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LONG TERM EFFECT OF PMSG ON RABBIT DOES REPRODUCTIVE PERFORMANCE

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ABSTRACT : This study was carried out to verify the long term effect of Pregnant Mare Serum Gonadotrophin (PMSG) treatments (20 IU three days before the induction of ovulation with GnRH) on the fertility and ovarian response of does artificially inseminated for at least 7 cycles. Twenty-five nulliparous does were submitted to recurring treatments at fixed intervals of 45 days. A total of 175 artificial inseminations were performed. The results confirmed previous observations about the progressive loss of efficacy of the PMSG in inducing pregnancy (from 80.9 to 60.9 %), above all in does classified as hyper-immune (84 % of animals). The lactation stage negatively influenced the reproductive performance especially in does at the first two treatments (fertility rate 53.9 *vs* 79.2 % ; litter size 7.5 *vs* 8.8 ; P≤0.05). These hormonal treatments altered the follicular growth causing a significantly higher number of haemmoragic follicles (1.7 *vs* 4.9) and also affected the quality of collected embryos (0 degenerated embryos *vs* 4.9).

RÉSUMÉ : Effet à long terme de PMSG sur les performances de reproduction des lapines.
Cette étude a pour but de vérifier l'effet à long terme des traitements par PMSG (20 UI trois jours avant l'induction de l'ovulation par GnRH) sur la fertilité et la réponse ovarienne des lapines artificiellement inséminées pendant au moins 7 cycles. Vingt cinq lapines nullipares ont été soumises à des traitements renouvelés systématiquement tous les 45 jours. Au total 175 inséminations ont été réalisées. Les résultats confirment les observations précédentes concernant une perte progressive de l'efficacité de PMSG à induire la gestation (de 80,9 % à 60,9 %) surtout pour les lapines classées hyper-immunes (84 % des animaux). La persistence de la lactation influence négativement les performances de reproduction particulièrement pour les lapines soumises aux deux premiers traitements (taux de fécondité 53,9 vs 79,2 % ; taille de la portée 7,5 vs 8,8 ; P≤0.05). Ces traitements hormonaux altèrent la croissance folliculaire provoquant un nombre significativement plus élevé d'hémorragies (1,7 vs 4,9) et affectent la qualité des embryons récoltés (de 0 à 4,9 embryons dégénérés).

INTRODUCTION

In Italy, artificial insemination (a.i.) of rabbit is widely used in industrial production for its benefits in genetic improvement, hygiene and management (FACCHIN, 1992). This technique permits the insemination of females independent of the oestrous cycle, but the resulting fertility and prolificacy depends mainly on the sexual receptivity and lactation stage of the does at insemination time (THEAU-CLEMENT and ROUSTAN 1992).

In the last 20 years many efforts have been made in order to define practical and efficient hormonal protocols to induce oestrous synchronization for a.i. of does, but the most commonly used are still based on the administration of exogenous gonadotrophins (PMSG, FSH, hCG).

However, many authors (ADAMS 1961, HEIDBRINK 1980, CASTELLINI *et al.* 1991, BONANNO *et al.* 1993) cautioned against the indiscriminate use of these hormones which were considered responsible for the reduction in fertility and prolificacy noted by many breeders. The cause of this negative reproductive performance has been ascribed to an immunization process in the does triggered by the strong immunological properties of the gonadotrophin employed and by the high dosages used (MAY and SIMPSON, 1975). However, only recently CANALI *et al.* (1989, 1990) and BOURDILLON *et al.* (1992), using ELISA methods, were able to identify circulating antibodies against PMSG in does treated three or more times consecutively with this gonadotrophin.

In previous trials, using 40 IU of PMSG for oestrous synchronization, a significant correlation between antibody titer against PMSG and both number

and treatment intervals ($r = 0.47$ and $r = -0.51$ respectively) was found (CANALI *et al.*, 1991). Considering the negative effect of high doses of PMSG, which have been especially detrimental when repeated at short intervals, the aim of this study was to assess the ovarian response and the reproductive performance of does treated with a reduced PMSG dose of 20 IU at fixed intervals of 45 days.

MATERIALS AND METHODS

Experiment A

The trial was carried out on 25, 140 days old, New Zealand White does reared in the experimental rabbitry of the University of Perugia. During the experiment, which started on May 1993 and lasted 1 year ; environmental temperature ranged from 16 ± 2.2°C in winter to 28 ± 3.0°C in summer, relative humidity from 55 % to 75 % and daylight was set at 16 hours/day. Animals were allocated to individual cages and fed *ad libitum* with a commercial pelleted diet.

Eleven days after each kindling, all the does received i.m. 20 IU of PMSG (Ciclogonina, PROCHENA) and, three days later, 20 μg of GnRH (Receptal, HOECHST) to induce ovulation. The females were then artificially inseminated independently of their sexual receptivity with 0.5 ml fresh semen, pooled from two bucks of tested fertility, diluted 1 : 8 with TRIS buffer. The non-pregnant does waited for the next treatment-insemination cycle. Before insemination two classes of sexual receptivity (R+ or R-) were established observing the behaviour of each female in the buck cage for 5 minutes.

Both fertility rate, expressed as the ratio between number of kindlings and inseminations, and litter size at birth were recorded. During the experiment 9 does were eliminated for pathological problems (3 having pneumonia, 4 endometritis and 2 mastitis) and the remaining females were submitted to at least seven cycles of treatment totalling 175 artificial inseminations.

Immediately before each treatment, a blood sample was collected from the marginal ear vein of each animal and the serum was stored at -20 °C until assayed for anti-PMSG antibodies with a direct ELISA method (CANALI *et al.*, 1990). In this test anti-PMSG antibodies, binding to purified PMSG coated on plastic microplates, are revealed by specific anti-rabbit-IgG HRP conjugate. The optical density (OD) from diluted sera of each treated rabbit, corrected by OD values from untreated control rabbits, was considered an estimation of the antibody anti-PMSG level. At the end of the experiment, on the basis of OD found at the third kindling, two groups of animals were arbitrarilly discriminated : the first (n = 21), having OD greater than 50, was classified as hyper-immune (I+), the other (n = 4), with OD less than 50, as hypo-immune (I-).

Experiment B

To evaluate ovarian response, 2 groups of 11 pluriparous does (kindling order was between 2 and 7) were sacrificed 2 days after a.i. The first one did not received PMSG, the second one received ≥3 treatments. The genital tracts were immediately removed and after dissection of the ovaries from surrounding tissue, the number of fresh corpora lutea (CL), anovulated follicles (AF) with an external diameter greater than 1 mm, haemorragic follicles (HF) and in vitro flushed embryos were recorded. Recovered embryos were then morphologically evaluated for development stage and assigned to a quality grade score (A = Excellent, B = Good, C = Fair, D = Degenerate) as outlined by LIDNER and WRIGHT (1983). Blood samples were also collected at a.i. time to evaluate the immunity classes (OD <50 or ≥50) of each doe.

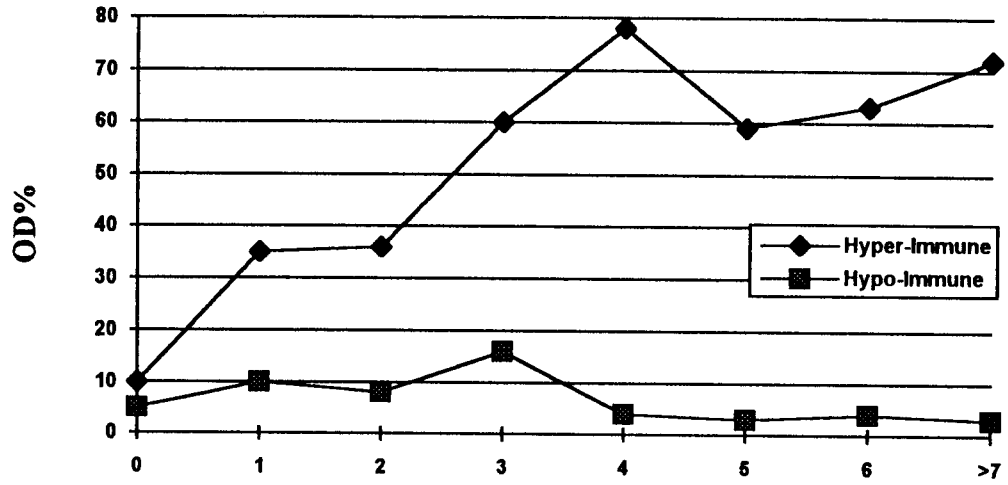
Statistical analysis

Statistical analysis was executed with SAS package (S.A.S., 1991). FREQ procedure was used for non-parametric variables and GLM for all the others with a linear model considering sexual receptivity (R+, R-), treatment order (0, 1-2, ≥3), immunity class (I+, I-), lactation stage (L+, L-), and interaction between treatment order and the other two effects. The date of insemination had no significant influence and therefore it was not included in the statistical model (Experiment A). For the experiment B, the effects analyzed were the number of PMSG treatments (0, ≥3) and immunity class.

RESULTS AND DISCUSSION

In figure 1 the mean ODs of does immunologically responsive and non-responsive to PMSG during repeated treatments are shown. Increasing the number of inoculations, 84 % of the does had a significant increase of anti-PMSG circulating antibodies, while the remaining ones developed no immunological response. The present findings are in accordance with those obtained in previous experiments (CANALI *et al.*, 1991), even if the trend of OD values was less marked (75 *vs* 237.6, respectively, in the 6th treatment) probably because of the longer interval between successive PMSG injections and the lower dosage employed (20 *vs*

Figure 1 : Antibody levels anti-PMSG following repeated treatments



40 IU). Also BOURDILLON *et al.* (1992), using 30 IU PMSG 48 hours before a.i., found an increase in anti-PMSG antibodies after three administrations (37.6 *vs* 22.3 % OD) which, however, was not significant.

As THEAU-CLEMENT and LEBAS (1994), the sexual receptivity was not affected by repeated PMSG treatments and an average 83.4 % of the does were R+, ranging from 95 % at the first kindling to 75 % at the fifth. ALABISO *et al.* (1994), evaluating the heat of does by the vulvar colour in a comparable experiment, always found a similar high percentage of receptive does (75.3 %).

The I+ and I– groups had 85.7 % and 83.5 % receptive does respectively, thus suggesting that the oestrous synchronization effect of PMSG was not negatively influenced by anti-PMSG circulating antibodies. Receptive does had a significantly higher fertility rate (71.4 % vs 31.1 %, P≤0.01) then non-receptive does, confirming the results reported by THEAU-CLEMENT and LEBAS (1994) in females treated with 25 IU of PMSG (75.9 % *vs* 53.9 %).

Table 1 summarizes the effects of treatment order (0, 1–2, ≥3), lactation stage (L+, L–) and immunity class (I+, I–) on the reproductive performance. Both fertility rate and litter size of hyper-immune does decreased with the repeated treatments (from 80.9 % to 60.9 % and 8.3 to 7.0, respectively), while those of the hypo-immune group showed no particular trend. However, after 3 treatments the hypo-immune does had higher litter size (10.3 *vs* 7.0, P≤ 0.01) and fertility (72.3 % *vs* 60.9 %) with respect to the hyper-immune group. The relation between treatment order and lactation stage showed a negative effect of lactation on reproductive performance which was very pronounced in the primiparous does (treatment order 1–2). Such effect could be attributed to the hormonal antagonism between lactation and reproduction (referred by MCNITT, 1992) and to the high metabolic requirements of does (especially primiparous) during the lactation period.

Table 2 summarizes the effects of multiple PMSG treatments and immunity class on ovarian structure and embryo quality (experiment B). The number of haemorrhagic follicles was significantly lower in untreated animals (1.70 *vs* 4.90, P≤ 0.01) while those of anovulated follicles (AF) and corpora lutea (CL) were not affected by treatment order. Also, the number of good quality embryos (grade A to C) was significantly higher in control rabbits (8.2 *vs* 6.9, P≤ 0.05) and that of degenerated embryos significantly lower (0 *vs* 4.9, P≤ .01). With regard to the immunity class, the number of CL was about three times higher in the hypo-immune group. The number of good quality recovered embryos reflected the litter size registered in experiment A.

The long term effects on rabbit doe reproductive performance which arise from the repeated treatments with PMSG are still debated. Field results, concerning litter size and fertility of does primed with 20 IU of PMSG, revealed no negative effects (BOURDILLON *et al.*, 1992), unless the gonadotrophin treatments were repeated three or more times consecutively (CANALI *et al.* 1990). According to these authors the non-pregnant does, receiving a greater number of treatments at closer intervals under intensive breeding management, have a higher probability of developing stronger

Table 1 : Effect of treatment order on conception rate and litter size in lactating (L+) and non-lactating (L–) does (Means, SE, χ^2) in relation to the immunity class (I+, I–).

	Number of A.I. (n)		Conception rate (%)		Litter size (n.)	
Treatment order	**I+**	**I–**	**I+**	**I–**	**I+**	**I–**
0	21	4	80.9	75.0	8.3	9.0
1 – 2	42	8	69.0	62.5	7.5a	9.1b
≥ 3	82	18	60.9	72.3	7.0A	10.3B
n.	145	30				
Overall mean			*64.3*	*73.3*	*7.3A*	*9.7B*
	L+	**L-**	**L+**	**L-**	**L+**	**L-**
0	–	25	–	78.0	–	8.6
1 – 2	26	24	53.9a	79.2b	7.5a	8.8b
≥ 3	61	39	60.7	73.9	8.2	8.9
n.	87	88				
Overall mean			*58.6a*	*76.3b*	*7.8a*	*8.8b*
χ^2			5.01			
SE					3.22	

In the same row : A...B : P ≤ 0.01, a...b : P ≤ 0.05.

immunological responses against PMSG which in turn is supposed to further impair the reproductive function. On the contrary, CECCHINI *et al.* (1992) observed no negative effect following multiple PMSG treatments of does undergoing a.i. under commercial management conditions. However, in this context, the lack of any negative effects on fertility may be due to the short longevity of does (about 4–6 kindlings/doe) and to the early replacement of does for low reproductive performance. CONTERA (1989) and SCHLOLAUT, (1989) recommended using PMSG occasionally and, if necessary, to reduce the doses, avoiding repeated treatments over a short time. BOURDILLON *et al.* (1992) suggested restricting the use of PMSG only for primiparous does.

More recently, THEAU and LEBAS (1994) found a significant improvement in the fertility rate (79.9 % *vs* 65.6 %) of does treated with 25 IU of PMSG only during the first period of experiment (until the 4th

Table 2 : Effect of PMSG treatments and immunity classes (I+, I–) on the number of ovarian structures and embryo quality of does (Means, SE).

	n.	Anov. Foll.	Corpora Lutea	Haemo Foll.	Embryos (A to C)	Degenerated embryos (D)	Total embryos
PMSG treatment							
0	11	19.2	8.8	1.7A	8.2b	OA	8.2
n ≥ 3	11	18.0	14.2	4.9B	6.9a	4.9B	11.8
Immunity classes							
I–	13	19.6	12.3	2.6	9.0B	0.9	9.9
I+	9	18.4	10.7	4.0	6.0A	4.0	10.0
SE		7.4	11.2	4.9	2.0	3.7	3.1

In the same column : A...B : P ≤ 0.01, a...b : P ≤ 0.05.

treatment order). In conclusion they suggest to use PMSG only in the lactating does, although only the non-receptive does had a significant improvement of fertility rate (53.9 % *vs* 38.1 %).

The contradictory results obtained by different authors with PMSG treatments in relation to the lactation stage, receptivity of the doe, number of treatments, dosage used suggest complex interactions of the exogenous gonadotrophin with the reproductive physiology. A direct effect of PMSG on the ovary must also to be taken into consideration. Exogenous gonadotrophins administered at higher doses, from 40 to 150 IU, may induce ovarian overstimulation resulting in abnormal follicular steroid production and severe environmental changes of the reproductive tract (FOOTE and ELLIGTON 1988). Furthermore, these hormonal treatments may alter follicular growth, cause failure to ovulate (KENNELLY and FOOTE 1965) or ovulation of premature oocytes (HYTTEL *et al.* 1991), interference of ovum transport mechanisms (GREENWALD 1961), delayed zygote development (CARNEY and FOOTE 1990).

The in vitro embryo development rate seems to be significantly affected also by the low dose of PMSG (20 IU) used to induce oestrous synchronization of rabbit (STRADAIOLI *et al.*).

However, considerable work remains to be done to define practical and efficient hormonal protocols to improve reproductive performance under normal management commercial rabbitry conditions.

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REFERENCES

ADAMS C., 1961. Artificial insemination in the rabbit. *J. Reprod. Fertil., 2, 251-254.*

ALABISIO M., BONANNO A., ALICATA M. L., PORTOLANO B., 1994. Trattamento differenziato con PMSG su coniglie inseminate artificialmente. *Coniglicoltura, 31 (1/2), 25-30.*

BONANNO A., ALABISO M., ALICATA M. L., PORTOLANO B., 1993. Prestazioni riproduttive di coniglie sincronizzate con 20 UI di PMSG e sottoposte ad I.A. *Coniglicoltura, 30 (2), 37-40.*

BOURDILLON A., CHMITELIN F., JARRIN D., PAREZ V., ROUILLERE H., 1992. Effect of PMSG treatment on breeding result of artificial inseminated rabbits. *J. Appl. Rabb. Res., 15, 530-537.*

CANALI C., BOITI C., CASTELLINI C., BATTAGLINI M., ZAMPINI D., 1989. Effetto di immunizzazione in coniglie trattate ripetutamente con PMSG. In : *Proc. II Meeting sullo studio della efficienza riproduttiva degli animali di interesse zootecnico, Bergamo, 24 Nov, 103-108.*

CANALI C., BOITI C., CASTELLINI C., ZAMPINI D., 1990. Effetti del PMSG sulle prestazioni riproduttive delle fattrici cunicole. *Coniglicoltura, 27 (3), 21-23.*

CANALI C., BOITI C., ZAMPINI D, CASTELLINI C., BATTAGLINI M., 1991. Correlazione tra fertilità e titolo anticorpale anti-PMSG di coniglie trattate ripetutamente con gonadotropine nel corso della loro carriera riproduttiva. *Atti Cong. ASPA, Roma 671-678.*

CARNEY E.W., FOOTE R.H., 1990. Effect of superovulation, embryo recovery, culture system and embryo transfer on development of rabbit embryos in vivo and in vitro. *J. Reprod. Fert., 89, 543-551.*

CASTELLINI C., CANALI C., BOITI C., BATTAGLINI M., 1991. Effetto del PMSG nelle prestazioni riproduttive di coniglie fecondate artificialmente *Atti Cong. ASPA, Roma, 679-683.*

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

CONTERA A. C., 1989. Management ed efficienza riproduttiva. *Coniglicoltura, 26 (6), 17-20.*

FACCHIN E., 1992. Artificial insemination in rabbit. *J. Appl. Rabb. Res., 15, 95-103.*

FOOTE R.H., ELLIGTON J.E., 1988. Is superovulated oocyte normal? *Theriogenology, 29, 111-123.*

GREENWALD G.S., 1961. A study of the transport of ova through the rabbit oviduct. *Fertility and Sterility, 12, 80.*

HEIDBRINK G., 1980. Artificial insemination in commercial rabbit production - *Bulletin 573S Colorado State University.*

HYTTEL P., CALLESEN H., GREVE T., SCHMIDT M., 1991. Oocyte maturation and sperm transport in superovulated cattle. *Theriogenology, 35(1), 91-108.*

KENNELLY J.J., FOOTE R.H., 1965. Superovulatory response of pre- and post-pubertal rabbits to commercially available gonadotrophins. *J. Reprod. Fert., 9, 177-188.*

LIDNER G. M., WRIGHT R.W., 1983. Bovine embryo morphology and evaluation. *Theriogenology, 20, 407-416.*

MAY D., SYMPSON K.B., 1975. Reproduction in the rabbit. *Animal Breeding Abstracts, **43 (6)**, 253-261.*

MCNITT J.I., 1992. Endocrinologica approaches for commercial rabbit production. *J. App. Rabbit Res. **15**, 364-392.*

SCHLOLAUT W., 1989. Fecondazione artificiale, le esperienze tedesche. *Coniglicoltura, **26 (4)**, 19-22.*

S.A.S., 1991. SAS/STAT_ User's Guide Statistics - Version 6. *SAS Inst. Inc., Cary NC, USA.*

STRADAIOLI G., MONACI M., VERINI SUPPLIZI A., CANALI C., VACCA C., BOITI C., 1993. Recovery rate and embryo quality in New Zealand White (NZW) rabbits treated with PMSG and PGF2a. 9th Scientific *Meeting of the European Embryo Tranfer Association. Lyon 10-11 settembre 1993.*

THEAU-CLEMENT M., LEBAS F., 1994. Etude de l'efficacité de la Ciclogonine (PMSG) pour induire la réceptivité chez la lapine. *Cuniculture, **115 (21)**, 5-11.*

THEAU-CLEMENT M., ROUSTAN A., 1992. A study on relationship between receptivity and lactation in the doe, and their influence on reproductive performances. *J. Appl. Rabb. Res., **15**, 412-421.*