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PRODUCTION OF ANTI-PMSG ANTIBODIES AND ITS RELATION TO THE PRODUCTIVITY OF RABBIT DOES

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ABSTRACT : A batch of 100 female rabbits of the Hyplus breed were subjected to artificial insemination (A.I.) for a period of 9 months. With the goal of inducing the sexual receptivity of the does, half of them received systematic doses of 25 I.U.. of PMSG (Ciclogonine PROCHENA), 48 hours before A.I.. The other half were not injected (Control group). Following this experimental period (40 days after the last injection), blood samples of all the does were taken, in order to assay the anti-PMSG antibodies and to study the relationship between the production of antibodies, and the receptivity and productivity of the does observed for the previous litter. For the PMSG group, we classified the results of the does according to their level of immunity which either has a low binding rate (≤ 4 %) or a high one (> 4 %). 30,6% of the does from the group treated with PMSG produced anti-PMSG antibodies with a high binding rate. In no way related to the level of production of the antibodies, the PMSG treated does were found to be significantly more receptive than the does in the control group (respectively 70.6 - 66.7 *vs* 42.0%, P = 0.023), while the fertility did not appear to have been either improved or decreased by the PMSG treatment. The total number of young born, born alive and weaned rabbits per litter did not vary according to the treatment or the intensity of antibody production. Likewise the weight of the litter and also the individual mean weight of the weaned young did not vary significantly according to the group. However, inside of the group treated with PMSG, the individual weight at weaning was the highest for the young from hyper- immune mothers (710 *vs* 639g, P = 0.035). For 29 does from the PMSG group, a second blood sample was taken 12 weeks after the previous one. Only 3 does showed a decreased level of antibodies, 4 months after the last injection. The study showed that one third of the does developed an immune reaction following injections of PMSG; however the proportion of antibodies produced did not appear related to either their sexual receptivity or productivity.

RESUME: Production d'anticorps anti-PMSG, relation avec la productivité des lapines.
Un troupeau de 100 lapines de souche Hyplus a été conduit en insémination artificielle (I.A) pendant 9 mois. Dans l'objectif d'induire la réceptivité sexuelle des lapines, la moitié d'entre elles recevait systématiquement 48 heures avant I.A, 25 u.i de PMSG (Ciclogonine PROCHENA), l'autre moitié ne recevait aucune injection (Lot témoin). A l'issue de cette période expérimentale (40 jours après la dernière injection), un prélèvement de sang a été réalisé sur toutes les lapines, afin de doser les anticorps anti-PMSG et d'étudier la relation entre la production d'anticorps, la réceptivité et la productivité des lapines lors de la portée précédente. Pour le lot PMSG, nous avons distingué les résultats des lapines selon leur niveau immunitaire qui se traduit par un taux de liaison faible (£4 %) ou fort (> 4 %). 30,6 % des lapines du lot traité à la PMSG produisent des anticorps anti-PMSG ayant un taux de liaison fort. Sans relation avec le niveau de production d'anticorps, les lapines traitées sont significativement plus réceptives, que les lapines du lot témoin (respectivement 70,6- 66,7 *vs* 42,0 %, P = 0,023), la fertilité ne semble ni améliorée, ni détériorée par le traitement PMSG. Le nombre de nés totaux, nés vivants et sevrés par portée ne varie pas selon le traitement ou selon l'intensité de la production d'anticorps. De même, le poids de portée ainsi que le poids moyen de lapereaux sevrés ne varient pas significativement en fonction du lot. En revanche, au sein du lot traité à la PMSG, le poids moyen au sevrage est plus élevé pour les lapereaux issus de mères hyperimmunes (710 *vs* 639 g, P = 0,035). Sur 29 lapines du lot PMSG, un deuxième prélèvement de sang a été réalisé 12 semaines après le précédent. Seules 3 lapines (sur 29 étudiées) ont eu une production d'anticorps diminuée, 4 mois après la dernière injection. Ce travail montre qu'un tiers des lapines développe une réaction immunitaire suite à des injections de PMSG cependant, le taux d'anticorps produit ne semble affecter ni la réceptivité sexuelle, ni la productivité des lapines.

INTRODUCTION

If the use of artificial insemination (A.I.) is to be widely developed, it may be necessary to master the induction of sexual receptivity in does. Among the hormonal treatments available, PMSG (Pregnant Mare Serum Gonadotropin) is the most widely used molecule in European rabbit producing countries. Indeed it stimulates an increase in the number of pre-ovulatory follicles (BONANNO *et al.* 1990), as well as sexual receptivity and litter size (MAERTENS *et al.* 1983, THEAU-CLEMENT and LEBAS 1994). However, CANALI *et al.* (1991) have shown, for a group of 20 does which received repeated treatments of PMSG

(40 I.U.. Ciclogonina, PROCHENA, 2 days before A.I.) on the one hand a progressive drop in fertility and on the other an increase in the level of anti-PMSG antibodies. Both of these phenomena could be detected since the 4th injection. This result, confirmed by STRADAIOLI *et al.* (1994), is not surprising given that PMSG, as far as rabbits are concerned, is both an exogenous hormone, and a large proteinic molecule. Moreover the treatment was repeated at relatively short intervals.

In order to test the efficiency of Ciclogonine (PMSG) in inducing sexual receptivity in does at the time of A.I. and more generally their productivity, we subject an initial batch of 148 female rabbits to artificial insemination for a period of 9 months, during which half of them systematically received injections of PMSG before each insemination. Following this experimental period, blood samples were taken from the 100 does which were still alive (in the treated group and the control group), in order to assay possible anti-PMSG antibodies for the purpose of studying the immunogenicity of the molecule and to try and establish a relationship between the production of antibodies and the receptivity and productivity of the does for their previous litter.

MATERIALS AND METHODS

Experimental design

The experimental approach has been described in more detail in a previous article in this same journal (THEAU-CLEMENT and LEBAS 1996). The test was done using Hyplus breed rabbit does which had undergone artificial insemination without hormonal treatment with PMSG for seven months. During the following nine months, the efficiency of the Ciclogonine was tested by comparing the reproduction performances of 2 identical groups of does, one of which was a control group which was not injected and the other, which was treated 48 hours before each A.I. (sub-cutaneous injection of 25 I.U.. of Ciclogonine PROCHENA diluted in 2.5 ml of a vitamin complex, "Vitatox" FATRO). The does were inseminated every 42 days, while those females which were found by palpation to be empty, were inseminated 21 days after the previous A.I. Each series of insemination was performed with a heterospermic mixture of fresh semen. Does which were empty after three consecutive inseminations without fertilisation were systematically eliminated. On the day of insemination, does from the 2 groups were individually placed in a cage with a male to test their sexual receptivity, immediately before the insemination itself. Forty days after the last injection, blood samples were systematically taken from the 100 does which were still present (51 control does and 49 treated does). Twelve weeks later, a second blood sample was taken from 29 treated does. The serum was frozen after centrifuging.

Antibody detection in plasma samples

The antibodies levels were expressed as percentage of radioactive PMSG bound by 10µl of human plasma. The highest was this percentage, the highest was the binding rate. Anti-PMSG antibodies in treated animals were assayed in serum of blood samples obtained 40 and 120 days after the latest injection of PMSG. Pregnant Mare Serum Gonadotropin hormone was purified according to CHRISTAKOS and BAHL (1979) starting from the Folligon preparation furnished by Intervet (Organon Oss.Netherland). The biological activity of the final purified preparation was 10 000 I.U.. per mg as determined by the STEELMAN and POHLEY (1971) bioassay.

The purified PMSG was radiollabeled using 125 Iodine (Amperes IMS-30) according to the enzymatic procedure of THORELL and JOHANSON (1971)

In order to obtain a high specific radioactivity and a reliable tracer, 12.5 µg of PMSG (lyophilised powder, dry weight) were iodinated using 1 milliCurie of 125I, 1 µg of lactoperoxydase (Boerhinger) and 10 µl of H_2O_2 (Perhydrol Merck 1/30 000). Immediately after the reaction, (4.5 min) the radiolabelled hormone was purified by chromatography on G-75 Pharmacia (column 0.9 x 35 cm) equilibrated in Tris-HCl buffer 0.01 M ; pH 7.6 containing 0.1% bovine Serum Albumin (bSA) (Fraction V of Cohn).

All dilution of sera and tracer were performed in Tris-HCl buffer 0.025 M, pH 7.6, bSA 0.1% and Neomycin sulphate.

The incubation volume was always 500 µl. Reagents were added in the following order:

- 300 µl buffer
- 100 µl of 1/10 diluted serum
- 100 µl of ^{125}I PMSG corresponding to 10 000 cpm (T) or 16 666 dpm (efficiency of the counter = 60 %) or to 0.14 ng of hormone (as determined by the self displacement method of ROULSTON (1979).
- Incubation was carried out for 16h at 20°C
- 100 µl of a sheep anti-rabbit immunoglobins was then added.
- Incubation was carried out for 1 h at 20°C
- 500 µl of 4 % (W/V) polyethylene glycol (PEG MW 10 000 Merck inc) diluted in distilled water was then added.

After 2 hours, the tubes were centrifuged at 2 500 g for 20 min and the supernatants aspirated

Table 1 : Immunitary response of rabbit does after 7 cycles of reproduction

	PMSG group		Control group		Signification
Binding rate	*Number*	Frequency	*Number*	Frequency	
≤4 %	34	69.4 %	50	98.0 %	P = 0.001
> 4 %	15	30.6 %	1	2.0 %	

carefully. The pellets were washed with 3 ml of Tris bSA buffer and centrifuged.

After aspiration of the supernatants, the pellets were counted for radioactivity in a gamma counter having an efficiency for ^{125}I of 60 %.

Non specific binding (NSB) of ^{125}I PMSG was determined in all experiments using 400 µl of buffer added to 100 µl of ^{125}I PMSG.

Statistical analysis

The data were statistically analysed using the SAS statistics library. We studied the percentages with Fisher's exact test or by means of variance analysis. The litter sizes, binding rates and weight variables were analysed by means of a fixed effect variance analysis with 3 levels for the group factor (control group, PMSG group with low immune response and PMSG group with high immune response)

RESULTS AND DISCUSSION

Assays of the anti-PMSG antibodies were performed on the 100 does still present when the PMSG experiment ended. It should be remembered that the number of does eliminated from the test because of infertility did not vary according to group; 3 does from the PMSG group were eliminated (due to 3 consecutive failed A.I. attempts) as opposed to 2 from the control group. The maximum threshold of binding rate judged to be "low" was set to 4% since more than 97.5% of the does in the control group had a proportion lower than this threshold. For the experimental group, it was assumed that the acquired immunity was significant when the proportion of antibodies exceeded this 4% threshold.

Proportion of anti-PMSG antibodies present after 7 reproductive cycles

Table 1 shows the results of antibodies level for each of the 2 groups. The group treated with PMSG has a significantly higher proportion of does with high binding rate (respectively 30.6% and 2.0%, P<0.001). In fact only the serum of a single doe from the control group has a binding rate (5.7%) greater than the accepted limit. It has therefore been eliminated from the following analysis of the results. Once again the variability of the immune response referred to by CANALI *et al.* (1991) was observed. Indeed, out of 20 does having received at least 6 treatments of PMSG, the authors found that 3 did not have a significant immune response, while on the other hand 11 had a response which increased in proportion to the number of injections received. The proportion of does which reacted to the PMSG was lower in our study (30.6% *vs* 55%, P = 0.053).

Does from the PMSG group received between 6 and 9 injections (Table 2) but females from extreme classes were not numerous (respectively 6 and 3 females). Since 7 injections, more than 30% of the does developed significant immunity. Beyond this number the binding rate did not appear to vary according to the number of injections received. It may be noted that there are no does with a binding rate greater than 4% in the group which only received 6 injections (systematically fertilised 10-11 days post-partum). However the low number in this group made it impossible to draw any conclusions.

Relationships between the anti-PMSG antibodies, receptivity and fertility

For the insemination which took place before blood sampling, we compared the percentage of

Table 2 : Relationships between the binding rate and the previous number of PMSG injections

Injections number	6	7	8	9	Signification
Total Does number	6	21	19	3	
Does with a binding rate higher than 4 % (%)	0	38.1	31.6	33.3	NS

Table 3 : Relationships between the antibodies binding rate (ABR), does sexual receptivity and fertility

Groups	Control	PMSG ABR <4%	PMSG ABR>4%	Signification
No of Does	50	34	15	
Receptivity (%)	42.0	70.6	66.7	P = 0.023
Fertility (%)	70.0	70.6	86.7	NS

receptive does and their fertility, both for the control group and the group treated with PMSG depending on whether, at the end of the experiment (40 days after the last injection), they had a low binding rate (≤ 4%) or a strong one (> 4%). The percentage of receptive females varies according to the group (Table 3, P = 0.023). In fact, the females in the control group were significantly less receptive than the does in the PMSG group which had only produced few antibodies (respectively 42.0 *vs* 70,6%, P = 0.014). On the other hand, within the PMSG group, receptivity at mating did not vary according to the intensity of the immune reaction (70.6% and 66.7% for low and high binding rate respectively). However, the number of does showing a high binding rate is too small to be able to conclude that there is no relation between the production of antibodies and the efficiency of PMSG in inducing sexual receptivity. Furthermore, we have found no references in the literature which could either refute or confirm this hypothesis.

When the sexual receptivity in each of the groups was studied as a function of the physiological stage reached by each of the does (Table 4), we only observed the favourable effect of PMSG on does which were lactating, an effect which had already been pointed out in the previous article (THEAU-CLEMENT and LEBAS, 1996).

On the other hand, after 7 series of injections, the fertility did not depend on whether the does had or had not been treated with PMSG, or on the level of intensity of the immune reaction (Table 3). This observation contradicted the results of CANALI *et al.* (1991), who had observed a gradual drop in fertility, in parallel with the increase in the level of antibodies from the 1st to the 7th injection. We did not find this negative relationship. However, it should be noted that the dose of PMSG administered in the study of CANALI *et al.*, was 40 I.U./injection while we have used 25 I.U../injection only. According to the observation of our experimental population, it appeared as if the PMSG no longer had any effect on fertility at the end of the experiment, no matter what the level of antibodies produced.

Moreover, the fertility did not vary significantly according to the physiological stage reached by the does (Table 4). In non-suckling does, the use of PMSG was unjustified, since the fertility was greater or equal to 84%. It is surprising to observe, at the end of the PMSG experiment, that there was no evidence of the effect of this treatment on lactating does, and also that this lack of effect was not associated with the presence of a particular level of anti-PMSG antibodies.

Effect of the anti-PMSG antibodies on the characteristics of numerical and weight productivity of does.

Productivity in terms of young born

The total number of young born and born alive per litter did not vary significantly according to the group (Table 5). Only the number of stillborn in the treated group, with a low level of antibodies, was higher; this

Table 4 : Relationships between anti-PMSG antibodies binding rate (ABR) , does sexual receptivity and fertility in relation to their physiological stage

		Number	Receptivity (%)	Fertility (%)
Control	Non Lactating	25	40.0a	84.0a
	Lactating	25	44.0a	56.0a
PMSG ABR <4%	Non Lactating	11	54.5a	90.9a
	Lactating	23	78.3b	60.9a
PMSG ABR > 4 %	Non Lactating	8	50.0a	100a
	Lactating	7	85.7b	71.4a

In the same column : a,b : P < 0,05

Table 5 : Relationships between the antibodies binding rate (ABR) and the numeric and weight productivity of rabbit does : mean and (standard error)

Groups	Control	PMSG		Signification
		ABR <4%	ABR >4 %	
No of litters	*36*	*24*	*12*	
Born alive / litter	8.08 (0.71)	8.54 (0.90)	7.67 (0.84)	NS
Stillborn / litter	0.28 (0.11)	1.46 (0.58)	0.08 (0.08)	P = 0.019
Total born / litter	8.36 (0.72)	10.00 (0.73)	7.75 (0.87)	NS
Litters at weaning	*36*	*22*	*12*	
Weaned / litter	7.08 (0.66)	7.09 (0.92)	7.17 (0.81)	NS
Litters weaned	*33*	*19*	*11*	
Litters weight (g)	4936 (313)	5118 (454)	5408 (223)	NS
Individual weight (g)	692 (27)	639 (17)	710 (29)	P=0.035 (1)

(1) Analysis inside the PMSG group only

effect was due to only 2 females which had completely lost their litters, with 8 and 12 stillborn respectively.

The number of young weaned per litter did not vary significantly according to the treatment or the intensity of antibody production.

For this latter series we did not find any marked effect of the PMSG tending to significantly increase the size of the litter at birth or at weaning.

Weight productivity

The litter weight as well as the individual mean weight at weaning did not vary significantly according to the group (treated does *vs* untreated does). On the other hand, within the PMSG group, the individual weight of the young rabbits varied significantly according to the quantity of antibodies produced (P = 0.035). Indeed there was a difference of 71 g in favour of young born to does which had developed a strong immune reaction (710 g *vs* 639 g, Table 5). We had already shown previously (THEAU-CLEMENT and LEBAS, 1996), that the PMSG treatment had the effect of decreasing the weight of weaned young. It appeared as if the presence of a strong concentration of antibodies neutralised this effect. However the low number of cases (11 litters) did not allow us to draw any conclusions concerning this hypothesis.

Evolution of the binding rate 12 weeks after interrupting the treatment with PMSG.

For 29 does, the assays of anti-PMSG antibodies were performed on serum samples taken 40 days and 124 days after the final injection. The percentage of does for which the binding rate was > 4% did not vary significantly between the 2 samplings (Table 6). Twenty six females (89.7%) retained their immunity (± 4% variation in the binding between the 2 series of samples). Only 3 does (10.3%) had a binding rate which decreased between the 2 samplings (respectively from 16 to 5, from 44 to 38 and from 37 to 6%). It should be noted that they correspond to females which had the highest levels of antibodies.

CONCLUSION

This study showed that one third of the does developed an immunity to PMSG. However, the level of antibodies produced did not appear to affect either the sexual receptivity, or the fertility, or the quantitative productivity. The young born from mothers with a higher proportion of antibodies than the control group, were significantly heavier at weaning time. The limited observations of acquired immunity are therefore still insufficient to be able to determine precisely the optimal use of this molecule. The study should therefore be continued, with at least a similar

Table 6 : Antibodies binding rate, 6 and 18 weeks after the last PMSG injection

Time elapsed since the last injection	6 Weeks	18 Weeks	Signification
Mean Binding Rate	6.9	4.6	P = 0.055
Standard deviation	10.2	6.9	
Propotion of does with a binding rate >4%	31	34	NS

number of does and injections number. The objective of such a study should be the simultaneous analysis of the antibodies kinetics of apparition with successive PMSG injections and of the kinetics of rabbit does reproduction parameters.

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