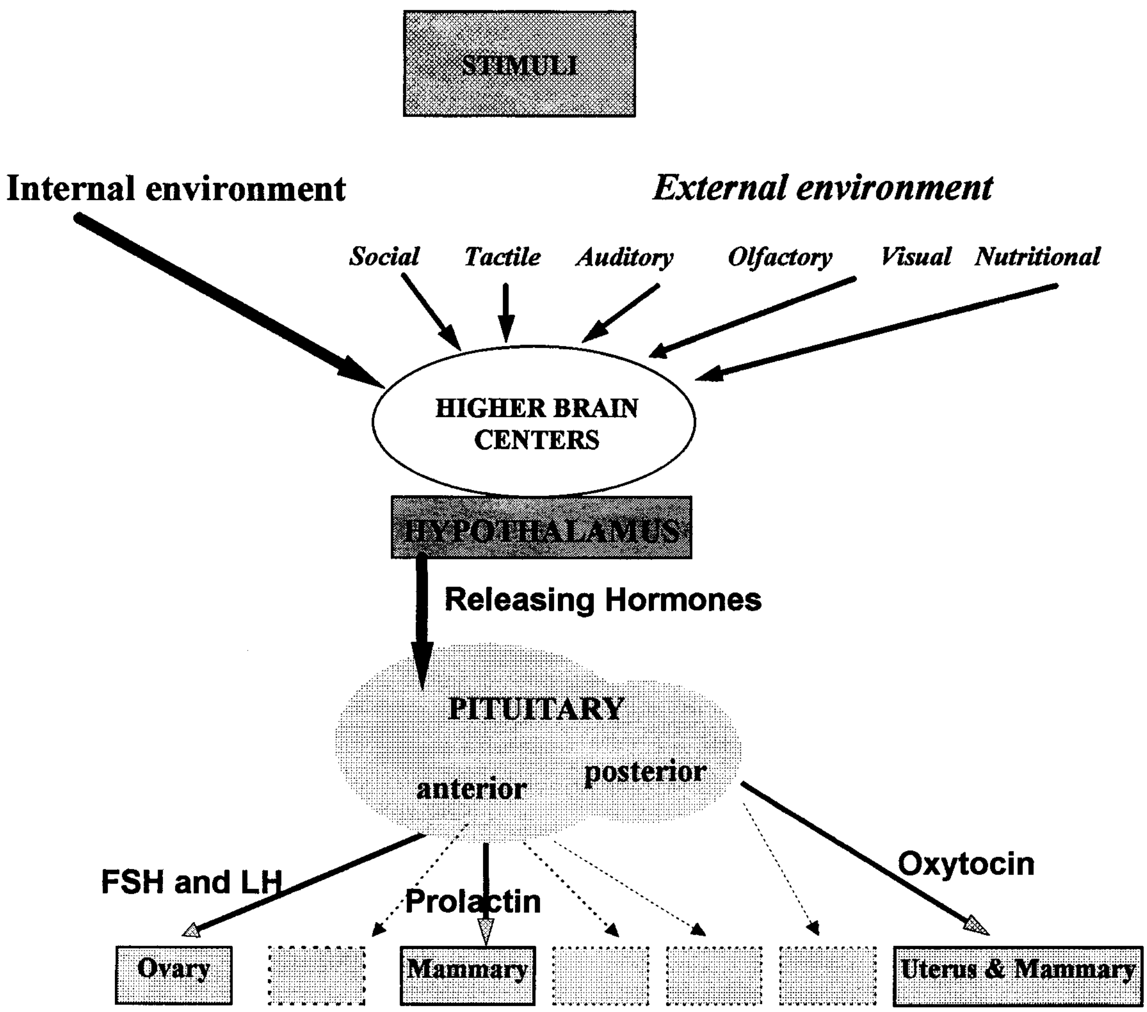
Although fundamental physiological differences exist between the rabbit (mating-induced ovulation) and some other domesticated species (cycled species for example), it is nevertheless of interest to consider some environmental factors which synchronise oestrus in other species.

ENVIRONMENTAL FACTORS USED IN BREEDING SPECIES

Photoperiod

♦ *Poultry.* Research work on photoperiod applied to poultry was largely developed between the thirties and the eighties, and has lead to numerous applications in poultry husbandry practice. The perception of light is through the skull (and not extra-retinal as in other species) and melatonin (a hormone secreted by the pineal gland with a well-defined day/night rhythm) has apparently a minor involvement. Photoperiod has a double role and acts first as a stimulant of reproduction and secondly as a daily synchronizer of the endocrine events which result in ovulation. With hens, asymmetrical interrupted lighting programs, keeping a

Figure 1 : Effects of environmental factors on the hypothalamo-pituitary axis



main night period (8 or 10 hours), can lead to some extra productivity without any modification of the egg characteristics (SAUVEUR, 1996).

♦ *Sheep and goats.* Breeds of sheep and goats from temperate latitudes exhibit seasonal variations of breeding activity which are controlled by annual photoperiodic changes. Short days stimulate sexual activity, but prolonged exposure results in refractoriness to short days and subsequent cessation of reproductive activity. The refractoriness can be broken by exposing the animals to long days, thus alternations between long days and short days are essential for the photoperiodic control of seasonal reproduction. Light pulses during the dark period can mimic a long day but it is necessary to use melatonin to mimic a short day. 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 sheep and goats has lead to the successful application of light treatments to control seasonal reproductive activity in field conditions (CHEMINEAU *et al.* 1992).

♦ *Pigs.* The primiparous sows have a delayed oestrus after weaning. KERMABON *et al.* (1995) showed that all sows subjected to a gradual decrease in photoperiod during pregnancy (from 12 to 8h/day) went into oestrus by 10 days post–weaning, in contrast to sows hold on a constant daylength.

♦ *Rabbits.* Within the European latitude, HAMMOND & 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 in increasing daylength. WALTER *et al.* (1968) showed that 16h/24h of constant lighting all the year round reduces the reproduction problems normally associated with decreasing daylength periods.

THEAU–CLÉMENT *et al.* (1990) found that a modification of the daylength performed 8 days before AI (from 8h continuously to 16h/day) significantly improved the sexual receptivity of the does : 71.4 % *vs* 54.3 % for the control (16hlight/day constant). However, the effect on fertility was not significant (61.4 %*vs* 48.9 %), as confirmed by MAERTENS and LUZI (1995). MIRABITO *et al.* (1994b), using a similar experimental 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 minimum between 2 fertile inseminations) obtained a significantly higher fertility in the experimental group (+9%). However, in these studies, the weight of the litter at weaning was significantly lower in the treated group, suggesting that the lighting program can adversely affect the suckling ability of females and/or the feeding behaviour of the young rabbits.

Interrupted lighting programs have been also studied on rabbits. UZCATEGUI and JOHNSTON (1992), concluded that Rex rabbits need at least 14 h of continuous light to meet their reproductive potential and 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 total hours of light. ARVEUX and TROISLOUCHES (1994), submitting commercial hybrid does to different lighting programs (continuous : 16h light/day or discontinuous : 8h light, followed by 4h dark and so on), increased fertility (82.6 *vs* 67.6 % for natural mating) without any reduction of litter weight at weaning.

In conclusion, these results illustrate the need to study more intensively photoperiodism in the rabbit. Moreover, lighting programs are easy to apply and do not need large manpower costs. They will be all the more efficient as rabbit does will be in the same physiological condition. So, lighting programs are perfectly adapted to cycled production.

Controlled lactation

In some zootechnical species, an hormonal mechanism is responsible for the *post partum* anoestrus: the persistence of prolactin, allowing continued lactation, delays or blocks the gonadotropin secretions and consequently the onset of oestrous. In the sow, the block is total: the decrease in the amount of circulating prolactin following weaning allows gonadotropin secretion, and a new reproductive cycle begins. For cows and ewes, the ovarian blocking intensity varies with the production mode and the management systems, being more severe for lactating than for milking females. For mares, suckling by the foal does not block the onset of the ovarian cycle, so the general hormonal mechanism mentioned above does not apply.

♦ *Sows*. A daily litter separation from 6 to 12 hours at 2 to 5 weeks *post partum* induces oestrus in 65% of sows, compared with 50% in the control group (STEVENSON and DAVIS, 1984).

♦ *Rabbits*. Zootechnical results show that immediately after kindling there is a short period (1–2 days) during which nearly all does exhibit good sexual receptivity. On the other hand, 3 to 5 days after littering, does show very little sexual activity. Few physiological studies allow us to understand the mechanisms involved: moreover, the conclusions drawn by different authors are sometimes contradictory.

PAVOIS *et al.* (1994), by preventing access to the nest box by the doe for 24 or 36 hours before the AI obtained an increased fertility of 13 and 11% respectively in comparison with a freely suckling control group. The litter weight at weaning being adversely influenced only for a 36 hours separation. Verifying the effect of this technique (with some modifications) in 33 commercial rabbitries, DUPERRAY (1995) concluded that a temporary doe–litter separation results in an 8.5 % increase of fertility (in 70% of rabbitries) without any harmful consequences to the doe or the litter.

Animal manipulation

♦ ***Males*** : *Goats*. Based on a literature review, CHEMINEAU (1987) concluded that the introduction of bucks among anovulatory goats, after a period of complete segregation (odor, sight, sound and touch), induces synchronous ovulations in the following days. The percentage of responding goats when the olfactory stimulus is suppressed is lower than for goats in direct contact with males, indicating that all senses are probably involved in the response of intact females. Direct contact between sexes increases the percentage of ovulating females, and an increase in the number of males increases the induced ovulation rate. So, when stimulation is sufficient and anoestrus not too deep, conception rate and litter size of females which were anovulatory before the introduction of males, are equivalent to those of previously cyclic females.

♦ ***Females*** : *Rabbit*. MIRABITO *et al.* (1994a), by putting three females together immediately before insemination, did not obtain any improvement of performance even in nulliparous does. REBOLLAR *et al.* (1994) showed that a change of cage in nulliparous does, 48 hours before insemination affected the fertility to the same degree as 25 IU PMSG (81.8 *vs* 79.6 %, respectively).

Feeding

♦ *Ewes*: The weight before mating reflects the mean nutritional status of the flock and has a determining influence on ovulation rate, fertility and prolificacy. 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 the embryo viability. Hence "flushing" is commonly practised. This consists of increasing the ewe's feeding level just before mating.

♦ *Rabbit*. PARIGI–BINI and XICCATO (1993) observed large energy losses (28%) by primiparous does during lactation due to large simultaneously requirements for lactation, body growth and pregnancy. This could 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 that, in primiparous does, the detrimental effect of lactation decreases with a smaller number of suckling young rabbits and showed that a negative nutritional balance of does depresses foetal growth. In nulliparous does, VAN DEN BROECK and LAMPO (1977) demonstrated that a flushing following a restricted feeding period, improves reproduction performance. With lactating does, some rabbit breeders claim to use flushing too before insemination, but no scientific study has really defined a nutritional program well adapted to artificial insemination and for various production systems (depending on reproductive rhythm) and for various physiological status of the doe. This program should be really well adapted to cycled production.

CONCLUSION

Physiological mechanisms can be different between zootechnical species and sometimes even within the same species, depending on the breed or breeding systems. Moreover, many hypotheses on the effect of these hypothetical stresses on endocrine balance (prolactin, gonadotrophins...) can be suggested but, as far as the rabbit is concerned, few scientific answers are available. In conclusion, certain observations may be made:

- Efficient and lasting improvements can be achieved only in a healthy herd.
- A better understanding of physiological mechanisms could help us to devise some "bio-stimulation" methods able to improve the sexual receptivity of rabbit does.
- Before applying these methods in rabbitries, we have to test whether they improve not only reproductive performance, but also the general productivity (which depends on milk production, welfare, health and young growth...) during the whole lifetime production.
- These methods have to be easy to apply, inexpensive (low manpower costs), consistent with animal welfare and adapted with cycled production. Even if they are not, they can offer alternative systems which can interest breeders. Lighting programs, temporary doe–litter separation and feeding programs seem to be worthwhile avenues to explore.

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