RECENT ADVANCES IN RABBIT ARTIFICIAL INSEMINATION

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Artificial insemination (a.i.) has been introduced in industrial rabbitries mainly to improve breeding management. Further development of reproductive techniques (deep freezing of semen and embryos, embryo transfer; (GARCIA *et al.*, 1991), as occurred in other zootechnical animals, could lead to important changes in selection methods and preservation of rabbit populations (JOLY AND RENARD, 1994).
A.i. permitted to develop a new system of production which consists in executing breeding operations on fixed days of the week; cycled groups should be created every week, but further significant improvements can be reached with only one group per rabbitry which corresponds to inseminations every 5 or 6 weeks. Cycled production (c.p.), which groups animals in the same physiological phase, permits better production planning, reduces the needed of man power (about 10-15%), and facilitates the planning of all in-all out.
The use of this technique requires some punctual considerations. C.p. requires a fixed reproductive rhythm: inseminating every 5-6 weeks non-pregnant does follow the same rhythm of insemination as pregnant ones (figure 1).
Comparing the c.p with the traditional system (t.s.), c.p. increases the unproductive period of non-pregnant does. Consequently, when pregnancy rate is low many does must wait until the next cycle of insemination, thus increasing the kindling interval.

Figure 1: Scheme of different production system (cycled and traditional).

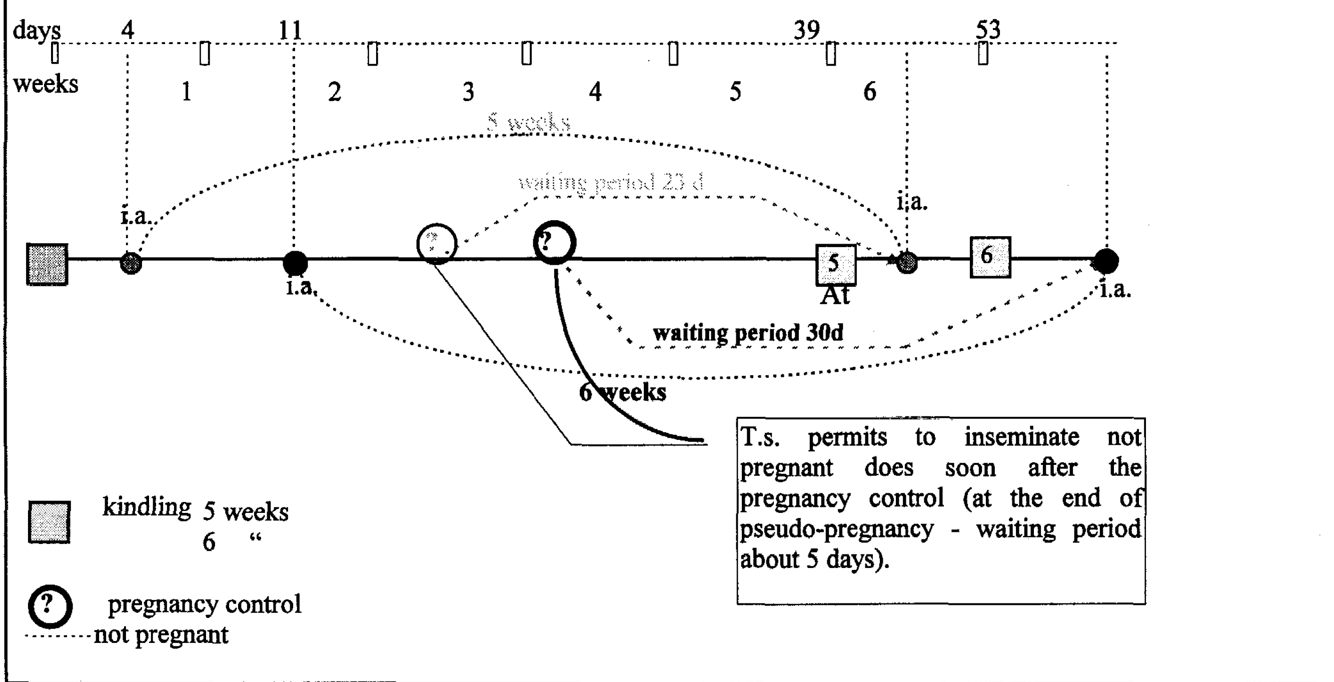
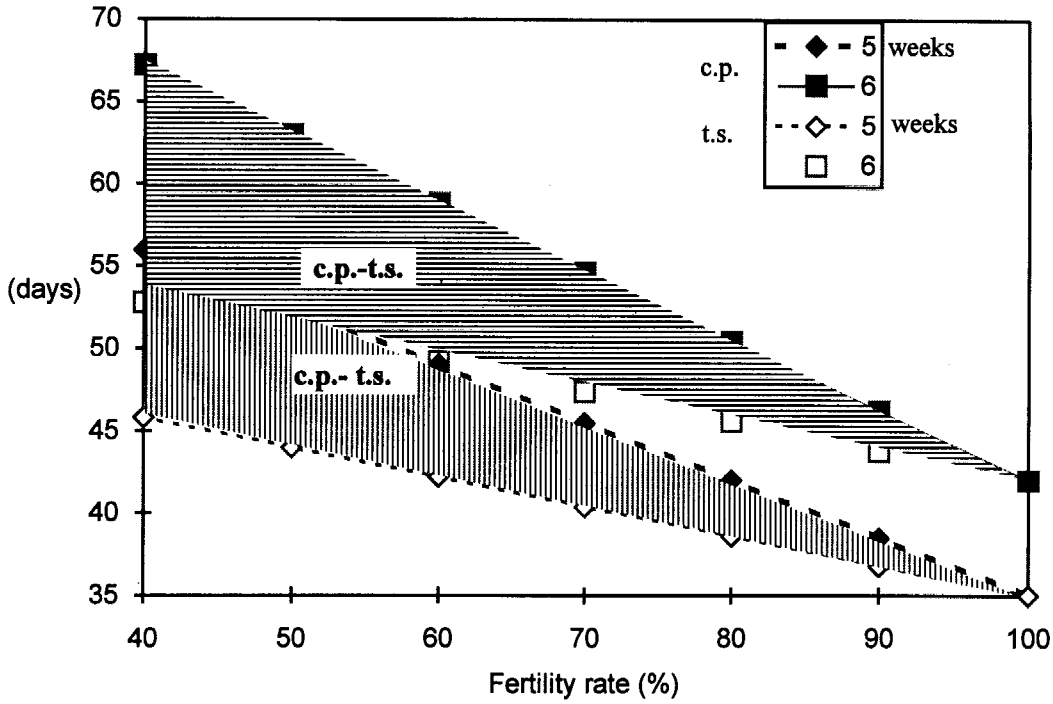


Figure 2 shows the relationship between fertility rate and theoretical kindling interval when different systems of production are compared (c.p. at 5 or 6 weeks vs t.s.).

Figure 2 : Relationship between fertility rate and kindling interval



When fertility rate is low (40%), kindling intervals are quite different in the two systems (c.p - t.s.: 10.2 and 14.4 days at 5 and 6 weeks, respectively), while as the fertility increases (90%), the differences become slight (1.7 and 2.4 days).
The c.p. at 5 weeks, hypothetically summarizes the positive effects of cycled production, reducing simultaneously the interval between successive a.i. However, to choice a suitable reproductive rhythm requires the analysis of other physiological factors.
Thus, it is very important to control all the factors affecting reproductive performance especially:

- FEMALE
- MALE
- TECHNOLOGICAL ASPECTS

FEMALE

One of the most important aspects of a.i. is the possibility of inseminating female rabbits regardless of their oestrus phase. This fact can be considered as a positive but, in view of a fixed cycled of production, the irregular alternation of oestrus, which strongly influences reproductive performance, can be disadvantageous.
To assure good and repeatable performance, some interacting factors, should be analyzed:

1. **KINDLING ORDER;**
2. **PHYSIOLOGICAL PHASE;**
3. **SEXUAL RECEPTIVITY**

1. KINDLING ORDER

Three categories of does with different reproductive responses can be defined: nulliparous, primiparous and pluriparous.
Many papers (BOURDILLON *et al.*, 1992; THEAU and LEBAS, 1994; DAVOUST *et al.*, 1994) concur that while nulliparous does generally show good performance (high fertility rate, medium litter size), primiparous, inseminated during lactation, give poor results.
Fertility rate of pluriparous lactating does are intermediate between nulliparous and primiparous, while litter size is generally higher.
This effect is probably related to hormonal antagonism between lactation and reproduction and to an energy deficit, particularly conspicuous for primiparous does (28% of energetic losses during lactation: PARIGI BINI

and XICCATO, 1993) which, simultaneously, have elevated requirements for lactation, body growth and gestation. FORTUN and LEBAS (1994), confirmed that, in primiparous does, foetal survival and foetal growth are negatively affected by lactation and concurrent pregnancy; the detrimental effect of lactation decreases with few suckling rabbits and the foetal growth is mainly dependent on the nutritional balance of doe.

2. PHYSIOLOGICAL PHASE (LACTATION OR NON LACTATION)

Rabbit does can be inseminated soon after parturition and are able to sustain contemporary lactation and pregnancy. However, it is well-known that lactation negatively influences all the reproductive functions of does, depressing sexual receptivity, ovulation rate (THEAU and POUJARDIEU, 1994; FORTUN and BOLET, 1995), fertilization, and embryo development.

Prolactin seems to be mainly responsible of this negative effect: it could act on the pituitary gland by depressing gonadotrophin secretion (FSH, LH), while in the ovary, it decreases *in vitro* follicular growth (HAMADA *et al.,* 1980), reduces LH receptors (KERMABON *et al.*, 1994), and consequently the efficacy of endogenous hormones.

High levels of prolactin, occurring during lactation, are responsible for the reduction of progesterone (FORTUN *et al.*, 1993) and for the increase of late foetal mortality.

3. SEXUAL RECEPTIVITY

A doe is defined as receptive if, in the presence of a buck she adopts a lordosis position and accepts the mate; the colour of the vulva is also a quite good indicator of receptivity and is easy to check (MAERTENS and LUZI, 1995a).

Sexual receptivity is the simplest index to detect the ovary status: a receptive doe generally exhibits a higher number of large follicles (KERMABON *et al.*, 1994) and a higher oestrogen level with respect to non-receptive ones (REBOLLAR *et al.*, 1992d; UBILLA and REBOLLAR, 1995).

Reproductive functions of rabbit does are not strictly related to sexual receptivity, and females can ovulate in every oestral phase, but the reproductive performance increases in receptive females.

Sexual receptivity is highest immediately after parturition. This fact is probably due to the inversion of the oestrogen/progesterone ratio occurring after parturition. Successively, sexual receptivity decreases to a value of about 40% at 3-4 days *post partum (p.p.)* and progressively increases in the following period (13-14 days *p.p.*). The initial level of receptivity is reached only after the weaning of young rabbits (THEAU-CLEMENT *et al.*, 1990a).

It should be considered that, apart from the first day *p.p.*, receptivity is affected by many factors (milk production and/or number of suckling rabbits, general condition of doe, environmental conditions) and shows a considerable variability. TORRES *et al.* (1987) showed that the number of suckling rabbits exerts a negative action on the mating acceptance of the doe.

Other factors can influence sexual receptivity. BOITI *et al.* (1996) showed that since rabbit does are susceptible to ovulate soon after parturition, ovulation can spontaneously occur in about 17% of does (probably caused by stress factors such as manipulation, controlled milking, etc.). These does, adopting a standard reproductive rhythm (6 weeks cycle), exhibit a high level of progesterone (> 2 ng/ml) and are not receptive and practically infertile.

Receptivity interacts with physiological phase and its effect is particularly marked in lactating does. As evidenced by THEAU and ROUSTAN (1992), the lowest reproductive performance are shown by lactating non receptive does.

Thus, to improve performance, considering that does are generally inseminated during lactation, it is necessary to increase sexual receptivity in lactating animals.

Many proposals have been made to synchronize sexual receptivity but the lack of regular oestrus cycle and the different ovarian conditions can produce multiple reproductive response.

However, the most studied effects are the followings:

- Inoculation of exogenous hormones (PMSG, combination of PMSG-hCG, prostaglandins);
- change of reproductive rhythms;
- bio-stimulations.

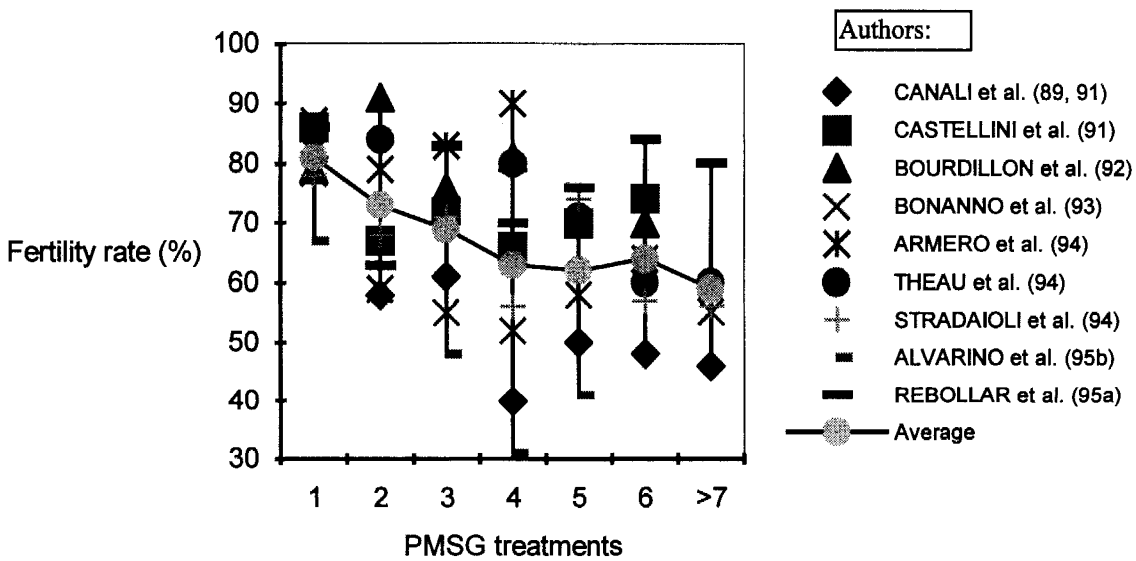
- **HORMONES**

The use of hormones to synchronize oestrus is widely diffused because of its simplicity and efficacy, it also requires fewer modifications of breeding operations (reproductive rhythms, raising structures; ALVARINO *et al.*, 1995b).
The treatment consists in administering a different dose and type of hormone, 2-3 days before the insemination.

PMSG (Pregnant Mare Serum Gonadotrophin).

PMSG is the most widely used hormone in industrial rabbit breeding.
The main action of this gonadotrophin is the rising of the oestrogen release of follicles present in the ovaries, subordinately PMSG should promote follicular growth with an increase of ovulatory response.
Many authors have reported its efficiency (increased receptivity, fertility and litter size) also suggesting the possibility of an immunity response due to successive inoculations (ADAMS, 1961, HEIDBRINK, 1980). The immune reaction triggered by PMSG are not surprising, taking into account its proteic nature and the high molecular weight.
CANALI *et al.* (1989) and BOURDILLON *et al.* (1992) found an increase of anti-PMSG antibodies in does repeatedly treated with this gonadotrophin. CANALI *et al.* (1991), administering 40 UI, verified that the immunization process, occurring in 55% of does, depressed reproductive performance and was negatively correlated with both number and interval between subsequent treatments: a doe which, after a negative treatment, is immediately re-inseminated frequently develops a relevant immune response.
BOITI *et al.* (1995) confirmed that the antibody response is subjective, and that hyper-immune animals, after 3 treatments, show smaller litter size and lower fertility rate. On the other hand, LEBAS *et al.* (1996), submitting does to 9 administrations (6 weeks interval) of PMSG, found that at the end of experiment the immune response (occurring in 30.6 % of does) cannot be related with sexual receptivity and fertility of the animals.
A dose response effect is probably involved in this process: anti-PMSG antibodies do not greatly increase when a low dose (20 UI) and a longer interval between subsequent treatments (6 weeks also for non-pregnant does) is used (STRADAIOLI *et al.*, 1994).
Analyzing the effect of repeated PMSG treatments reported by different authors, a reduced efficacy with the increase of treatment number order seems evident (Figure 3). This reduction does not depend only on treatment; the effect of parity and the aging of the doe can progressively reduce productive performance, even in non treated animals.

Figure 3: Fertility rate following PMSG treatments.



A positive effect is observed exclusively in the initial administrations. In a long-term experiment, THEAU and LEBAS (1996) pointed out that PMSG significantly improves performance only in the first 4 cycles of inseminations (+12.5% fertility rate; +8% litter size) and only in lactating animals (+18.4%).

To reduce the number of PMSG treatments, only non receptive does could be treated. ALABISO *et al.* (1994) and BONANNO *et al.* (1995), pointed out a differentiated reproductive protocol: receptive does were inseminated without any synchronized treatment, while non receptive ones were treated and inseminated 3 days later. This 'alternative' protocol increased reproductive performance but, unfortunately, it is not well adapted to c.p. and requires a large amount of manpower to detect receptivity.

Some authors have investigated the effect of this gonadotrophin on the genital apparatus with particular attention given to the ovarian arrangement, the quality of follicles and embryos. SCHMIDT *et al.* (1992) found that this gonadotrophin is responsible for hyperemia of the genital tract and for a significant increase in ovary weight. GOSALVEZ *et al.* (1994) found a negative influence of a super-ovulation dose of PMSG (100 UI/animal) on the quality of growing follicles in primiparous does.

GARCIA-XIMENES and VICENTE (1990), treating does with 50 UI, observed an abnormal number of cystic and haemorrhagic follicles probably due to a secondary LH-activity of PMSG on immature antral follicles which did not receive enough LH to ovulate and therefore degenerated into cysts or haemorrhagic follicles (BOMSEL-HELMREICH *et al.*, 1989). The same authors (GARCIA-XIMENES and VICENTE, 1992), analyzing the embryo recovery rates and the survival of frozen-thawed embryos, pointed out that the above mentioned defects negatively affect the number of embryos recovered (-7%) and the pregnancy rate of the recipient doe (-45%).

STRADAIOLI *et al.* (1993; 1994) and BOITI *et al.* (1995), in does repeatedly treated with 20 UI of PMSG, also reported an abnormal ovarian response and a higher number of haemorrhagic follicles which could be the result of ovarian disarrangement; in addition, the quality of recovered embryos and their *in vitro* development is significantly lower with respect to non treated does.

PMSG probably achieves opposite effects: it increases follicular growth and consequently the oestrogen release and the sexual receptivity (ranging from about +10 to +40%) and simultaneously modifies ovarian equilibrium, embryo development and foetal survival. In fact, when PMSG does not increase sexual receptivity or when only receptive females are compared the fertility rates are the same or a little worst (treated vs non treated does - ALABISO et al., 1994: -8.4%; MAERTENS *et al.*; 1983: -16.7%; THEAU and LEBAS, 1994: -9.9%; ARMERO *et al.*, 1994: +0.7%).

Direct comparisons between positive results obtained with the systematic application of PMSG in commercial rabbitries (CECCHINI *et al.*, 1992; COLIN *et al.*, 1992; PAREZ, 1992) and the above mentioned disadvantages cannot be easily done. The experimental conditions (dose, intervals between treatment and a.i., early replacement of does, genetic strain) and the receptivity and fertility level of females at the beginning of treatment can strongly affect the results. PMSG has less effect when the comparison is done in groups with high productivity (THEAU and LEBAS, 1994); in particularly severe conditions (primiparous does, lactating does, inseminated at 3-5 days of lactation) PMSG induces significant improvement.

Thus to determine, as completely as possible, all the advantages and disadvantages, the protocol of using must be standardized.

Some authors have experimented with low doses: BONANNO *et al.* (1991a) indicated that 10 UI are not sufficient to determine a positive effect on reproductive performance; when ovarian follicles have different degrees of maturation, this level does not permit standard response (BONANNO *et al.*, 1990). The administration of 20 UI, evaluated at both the ovarian level (BONANNO *et al.*, 1990) and in rabbitry experience (BONANNO *et al.*, 1991b, 1993), assures better performance.

PMSG associated with hCG permits further reductions of the dose; the administration of 8 UI + 4 UI respectively, during an unfavorable period of lactation (4-5 days after kindling), obtained good performances (DAVOUST *et al.*, 1994).

Only a few papers have analyzed the relationship between the dose and the interval treatment-insemination; it seems that the lowest doses of PMSG should require a longer time to develop large follicles (from 48 to 72 hours, BONANNO *et al.*, 1991a; from 72 to 96 - FACCHIN *et al.*, 1992). Increasing the dose (50 UI), GARCIA-XIMENES and VICENTE (1990), found that the most favorable interval was 48 hours; MANCHISI *et al.* (1988) showed that after only one day from the administration of 40 UI, 100% of the does accepted the mate.

The effect of PMSG on litter size is generally more evident (about +1 young/litter; MAERTENS and LUZI, 1995a) but in some experiments the increase was also associated with higher mortality at birth (ALABISO *et al.*, 1994; MAERTENS *et al.*, 1983) and an abnormal distribution of litter size (< 5 and >12 pups/litter; MAERTENS and LUZI, 1995b). THEAU and LEBAS (1996) did not find any effect of PMSG on the distribution of litter size, but reported a reduction of milk production, probably related to increased receptivity. The same argument was used by BONANNO *et al.* (1995) to explain a similar reduction of milk output (about 400 g/lactation). This reduction in milk can be further explained by the higher pregnancy rate registered in PMSG treated does and the reciprocal antagonism between gonadotrophin hormones and prolactin.

The results obtained by the different authors do not permit to reach any final conclusions regarding PMSG, however, the following recommendations can be suggested:

- reduce the dose (20 UI or less if associated with hCG) and avoid short intervals between subsequent treatments;
- do not use PMSG for all females; treat only the does where the treatment promises to be very effective (lactating does, THEAU and LEBAS, (1994); MAERTENS *et al.*, 1995) or when the litter size is particularly low;
- use the gonadotrophin in seasons particularly negative for reproductive performance;
- regarding the efficacy on the primiparous, it should be considered that, although the treatment gives a significant increase in performance (BOURDILLON *et al.*, 1992; PAREZ *et al.*, 1992), the nutritive requirements of a young doe remain unsatisfied even if particular feeding programs are adopted (PARIGI-BINI *et al.*, 1995). As a consequence, the future reproductive activity could be negatively affected and the female could present, during its reproductive life, worse health and poorer performance. Primiparous does could be re-inseminated with a more extensive rhythm or, alternatively, their litter size could be reduced.

Prostaglandins (pgs)

The luteolytic effect of PGs in the rabbit has been widely reported since 1970, but only recently natural or synthetic PGF2α have been used for breeding purposes (McNITT, 1992) to reduce the functional life-span of *corpora lutea* in pseudo-pregnant does (NAVA *et al.*, 1992, REBOLLAR *et al.*, 1992b) and to induce parturition.

Regarding the first use it is important to note that GnRH administration at a.i. induces ovulation in almost all the females determining, in non-pregnant does, a phase of pseudo-pregnancy lasting about 16-18 days. The treatment with PGs is therefore appropriate but not widely used because, as affirmed above, the c.p. at 5-6 weeks requires an unproductive period longer than pseudo-pregnancy.

Regarding the second effect, the synchronization of parturition is a positive factor in view of the simultaneous care of nest boxes, born alive, weaned rabbits. Synthetic (100-200 μg) or natural (> 700 μg - ALVARINO *et al.*, 1995a) PGS, injected at 28 or 29 d of pregnancy, induce "natural" parturition approximately 64 hours later, reducing the mortality at birth without any negative effect on maternal behaviour (UBILLA and RODRIGUEZ, 1989; FACCHIN *et al.*, 1991; REBOLLAR et al., 1992c).

A indirect positive effect of F2α used to synchronize parturition is an increased sexual receptivity and fertility rate when the does are inseminated about 7 days *p.p.* (+16% UBILLA and RODRIGUEZ, 1989; +20% REBOLLAR *et al.*, 1992a).

Many data suggest that, besides the luteolytic effect, PGs may be involved in the ovulatory process by stimulating the enzymatic proteolytic cascade which leads to the disruption of the follicular wall (MYAZAKI *et al.*, 1991). However, this physiological role has also been modulated by many other substances including histamine, kinins, leukotriens, plasminogen activator which are involved in the process. Systemic PGs can also release pituitary LH, but the large doses required to induce the effect, undermine their physiological role during the normal ovulatory process.

SINKOVICS *et al.* (1989) and FACCHIN *et al.* (1992), using 200 μg of $F_{2\alpha}$ 64 hours before a.i., for synchronizing oestrus of pluriparous does, showed a positive effect on the performance (+ 6.4% fertility rate; + 0.7 litter size compared to PMSG treated group). In a comparable trial, ALVARINO *et al.* (1995c) concluded that PGs improve the fertility rate in nulliparous (83.9 vs 76.9 PGs vs PMSG 25 UI) and pluriparous does inseminated at 11 days *p.p.* (82.6 vs 80.2 respectively) while at 4 days *p.p.*, PMSG resulted the best.

The physiological action of PGs is not obvious because rabbit does are not cyclic females and the reproductive response could be mediated by the ovary: if the doe presents different ovary situations, the effect of PGS could be very different (luteolytic or luteotrophic).

However, other physiological research is required before prostaglandins should be considered as an appropriate synchronizing molecule.

- **REPRODUCTIVE RHYTHM**

The interval between kindling and a.i. represents one of the most important factors affecting the economy of a rabbitry.

The effect of reproductive rhythm on the performance of does has been widely studied with controversial results (MAERTENS and OKERMAN, 1987; PARIGI-BINI *et al.*, 1989; LAMB *et al.*, 1988, 1991). Many rearing conditions have now changed and the reproductive rhythm also depends on the c.p. scheme adopted.

As affirmed, rabbit does do not show a regular cyclic evolution of sexual receptivity after kindling; modifying the day of insemination it is possible to improve the percentage of receptive does and consequently the reproductive performance. The antagonism gestation-lactation depends on the day of lactation; there are particularly negative periods for reproductive activity (3-5 days *p.p.*) and others where the depressive effects are smaller (THEAU CLEMENT and ROUSTAN, 1992).

Post partum fecundation is physiological for rabbit does, but selection for increased litter size and milking ability make more difficult the full concomitance lactation-pregnancy. During the reproductive season, the wild rabbit and the hare, recurrently mate before (super-foetation) or immediately after kindling (about 55%, CASTIGLIONE, 1994). In domestic rabbit, when a low number of suckling pups is present (GARCIA and PEREZ, 1989) the effect of lactation on sexual receptivity and fertility is negligible.

Many papers (PARIGI-BINI *et al.*, 1989; CASTELLINI and BATTAGLINI, 1991), comparing a *p.p.* vs semi-intensive rhythm (8-11 days), showed a non proportional reduction of kindling interval due to a progressive reduction of mating acceptance and fertility rate. Similarly CERVERA *et al.* (1993) found that the reduction of kindling interval is only 59% of the expected value (5 vs 8.5 days).

Generally, as the interval from a.i. and parturition become longer, there is a progressive increase of fertility rate; REBOLLAR P. *et al.* (1994) comparing fertility of does inseminated at 3-4, 10-11 or 24 days *p.p.* with respect to non lactating does, found a reduced effect of lactation (-18.1 vs -20.2 vs -9.3% respectively).

The c.p. at 5 weeks (a.i. 3-4 days *p.p.*), in a particularly unfavorable phase for reproductive activity, probably cannot be planned without the help of exogenous stimulation (DAVOUST, 1994; fertility rate: 19.7 vs 47% - control vs PMSG).

FANTUZZI, (1993), comparing an extensive rhythm (a.i. after weaning) with the most common semi-intensive (11 days *p.p*), estimates that extensive system is more economic. More data about the "expected" better sanitary conditions of the rabbitry (does and young rabbits) and higher production/cage, are necessary to conclude about this topic.

An optimum reproductive rhythm probably does not exist and multiple protocols can be established taking into account various factors of production (number of cages, cost of rabbitry, type of man-power, etc.).

- **BIO-STIMULATIONS**

In the near future, EC policy (regarding meat residues, animal welfare) and the exigency to maintain a "natural" image of rabbit meat, will determine a reduction in the use of hormones.

The induction of punctual stresses, modifying the endocrine balance of the doe, can improve reproductive activity without any hormonal treatment. The most studied factors are lighting programs, the control of lactation and the manipulation of doe.

Lighting programs

THEAU *et al.* (1990b), modifying the light program (8 hours light/day until 8 days before insemination and 16 hours/day immediately after), found significant improvement of sexual receptivity of the does (71.4 vs 54.3%) without any effect on fertility as confirmed by MAERTENS and LUZI (1995a).

MIRABITO *et al.* (1994), using a similar photoperiod but with a longer re-mating interval (6 vs 5 weeks), obtained a higher fertility in the experimental group (+9%); the weight of litter at weaning was significantly lower in the treated group, indicating that the light program negatively modified the milking ability of females and the feeding behaviour of the animals.

ARVEUX and TROISLOUCHES (1994), submitting does to different lighting programs (continuous: 16 hours/day light, discontinuous: 8 light - 4 dark - 8 light - 4 dark), increased fertility (82.6 vs 67.6 % natural mating) without any reduction of litter weight at weaning.

Comparing the effect of light duration in tropical conditions (16 hours/day vs natural light), DEPRES *et al.* (1994) concluded that the light program can improve performance only when other limiting factors (climate: hot and wet season) are favorable.

It is necessary to note that different lighting programs can be used only with one group per rabbitry.

Controlled lactation

PAVOIS *et al.* (1994), avoiding access to the nest by the doe for 24 or 36 hours before a.i., obtained an increase of fertility of 14 and 11% respectively; the litter weight at weaning was negatively influenced only in the second case. Examining the effect of this technique (with small modifications) in 33 commercial rabbitries, DUPERRAY (1995) concluded that the separation doe/litter caused an 8.5% of fertility gain (in 30% of rabbitries no effects) without any negative consequence to the doe or the litter.

Doe manipulation

REBOLLAR *et al.* (1995b) showed that the change of cage of nulliparous does, 48 hours before a.i., determined the same fertility rate as 25 UI PMSG (81.8 vs 79.6%). MIRABITO *et al.* (1994) gathering three females together, immediately before a.i., did not obtain any significant improvement of performance even in nulliparous. Probably the short duration of stress (6 minutes) and stress type is not sufficient.

Many hypothesis on the effect of these stresses on endocrine balance (prolactin, gonadotrophins, oxitocine, etc.) can be suggested, but no scientific answers can be offered. To date bio-stimulation is only a proposal for the future and not yet an alternative technique of reproductive management; more details about the effect of stress factors on milk production, welfare and health of the doe, litter weight at weaning must be developed.

MALE

Few studies have analyzed the physiology of rabbit bucks and the effect of management and environmental conditions on the semen production.

In general, when the semen characteristics of bucks are compared both *in vitro* (BATTAGLINI *et al.,* 1992, PANELLA and CASTELLINI, 1990) and *in vivo* (fertility rate, litter size - MONFREDINI and CAVAZZONI, 1992; BLOCHER, ITAVI, 1992), a large variability is always shown. This high variability (between and within bucks) causes a reduced repeatability and heritability for semen traits and suggests that genetic improvement of these characteristics would probably not be easy to achieve.

However the study and the standardization of factors which affect semen production will surely reduce this variability.

One of the most significant works which analyzes the effect of collection frequency on semen traits (volume, pH, number of spermatozoa, motility, live sperms) is that of BENCHEIKH (1993, 1995). In two different trials, 3 collection rhythms (extensive: 2 successive ejaculates (within 15 min.) once a week; intermediate: 2 successive ejaculates 2 times/week; intensive: 2 successive ejaculate 3 times/week) were compared. The "extensive" frequency of collection significantly improved all the examined traits. Nevertheless, the weekly sperm output was greater with the intermediate or intensive rhythms, but the increase was very light (only 28% of total sperms and 16 % of live sperms) with respect to the multiple collections.

These results are in agreement with the collection rhythm already practiced by the majority of rabbit semen centers (KIPRIANIDIS and FACCHIN, 1994).

Only a few references exists regarding the establishment of an appropriate photoperiod for rabbit bucks.

Because of the results of WALTER *et al.* (1968) were in opposition with optimal natural photoperiod of wild rabbit, THEAU-CLEMENT *et al.* (1994) studied the effect of light programs (8 and 16 hours light/day) on reproductive activity of young bucks. Throughout most of the duration of the experiment (6 months) they showed that the photoperiod of 16 hours significantly increased sperm production (+24% live sperms per ejaculate) as well as motility.

Few studies analyze the effect of specific feed supplementation on the reproductive activity of bucks. EL-MASKRY *et al.* (1994), showed that addition of Selenium and vitamin E, or Zn, during the hot season, increased the sperm concentration and, above all the addition of Zn, significantly improved the seminal traits of heat stressed bucks.

Analyzing the extension of sperm defects, COURTENS *et al.* (1994), sampling 27 bucks of two strains, showed that the most common abnormalities regard the acrosome (about 12% of samples) which was negatively correlated with fertility rate ($r = -0.55$: $P < 0.01$). Moreover, the decondensation of the nucleus chromatin (5.8% of samples) negatively affects litter size ($r = -0.26$).

TECHNOLOGICAL ASPECTS

The material and the procedures used for collection (type of artificial vagina) and insemination (type of straw) and the semen processing is of interest in order to assure good reproductive performance.

Semen collection and insemination

BATTAGLINI *et al.* (1991) suggested that the model of artificial vagina influenced the adaptation of the buck to the collection and that a vagina with a wider collection cavity increased the number of bucks adapted to the collection.

Many different models (commercial or artisan) have been created (aluminum, CONTERA *et al.*, 1994, *glass*, plastic). The most important characteristics of an artificial vagina regard: suitability for semen collection, easy of use and the possibility of hygienically collecting semen samples (SYNKOVICS *et al.* 1994, DAL BOSCO *et al.*, 1996). Although correlation between biological and microbial traits has never been found, the reduction of pathogenic or potentially pathogenic germs in the semen is still important. In fact, a large addition of antibiotic to the medium may reduce the storage time of refrigerated semen (MERCIER and RIDEAU, 1992).
Regarding the transmissible diseases, CASTELLINI *et al.* (1994) demonstrated that myxomatosis virus can be found in semen of rabbit bucks experimentally infected and that the disease can be spread by means of insemination; the presence of the virus in the semen, which also occurs in the asymptomatic animals, is a risk involved in the use of a.i. Considering that virus can also be isolated in semen of vaccinated bucks (CENCI *et al.*, 1996), serious attention must be given to the sanitary conditions of bucks reared in semen centers and some standard hygienic protocols must be adopted when semen is to be sold.
LE RUYET *et al.* (1994) studied the effect of the material used for a.i. and the depth of insemination on doe performance. The author conducted four successive trials demonstrating the importance of depth of insemination on litter size and fertility rate. Inseminations at 15 cm of depth with the doe the back position gave the best results. THEBAULT *et al.* (1994) confirmed that deep insemination with a glass straw and the back position obtained the best result; a question still remains unsolved regarding the distinct effect of the two factors.

Semen processing

A further diffusion of a.i. for genetic purposes requires semen storage. To date a.i. with refrigerated semen obtains results comparable to those with fresh semen, (MAERTENS and LUZI, 1995a) while deep freeze technique is not yet usable in the open field.
One of the most important factors which affects results is the medium used in semen dilution and the dilution rate. Surely TRIS-buffer and other commercial extenders, although not specific, are well adapted to extend rabbit spermatozoa. GOTTARDI *et al.* (1993) comparing the response of four different media at various times (0, 24 and 48 hours) and temperatures (5, 15, 25 °C) concludes that IMV (commercial extender) and TRIS media are the best and 15 °C is the optimal condition to store rabbit semen.
Another comparative study, carried on five media maintained at various temperatures, showed analogous results. After 50 hours of conservation, live sperms decreased in all the media and temperatures. High temperatures (30 °C) reduce at 4-6 hours the storage of semen; while at 5 °C a residue of live sperms (about 30%) was maintained (BF5 and TRIS buffer) even after 3 days. Also in this trial the best temperature for storing rabbit semen was 15 °C.
In vivo results revealed the same trend: TRIS-buffer and saline solution with respect to Salisbury medium showed higher fertility rate (69.4 vs 63.2 and 44.5% respectively; LAZZARONI *et al.*, 1992).
Analyzing the effect of the qualitative characteristics of rabbit semen (estimated by a Cellsoft®-CASA) on pregnancy rate, PIZZI *et al.* (1995) showed a significant effect due to sperm motility and concentration. Fitting an exponential model, the authors found that the maximum fertility rate (95%) can be reached with $5x10^6$ motile sperm/ml. Considering that a standard sperm concentration ranges between 300 and 600 x 10^6 with a motility of about 75%, a dilution of about 45-90 fold could be used. These results are in agreement with these of THEAU and ROUSTAN (1982) who showed that, using deep frozen semen, dilution rate (from 1/10 to 1/100) had no significant effect on pregnancy. Respecting the standard dilution currently used (1/5 and 1/10), when correct qualitative evaluations of semen are performed, a higher dilution rate is suggested.
As already affirmed the results obtained with deep freeze semen are not yet satisfying. To increase this scarce performance an improvement of medium and freezing-thawing procedures are necessary.
In a short paper, COURTENS (1995) showed that the most commonly used media, which adopt as cryoprotectants DMSO and glycerol (ANDRIEU and COUROT, 1976) or acetamide (CHEN *et al.*, 1989) are not perfectly adapted to rabbit spermatozoa. Although the two extenders are hyper-osmotic with respect to the sperm, the second demonstrates a physiological hypo-osmoticity. Thus, in the first case, negative effects are concentrated during thawing and in the second during dilution and freezing.
In a field trial FARGEAS (1995) confirmed that frozen-thawed semen, with the same above mentioned media, reduces fertility rate by about 16-28% and 2 pups at birth with respect to natural mating.
Some small scale experiments (CHEN and FOOTE, 1994) showed quite good results using the egg yolk-acetamide extender with a seeding at -6 °C during freezing. The successive cooling and thawing rate did not show significant effect on post-thawing sperm motility. The conclusion, although there are great differences in the resistance of different bucks to freezing procedures, is that seeding at sub-zero temperature should permit the spermatozoa to equilibrate their own osmolality and to loose enough water.

Some studies have analyzed the effect of freezing-thawing protocols but only few data on biological behaviour of rabbit sperms are available. The study of bio-physical traits, although difficult, remains one the more objective methods for developing a freezing-thawing procedure. However, the comparison of results obtained with bio-physical parameters and real freezing processes, shows some discrepancies (CASTELLINI and BURRINI, 1995) and probably other more direct procedures to estimate the freezing behaviour of rabbit sperm must be developed.

CONCLUSION

A.i. and c.p. changed the management of rabbit breeding and affected the reproductive protocols previously used. The fixed rhythm of insemination increased the unproductive periods of non pregnant does and consequently the importance of fertility rate. Thus, the physiological conditions of the does and their influence on reproductive performance assume big importance.

Taking into account the current limiting factors (inadequate feed intake of primiparous doe, *p.p.* problems, etc.) it is reasonable to manage them to obtain standard production (reduction of ups and downs) and higher incomes.

In conclusion different reproductive protocols should be worked out with respect to different categories of does:

NULLIPAROUS	Change of cage
PRIMIPAROUS	Use of more extensive rhythms, or reduction of litter size.
PLURIPAROUS	Choice of reproductive rhythms adapted to rabbitry conditions (general condition of environment, feeding plan, genetic strain); use of different protocols for L+ and L- does.

Regarding PMSG, the following recommendations are suggested:

- reduce the dose, avoiding short intervals between subsequent treatments;
- do not provide a general use of PMSG but treat only does where the treatment promises to be effective (L^+ R^2 does);
- use the gonadotrophin in seasons particularly negative for reproductive performance.

Regarding the semen traits, a large variability is recorded between and within bucks.

Although single rabbitry is not really concerned to reduce this variability, semen centers are interested to standardize all the factors (environment, management, hygiene) which affect seminal parameters, reproductive performance and sanitary risks.

A frequency of collection of 2 successive ejaculates (within 15 min) once a week and a photoperiod of 16 hours significantly improves all the reproductive activity of bucks (libido, semen volume, pH, number of spermatozoa, motility, live sperms).

Considering that virus can be isolated in semen of rabbit bucks (vaccinated or not), specific hygienic protocols and great attention to the sanitary condition of bucks reared in semen centers are required.

Analyzing the effect of live sperm/ml on fertility rate, it seems that a value of about $5x10^6$ is already capable to assure the maximum fertility rate, suggesting to elevate the dilution rate currently used.

Regarding semen storage it is possible to affirm that refrigerated semen obtains results comparable with fresh semen; TRIS-buffer and some commercial extenders (IMV, Minitüb) are the most appropriate to refrigerate (at 15 °C) rabbit semen. Deep freezing technique is not yet usable in open field; in comparison with natural mating a reduction of about 20-30% of fertility rate and litter size is showed.

More investigations on biological behaviour of rabbit sperms are necessary to further develop convenient media and freezing-thawing procedures. Moreover, taking into account a different individual resistance to freezing damages, a selection for this characteristic could be attempted.

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