WORLD RABBIT SCIENCE, 2 (3), 107-113.

CYCLE SYNCHRONIZATION OF RABBIT DOES NATURALLY MATED OR ARTIFICIALLY INSEMINATED

ARMERO E., GARCIA-XIMÉNEZ F., VICENTE J.S., BASELGA M.

Departamento de Ciencia Animal,
Universidad Politecnica de Valencia
P.O.Box 22012, VALENCIA 46071 - Spain.

SUMMARY : Effects of Gonadorelin and PMSG on a cycle synchronization of 42 days were evaluated. Three hundred and seventy five does were used in two experiments.
In the first, does were split in two groups, a group of 120 does received 25 IU PMSG 48 hours before mating and a Gonadorelin i.m. injection (20 μg) just after mating. The other 120 does group received only the same dose of Gonadorelin i.m. just after mating.
In the second experiment all does were injected with 25 IU of PMSG 48 hours before mating (65 does) or artificial insemination (70 does). Pregnancy was checked 12 days later in both experiments and non pregnant does were treated with 200 μg of $PGF_{2\alpha}$ and mated or A.I. Mating acceptance rate was higher in does treated with 25 IU of PMSG than in non treated does (98.1 % *vs* 87.2 % ; P<0.001). This positive effect was supported along the experiment (at least eight months or four cycles). Significant differences were observed in fertility rate between PMSG treated group and non treated in unfavourable season (92.0 % *vs* 79.4 % ; P<0.05). No significant effect was observed in litter size between these groups. In the second experiment, fertility rate of does artificially inseminated was lower than naturally mated (80.5 % *vs* 64.3 % ; P<0.05).

RÉSUMÉ : Synchronisation du cycle de lapines saillies naturellement ou artificiellement inséminées .
Les effets de la Gonadorelin et de PMSG sur la synchronisation d'un cycle de 42 jours ont été évalués. 375 lapines ont été utilisées en deux expérimentations.
Dans la première expérimentation, les lapines étaient réparties en deux groupes de 120 lapines chacun, l'un recevant 25 UI PMSG 48 heures avant la saillie et une injection i.m. de Gonadorelin juste après la saillie ; l'autre ne recevait que la même dose de Gonadorelin juste après la saillie.
Dans la deuxième expérimentation, toutes les lapines ont reçu une injection de 25 UI de PMSG 48 heures avant, soit la saillie naturelle (65 lapines) soit l'insémination artificielle (70 lapines). La gestation a été contrôlée 12 jours plus tard et les lapines non gestantes ont été traitées par 200 μg de $PGF_{2\alpha}$ puis saillies ou I.A.. Le taux d'acceptation de la saillie était plus élevé pour les lapines traitées avec 25 UI de PMSG que pour celles non traitées (98.1 % vs 87.2 % ; P<0.001). Cet effet positif s'est maintenu tout au long de l'expérimentation (près de 8 mois ou 4 cycles). Des différences significatives dans le taux de fertilité sont apparues, entre les lots traités et non traités par PMSG, pendant une saison défavorable (92.0 % vs 79.4 ; P<0.05). Dans ces même lots, il n'a pas été observé d'effet significatif sur la taille de la portée. Dans la deuxième expérimentation, le taux de fertilité des lapines inséminées artificiellement était plus bas que celui des lapines naturellement saillies (80.5 % vs 64.3 % ; P<0.05).

INTRODUCTION

At the present time three types of hormonal treatments can be used in rabbit farms to improve reproductive performance of does. PMSG, with both FSH and LH hormonal activities, promotes follicular growth and oestrogen synthesis. PMSG improves sexual receptivity (KHALIFA *et al.*, 1989 ; THEAU-CLÉMENT and LEBAS, 1994) when utilized at low doses, but when used at high doses induces superovulation. First treatment with PMSG generates specific antibodies which, in repeated treatments, can block completely its hormonal effect, specially when high doses are applied at short time intervals (CANALI *et al.*, 1991).

GnRH and synthetic analogues provoke ovulatory discharge of LH and consequently ovulation in all receptive does (THEAU-CLÉMENT *et al.*, 1990). However, ovulatory efficiency in lactating and in non receptive does is lower (72.5 to 88 %, RODRIGUEZ and UBILLA, 1988 ; THEAU-CLÉMENT *et al.*, 1990).

Finally, $PGF_{2\alpha}$ and its analogues induce luteolysis and subsequent sexual receptivity. So, these hormones can be utilized after negative palpation on day 11 of pseudopregnancy (RODRIGUEZ *et al.*, 1989).

The aim of this work is to study in industrial farms the effects of routine application of these three hormonal treatments on sexual receptivity (mating acceptance rate) and fertility rate of rabbit does when they are mated or artificially inseminated.

MATERIAL AND METHODS

A x V crossbreed rabbits were used (strain A and V were described by ESTANY *et al.*, 1988).

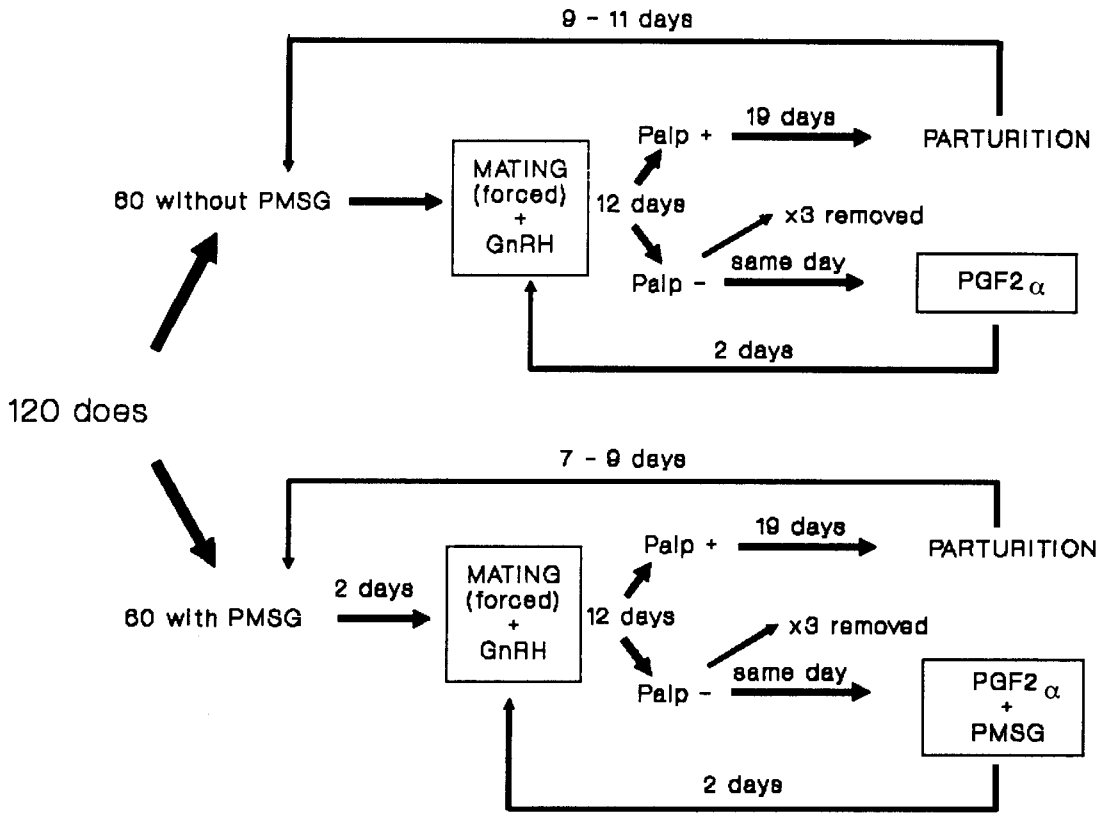
Three hundred and seventy five does were chosen at random in three farms and used in two experiments. All does were fed ad libitum with a commercial feed. The experiments (I and II) were carried out over a 8-month reproductive period. The first mating or artificial insemination (AI) took place in January 1993 and the last kindling was in September 1993. Does were mated or inseminated 11 days after parturition (first presentation) and the set of events taking place to produce a pregnancy is called a cycle. The weaning was between 28-35 days after kindling.

Experiment I : Effect of repeated PMSG injections on reproductive performance.

This experiment was carried out in two farms. In each one, experimental does were split into two groups of sixty females. A group received a subcutaneous injection of 25 IU PMSG (Serigan, Ovejero) 48 hours before mating and an intramuscular injection (i.m.) of 20 μg of GnRH synthetic analogue (Gonadorelin, Fertagyl, Intervet). A time interval of 48 hours between PMSG treatment and mating was defined as the most favourable (GARCIA-XIMENEZ and VICENTE, 1990). The females of the other group received only a i.m. injection of 20 μg of Gonadorelin. Does which refused the mating were forced to mate. Gonadorelin treatment was used to overcome a possible blockage of hypotalamic discharge of GnRH, mainly in does forced to mate.

Pregnancy was checked by abdominal palpation on day 12 after mating. Non pregnant rabbits were immediately injected with 200 μg of a synthetic prostaglandin analogue (Etiproston, Prostavet, Virbac) and, furthermore, the does from PMSG group were injected again with 25 IU PMSG. Forty eight hours later (25 days after parturition : first return ; 39 days after parturition : second return), these does were mated and treated with Gonadorelin. Females which were not pregnant at second return or showed health troubles were eliminated of the experiment. The remaining does, 9-11 days after kindling began a new cycle (Fig. 1).

Figure 1 : Scheme of Experiment I.



Experiment II : Artificial insemination *versus* natural mating (voluntary and forced).

This experiment was carried out in one farm. Females were split in two groups, one for natural mating (65 does) and the other for AI (70 does). All females were treated with 25 IU PMSG 48 hours before mating or AI. Does that refused the mating were forced to mate. All does were i.m. injected with Gonadorelin (20 μg) at the moment of mating or A.I.

Semen was collected using an artificial vagina. After checking the semen quality (blank colour and global motility > 70 %), this was diluted at rate 1:10 with skim milk + antibiotic (500 IU penicillin procaine + 500 IU penicillin benzatine + 0.1 g streptomycin sulphate / ml diluted semen). Each does was inseminated with 1 ml diluted semen using a long glass pipette (DIAZ *et al*, 1991).

Non pregnant does at 12th day post mating or AI were immediately injected with 200 μg of Etiproston and 25 IU PMSG and 48 hours later the females were mated or inseminated again. Elimination criteria were the same that in experiment I (Fig. 2).

Statistical analysis.

Chi–square tests were performed to analyse the effects of farm, type of mating (voluntary or forced), type of reproduction (natural mating or artificial insemination), interval kindling–mating and season on mating acceptance and fertility rates.

The same effects on litter size and inter–parturition intervals were estimated by an analysis of variance. In previous analysis, farm and interactions were not significant.

RESULTS

Experiment I.

Mating acceptance rate :

Using 24 IU of PMSG 48 hours before mating, male acceptance rate was increased (87.2 % *vs* 98.1 %, P<0.001, Table 1.)

Figure 2 : Scheme of Experiment II.

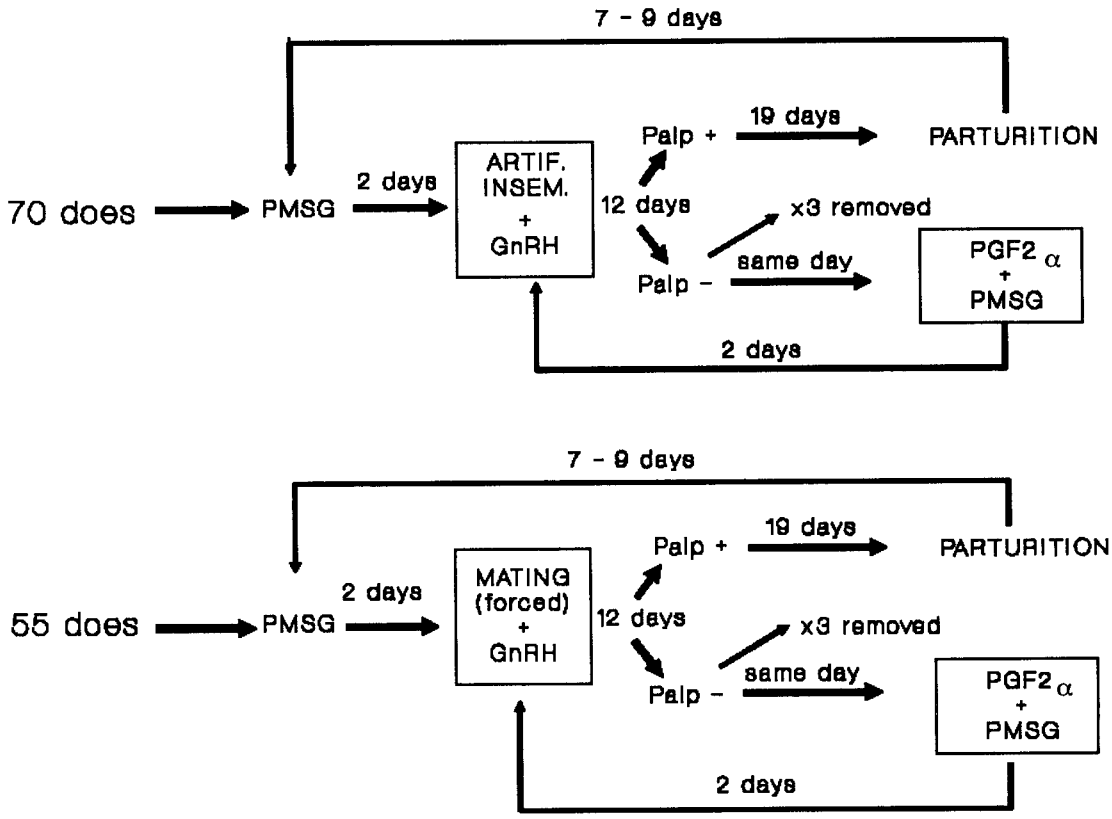


Table 1 : Effect of PMSG on mating acceptance rate according to the number of cycle of treatment.

	1	2	3	4	All cycles including 5 and 6
GnRH	87.3 % ^{ab} (150)	93.1 % ^{b} (131)	79.3 % ^{a} (82)	78.2 % ^{a} (55)	87.2 % ^{x} (470)
GnRH + PMSG	97.8 % ^{c} (139)	100 % ^{c} (127)	100 % ^{c} (60)	97.1 % ^{c} (35)	98.1 % ^{y} (409)

a,b,c, : Values with different superscript are statistically different (P<0.05) ; x,y : Values with different superscript are statistically different (P<0.001) ; ( ) : Number of matings (voluntary or forced).

The effect of PMSG increasing mating acceptance was held on during, at least, four repeated cycles of treatments (Table 1).

Table 2 : Effect of PMSG on mating acceptance rate according to the day of mating after parturition.

	1st presentation	1st return	2nd return
Without PMSG	88.5 % ^{b} (373)	76.7 % ^{a} (73)	100 % ^{c} (24)
With PMSG	97.7 % ^{c} (353)	100 % ^{c} (46)	100 % ^{c} (10)

a,b,c : Values with different superscript are statistically different, P<0.05 ; ( ) : number of matings (voluntary or forced).

Although 5a and 6a cycles were achieved for some does, its number was insufficient to establish significant conclusions. Mating acceptance rate was higher using PMSG in any season (January – May 99.0 % *vs* 88.7 %, P<0.001 ; June – September 94.7 % *vs* 83.7 %, P<0.05). PMSG improved mating acceptance in does on day 11 or day 25 post partum (97.7 % *vs* 88.5 %, P<0.001 ; 100 % *vs* 76.3 %, P<0.05. Table 2).

Fertility rate :

Fertility rate was not different in does accepting the mating treated with PMSG + GnRH and the ones treated only with GnRH (82.1 % and 81.2 %). Repeated treatments with PMSG + GnRH or GnRH did not provoke a fall of fertility of does accepting the mating (Table 3).

No significant differences of fertility rate were found in favourable season (January-May), between both treatments, PMSG + GnRH and GnRH (79.4 % vs 83.1 %). However in unfavourable season (June-September), PMSG + GnRH treatment improved fertility rate (92.0 % vs 79.4 %, P<0.05).

Fertility rate was lower in the first return than in the first presentation (65.3 % *vs* 84.2 %, P<0.001, Table 4). PMSG treatment did not overcome this differences.

Does forced to mate showed a lower fertility rate than does accepting the mating (40.9 % *vs* 81.2 %, P<0.001). Fertility rate of forced does treated with PMSG and GnRH was higher than does treated only

Table 3 : Effect of repeated cycles of treatments on fertility rate of does accepting the mating.

	1	2	3	4	All cycles including 5 and 6
GnRH	78.8 % (131)	87.9 % (132)	79.0 % (62)	73.2 % (41)	81.2 % (404)
GnRH + PMSG	78.5 % (135)	79.3 % (126)	83.1 % (59)	90.9 % (33)	82.1 % (397)

( ) : number of matings

Table 4 : Influence of day of mating on the effect of GnRH + PMSG or GnRH on fertility rate in does accepting the mating.

	1st presentation	1st return	2nd return
GnRH	83.8 % ^{a} (327)	66.0 % ^{b} (53)	79.2 % ^{ab} (24)
PMSG + GnRH	84.5 % ^{a} (343)	64.4 % ^{b} (45)	83.3 % ^{ab} (12)
Total	84.2 % ^{a} (670)	65.3 % ^{b} (98)	80.6 % ^{ab} (36)

a, b : Values with different superscript are statistically different, P<0.05 ; ( ) : number of matings.

Table 5 : Influence of type of mating on total litter size and youngs born alive.

	Voluntary LSMEAN ± S.E.M.	Forced LSMEAN ± S.E.M.
Total born/litter	8.7 ± 0.4 (604)	7.6 ± 0.7 (23)
Born alive/litter	8.3^{a} ± 0.4 (604)	6.3^{b} ± 0.8 (23)

a, b : Values with different superscript are statistically different, P<0.05 ; ( ) : number of matings. LSMEAN : Least Square Means. S.E.M. : standard error of mean.

with GnRH (87.5 % *vs* 34.5 %, P<0.05). However, the number of forced females in PMSG group (n=8) is not high enough to let us draw any relevant conclusion

Litter size :

Only the effects of type of mating and presentation showed significant differences between experimental groups in the number of rabbits born alive. These were less in forced does (6.3 *vs* 8.3, P<0.05 ; Table 5) and also in first return than in second return (6.7 *vs* 8.3, P<0.05 ; Table 6).

The annual replacement rate to GnRH group was 110 % and to GnRH + PMSG group was 117 % . Only diseased and dead does were removed and replaced.

Experiment II.

Fertility rate was higher in hormonal treated does naturally mated than in hormonal treated does artificially inseminated (80.5 % *vs* 64.3 %, P<0.05 ; Table 7). When fertility rate was compared between experimental groups according to the day of mating after parturition, it was found lower at first presentation and first return in AI and it was not different at second return.

Hormonal treated does, naturally mated or forced and artificially inseminated did not present a seasonal effect on fertility rate (Table 8).

No significant differences were found between experimental groups neither in interval between parturitions (45.5 days with natural mating *vs* 47.4 with artificial insemination ; Table 9), nor in rabbits born alive (7.5 with natural mating *vs* 8.1 with artificial insemination, table 9).

DISCUSSION

A positive effect of PMSG on mating acceptance rate was observed at least during four repeated treatments. In addition, the negative effect of

Table 6 : Influence of day of mating on total litter size at birth and number of youngs born alive.

	1st presentation LSMEAN ± S.E.M	1st return LSMEAN ± S.E.M	2nd return LSMEAN ± S.E.M
Total born /litter	7.9 ± 0.4 (542)	7.8 ± 0.5 (62)	8.9 ± 0.7 (23)
Born alive /litter	7.1 ^{ab} ± 0.4 (542)	6.7 ^{b} ± 0.6 (62)	8.3 ^{a} ± 0.8 (23)

a, b : Values with different superscript are statistically different, P<0.05 ; ( ) : number of matings. LSMEAN : Least Square Means. S.E.M. : standard error of mean.

Table 7 : Influence of type of reproduction (NM : voluntary or forced mating, or AI : artificial insemination) on fertility rate at first presentation, first return and second return.

	1st presentation	1st return	2nd return	Total
N M (voluntary or forced)	81.5 % a (222)	74.2 % a (31)	75.0 % a (4)	80.5 % a (257)
A. I.	67.1 % b (213)	51.9 % b (54)	68.4 % ab (19)	64.3 % b (286)

a, b : Values with different superscript are statistically different, P<0.05 ; ( ) : number of inseminations or matings.

lactating does on sexual receptivity was overcome. These results agreed with the ones of THEAU-CLÉMENT and LEBAS (1994). However, fertility rate was not increased by the treatment with GnRH or with PMSG + GnRH in does accepting the mating. This suggests that oestrogen release was increased but, perhaps, hypophisary blockage of ovulation during lactation was not overcome by GnRH. This increment of oestrogen release could explain the improvement of acceptance rate and subsequent higher fertility rate during unfavourable season of does treated with PMSG.

The main action of 25 IU of PMSG was to rise the oestrogen release of present follicles. A second action promoting follicular growth, so that produce an increment of ovulatory response, was not detected through an increment of litter size.

On the other hand, the fertility rate of forced does (non receptive) treated with PMSG was higher than does treated only with GnRH. THEAU-CLÉMENT and LEBAS (1994) observed a positive effect of PMSG on fertility rate of non receptive lactating does (38 *vs* 54 %).

Antibodies generation by repeated PMSG treatments, specially in animal species with short reproductive cycles, has been demonstrated in rabbits by CANALI *et al.* (1991) and BOURDILLON *et al.* (1992). However, in this experiment, mating acceptance and fertility rates were not affected by repeated PMSG treatments in contradiction with CANALI *et al.* (1991) and in accordance with BOURDILLON *et al.* (1992) and THEAU-CLÉMENT and LEBAS (1994).

With the utilized artificial insemination technique, fertility rate was lower than for natural mating with a similar rate of receptive/non receptive rabbit does. So, decrease in fertility rate was not due to receptivity differences. Lower fertility and prolificacity

Table 8 : Fertility rate according to the season and management : hormonal treatment and mating (NM, voluntary or forced), hormonal treatment and artificial insemination (AI).

	N M (voluntary or forced)	A. I.
January – May	81.9 % a (166)	64.0 % b (186)
June – September	78.0 % ab (91)	65.0 % b (100)

a, b : Values with different superscript are statistically different, P<0.05 ; ( ) : number of inseminations or matings.

Table 9 : Interval between parturitions and rabbits born alive with natural mating (NM) or artificial insemination (AI) in cycle synchronized female rabbits.

	Interval between parturitions LSMEAN ± S.E.M.	Rabbits born alive LSMEAN ± S.E.M.
N M (voluntary or forced)	45.5 ± 1.1 (190)	7.5 ± 0.2 (191)
A. I.	47.4 ± 1.3 (150)	8.1 ± 0.3 (164)

( ) : number of parturitions. Nulliparous does were not considered for "interval between parturitions". LSMEAN : Least Square Means. S.E.M. : standard error of mean.

rates in inseminated does than in natural mating does had been observed by BLOCHER and FRANCHET (1990). However, their results were not absolutely comparable (80 % *vs* 60 %, 10.2 *vs* 8.9 live youngs), because does in control group were not forced to mate and injected with GnRH. Ours records could be enhanced by improving the artificial insemination technology used in this experiment. Diluent, dilution and working temperature could have affected the results negatively (CASTELLINI *et al.*, 1990 ; CECCHINI *et al.*, 1992).

PMSG treatment on all does for cycle synchronization could not be of economical interest because of hormonal, material and labour costs, but PMSG selective treatment of some female rabbits could be rather interesting. The does that refused mating and those non pregnant (first return) could be treated with PMSG and $PGF_{2\alpha}$ + PMSG respectively, 2-3 days before mating or artificial insemination.

Acknowledgments : This study was supported by NANTACOPIVA.

Received : May 8, 1994.
Accepted : August 25, 1994.

BIBLIOGRAPHY

BOURDILLON A., CHMITELIN F., JARRIN D., PAREZ V., ROUILLERE H., 1992. Effects of PMSG treatment on breeding results of artificially inseminated rabbits. *J. Appl. Rabbit Res., 15, 530-537.*

BLOCHER F., FRANCHET A., 1990. Fertilité, prolificité et productivité au sevrage en IA et saillie naturelle ; influence de l'intervalle mise bas–saillie sur le taux de fertilité. *5èmes Journées de la Recherche Cunicole, Paris, Com. n°2*

CANALI C., BOTI C., ZAMPINI D., CASTELLINI C., BATTAGUNI M., 1991. Correlazione tra fertilita et titolo anticorporale anti PMSG di conigli trattate ripetutamente con gonadotropine nel corso della lora carriera reproduttiva. *Atti IX Congresso Nazionale ASPA, 671-678.*

CASTELLINI C., FACCHIN E., CANCELLOTI F.M., 1990. Diffusion de l'IA chez les élevages de lapins en Italie : résultats, problématiques et perspectives. *5èmes Journées de la Recherche Cunicole, Paris, Com. n°2.*

CECCHINI G., COLIN M., VACCHELLI M., 1992. Artificial insemination in rabbit : routine application to industrial breeders in Italy. *J. Appl. Rabbit Res., 15, 633-636.*

DIAZ M., REBOLLAR P.G., RODRIGUEZ J.M., 1989. Técnica de inseminación artificial en el conejo. *Hoja Divulgadora del Ministerio de Agricultura, Pesca y Alimentación, 17, 24 pp.*

ESTANY J., BASELGA M., BLASCO A., CAMACHO J., 1988. Mixed model methodology for the estimation of genetic response to selection in litter size of rabbit. *Livest. Prod. Sci., 21, 67-75.*

GARCIA-XIMENEZ F., VICENTE J.S., 1990. Effect of PMSG treatment to mating interval on the superovulatory response of primiparous rabbits. *J. Appl. Rabbit Res., 13, 71-73.*

KHALIFA R.M., MADY M.E., EL-ALAMY M.A., 1989. Effect of PMSG on sexual receptivity of low reproductive female rabbits. *J. Appl. Rabbit Res., 12, 239-240.*

RODRIGUEZ J.M., UBILLA E., 1988. Effect of sexual receptivity on ovulation response in rabbit does induced with GnRH. *IV World Rabbit Congress, Budapest, 504-507.*

RODRIGUEZ J.M., REBOLLAR P.G., DIAZ M., UBILLA E., 1989. Utilización de $PGF_{2\alpha}$ para sincronizar la inseminación de conejas pseudogestantes. *III Jornadas de Producción Animal, ITEA, 9, 259-261.*

THEAU-CLÉMENT M., BOLET G., ROUSTAN A., MERCIER P., 1990. Comparaison de différents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise à la reproduction. *5èmes Journées de la Recherche Cunicole, Paris, Com. n° 7.*

THEAU-CLÉMENT M., LEBAS F., 1994. Etude de l'éfficacité de la Ciclogonine (PMSG) pour induire la réceptivité chez la lapine. *Cuniculture, 115-21, 5-18.*