

# INFLUENCE OF AGE, STIMULATION BY PMSG OR FLUSHING ON THE OVARIAN RESPONSE TO LHRHa IN YOUNG RABBIT FEMALES.

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**SUMMARY** : Fifty young Californian rabbit females have been used to study the effect of age (14 or 17 weeks) and ovarian stimulation (nutritional flushing or PMSG) on the ovarian response to LHRHa injection. Stimulation by nutritional flushing showed, in the 14 and 17 week-old animals, a positive effect on live weight after 8 days, ovulation rate was satisfactory in females aged 17 weeks but not in the 14 week-old group, nevertheless 14 weeks old does showed an increase in the number of follicles larger

than 0.6 mm ( $P < 0.05$ ). Stimulation by PMSG (100 IU) provoked a six folds increase in the number of follicles larger than 0.6 mm (90.6 vs 15.1;  $P < 0.001$ ), but failed to increase the proportion of ovulating females, thus suggesting that a too high dose had been used. These results indicate that introduction of rabbit females to reproduction can be advanced to 17 week-old, provided that previous ovarian stimulation by nutritional flushing is done.

**RÉSUMÉ** : Influence de l'âge, de la stimulation par PMSG ou de la suralimentation sur la réponse ovarienne à LHRHa chez la jeune lapine. Cinquante jeunes lapines de race californienne ont été utilisées pour étudier l'effet de l'âge (14 et 17 semaines) et de la stimulation ovarienne (par suralimentation ou PMSG) sur la réponse de l'ovaire à l'injection de LHRHa. La stimulation par suralimentation pour les animaux âgés de 14 ou 17 semaines a un effet positif sur le poids vif, après 8 jours ; le taux d'ovulation était satisfaisant pour les femelles âgées de 17 semaines mais pas pour celles âgées de 14 semaines, bien que les femelles

âgées de 14 semaines montraient une augmentation du nombre de follicules d'une taille supérieure à 0,6 mm ( $P < 0.05$ ). La stimulation par PMSG (100 U.I.) multipliait par 6 le nombre de follicules de taille supérieure à 0.6 mm (90.6 vs 15.1 ;  $P < 0.001$ ) mais n'augmentait pas le nombre de femelles qui ovulaient, ce qui laisse à penser que la dose utilisée était trop élevée.

Ces résultats montrent que l'âge de la reproduction pour les lapines peut être avancé à 17 semaines à condition que la stimulation ovarienne ait été provoquée par une suralimentation.

## INTRODUCTION

Current information about factors influencing the onset of reproductive activity in rabbit females is available in recent literature. Taking into account sexual receptivity and ovulatory response, puberty is reached at 11 to 14 weeks of age (HULOT *et al.*, 1982). The number of preovular follicles increases with age to get similar values at 17 weeks to those found in multiparous females (GOSALVEZ *et al.*, 1989).

Plasma levels and pulsatility of FSH increases from 14 to 17 and 20 weeks of age (DIAZ *et al.*, 1991a), reaching similar levels to multiparous does (TSOU *et al.*, 1977; OSTEEEN and MILLS, 1979; MEUNIER *et al.*, 1983; RODRIGUEZ *et al.*, 1989).

Several works focus on the effect of ovarian stimulation in rabbit females at puberty. A positive

influence of previous presentation to the male has been found (DIAZ *et al.*, 1991b) as well as stimulation with PMSG (PINGEL *et al.*, 1981; GARCIA and VICENTE, 1990; REBOLLAR, 1993), nutrition level (VAN DEN BROECK and LAMPO, 1977; HULOT *et al.*, 1982; MANCHISI *et al.*, 1988) and photoperiod (KAMWANJA and HAUSER, 1983).

For some authors the reproduction start age can be a limiting factor on the reproductive results. None very good results have been obtained in rabbit females at 14 or 17 weeks of age (DIAZ *et al.*, 1988) or when body weight is under 3000 gr (HULOT *et al.*, 1982). On the contrary, VAN DEN BROECK and LAMPO in 1979 suggest that rabbit does beginning their reproductive life older than 16 weeks of age have not showed differences on their performance.

Most work has been carried out under natural reproductive schemes. Few works have considered

**Table 1 : Body weights (means  $\pm$  SEM) of rabbits used in the experiment 1.**

		Start day (g)	LHRHa day (g)	Slaughter day (g)
14 weeks	Without flushing	2421 $\pm$ 182	2501 $\pm$ 175	2685 $\pm$ 164
	With flushing	2438 $\pm$ 149	2622 $\pm$ 166	3013 $\pm$ 180
17 weeks	Without flushing	2886 $\pm$ 158	2902 $\pm$ 155	2990 $\pm$ 157
	With flushing	2812 $\pm$ 90	2899 $\pm$ 107	3038 $\pm$ 132

induced ovulation as needed in artificial insemination (AI) usually induced by an LHRH analog (REBOLLAR, 1993). The aim of this work has been to study the ovarian response to LHRHa in young females with or without previous stimulation by PMSG or increased nutritional level.

## MATERIALS AND METHODS

### Animals:

Fifty Californian rabbit females were used in this work. Animals were chosen randomly at 4 weeks of age and kept individually in cages under controlled illumination (16L:8D), fed and watered *ad libitum*.

### Experimental Designs:

#### Experiment I:

The influence of age (14 or 17 weeks) and nutritional flushing on the ovarian response was determined using 20 females in a 2 x 2 factorial design. Animals were submitted to a restricted feeding (70% of their ingestion in the prior week) for two weeks before the beginning of the experiment. Afterwards half of the animals in each age group received full feed whereas the other half was kept under restricted ingestion. Four days later all females were injected with 20  $\mu$ g LHRHa (Fertagyl,

INTERVET Lab., Leon, Spain). Four days later the females were slaughtered.

The reproductive tract was removed, determining for each ovary (left and right): weight, number of follicles with diameter higher than 0.6 mm and number of *corpora lutea*. Diameter determination was made using a graduated 1 mm ruler attached to a magnifying glass. When a large number of follicles present, to avoid duplication in counting, each was individually identified by an ink mark using a needle.

#### Experiment II:

Thirty, 17 week-old females were used to measure the influence of ovarian stimulation by PMSG. Half were injected with a saline solution and the other half received 100 IU of PMSG; this dose was adopted in order to avoid a lack of response in very young animals. Two days later all females were injected with 20  $\mu$ g of LHRHa. Reproductive tract measurements were made as in experiment I.

### Statistical Analysis:

Comparison of means were performed by the Mann-Whitney non-parametric test (SIEGEL, 1956). Distributions were compared with the Kolmogorov-Smirnov test (MASSEY, 1951). The test X2 corrected by YATES (1937) was utilized for proportions comparison. When body weights were considered, the comparison was made between the increases of weight during the periods from "start day" to "LHRHa day" or "slaughter day".

**Table 2 : Ovarian response to stimulation by nutritional flushing (mean  $\pm$  SEM).**

		Ovarian weight (g)	Ovulating does %	C.L. no./ovul. doe	Fol. no. $\phi$ > 0.6mm/ doe
14 weeks	No flushing	0.10 $\pm$ 0.01	40 (2)	4.5 $\pm$ 2.50	8.0 $\pm$ 2.4 *
	Flushing	0.12 $\pm$ 0.02	60 (3)	5.0 $\pm$ 1.15	16.0 $\pm$ 2.6 *
17 weeks	No flushing	0.18 $\pm$ 0.06	40 (2) *	9.5 $\pm$ 0.50	11.2 $\pm$ 3.3
	Flushing	0.19 $\pm$ 0.05	100 (5) *	7.8 $\pm$ 0.86	15.8 $\pm$ 2.7

\* P&lt;0.05

**Table 3 : Ovarian response to PMSG treatment in the 17 weeks old females (mean  $\pm$  SEM).**

	without PMSG	with PMSG
Ovarian weight (g)	0.18 $\pm$ 0.02***	0.60 $\pm$ 0.08***
Ovulating females % (n)	80 (12)	67 (10)
C.L./ovulating doe (no)	7.92 $\pm$ 0.65 *	11.00 $\pm$ 3.79
Fol. $\Phi$ > 0.6mm/doe (no)	15.13 $\pm$ 2.17***	90.67 $\pm$ 9.28

\* P&lt;0.05 ; \*\*\* P&lt;0.001

## RESULTS

The mean body weights in each group at the beginning of experiment I, at the time of LHRHa injection, and on slaughter day are shown in Table 1. The nutritional flushing had a significant positive effect (P<0.001) increasing live weight in 14 and 17 weeks old does (24% and 8% respectively). No significant effect on body weight was detected four days after the beginning of nutritional stimulation.

The effect of flushing on the ovarian response is shown in Table 2. At both 14 and 17 weeks of age the ovaries were sensitive to the previous stimulation by a short period of flushing. There was a higher number of antral follicles with diameters over 0.6 mm at 14 weeks of age (P<0.05). At 17 weeks of age a higher proportion (P<0.05) of ovulating females was found in the flushed group.

Stimulation by 100 IU of PMSG (Table 3) was effective in increasing the ovarian weight (P<0.001), the number of *corpora lutea* per ovulating female (P<0.05) and the number of antral follicles, larger than 0.6 mm (P<0.001), even there was not effect on the proportion of females which ovulated. The number of

*corpora lutea* per ovulating female treated with PMSG is shown in Figure 1, the difference between both distributions is very significant (P<0.001), showing PMSG an dispersing effect when compared with natural conditions.

Left and right ovaries had been individually considered, but no significant differences were found between them. In table 4, mean values of weight, number of *corpora lutea* and follicles larger than 0.6 mm, for both ovaries is shown.

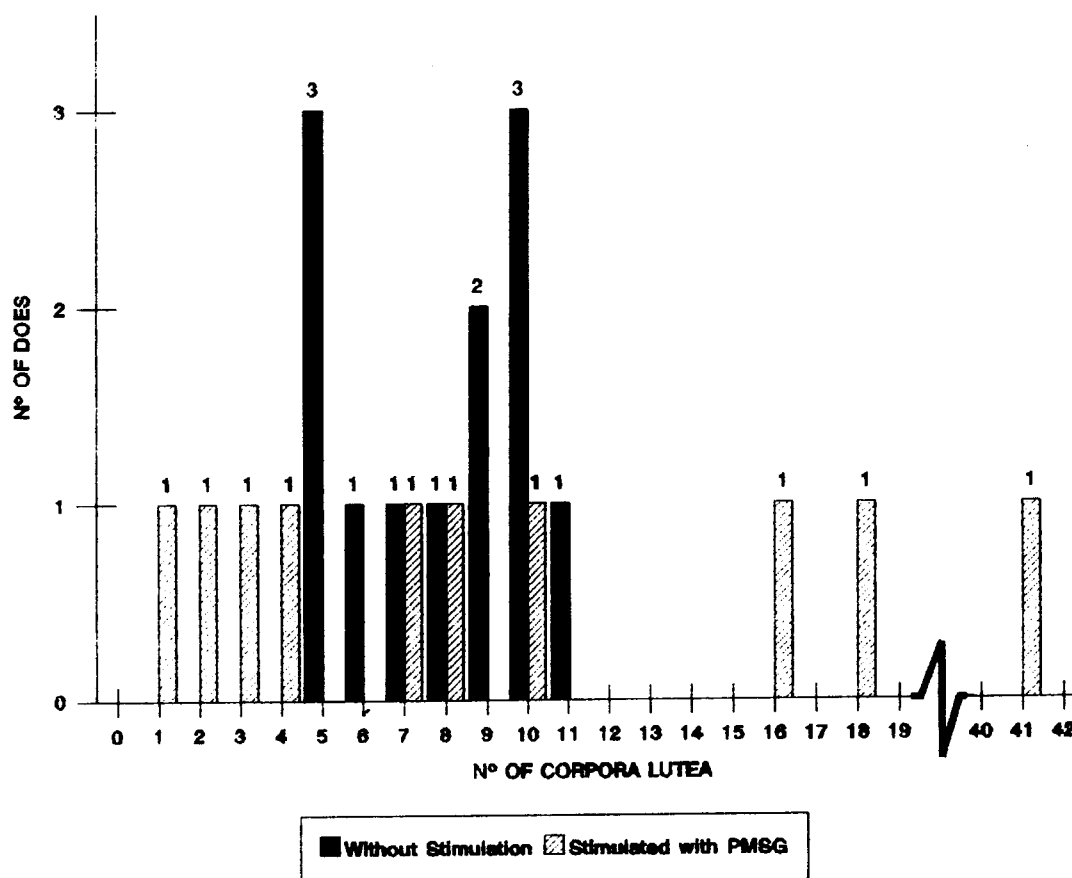
## DISCUSSION

Flushing increase the growth of follicles in 14 week-old females and increase the proportion of ovulating females in the 17 week-old does. The positive effect of flushing on reproductive performance of young rabbit females agrees with previous data (HULOT *et al.*, 1982), on the other hand, VAN DEN BROECK and LAMPO (1977) with older females found still better results with feeding ad libitum. Nevertheless, the response observed in the youngest of the studied groups is not satisfactory from the point of view of ovulation rate. Although VAN DEN BROECK and LAMPO (1979) do not found differences in fertility and prolificacy between females of 16, 18 and 20 weeks ; in our case, age or live weight seems limiting factors similar to observed in other works (HULOT *et al.*, 1982; DIAZ *et al.*, 1988). Consequently, our data suggest that nutritional flushing could be recommended in females not younger than 17 weeks to obtain a similar performance to that observed by DIAZ *et al.*, (1988) in 20 week-old females.

The main effect of PMSG stimulation was a wave of follicular growth (the number of follicles higher than 0.6 mm was multiplied by 6) as well as an increase (38.9 %) in the number of *corpora lutea* by ovulating female. Nevertheless, no improvement in the proportion of ovulating females was detected nor the

**Table 4 : Mean values of the studied variables in the left and right ovaries (mean  $\pm$  SEM).**

		14 weeks		17 weeks		17 weeks	
		No flush	Flush	No flush	Flush	No PMSG	PMSG
Ovarian weight (g)	Left	0.10 $\pm$ 0.01	0.14 $\pm$ 0.02	0.18 $\pm$ 0.07	0.19 $\pm$ 0.04	0.18 $\pm$ 0.02	0.61 $\pm$ 0.01
	Right	0.10 $\pm$ 0.01	0.11 $\pm$ 0.02	0.18 $\pm$ 0.06	0.19 $\pm$ 0.06	0.18 $\pm$ 0.02	0.59 $\pm$ 0.08
Corpora lutea (no)	Left	0.7 $\pm$ 0.5	1.6 $\pm$ 1.0	2.4 $\pm$ 2.0	3.6 $\pm$ 1.3	2.8 $\pm$ 0.5	3.3 $\pm$ 1.4
	Right	0.8 $\pm$ 0.6	1.4 $\pm$ 0.6	3.2 $\pm$ 2.7	4.2 $\pm$ 1.3	3.5 $\pm$ 0.6	4.1 $\pm$ 1.6
Follicles $\Phi$ > 0.6mm (no)	Left	3.2 $\pm$ 1.3	9.2 $\pm$ 1.3	5.8 $\pm$ 3.4	7.0 $\pm$ 1.9	6.9 $\pm$ 0.8	46.5 $\pm$ 5.1
	Right	4.8 $\pm$ 1.7	7.0 $\pm$ 1.8	5.3 $\pm$ 2.6	8.8 $\pm$ 3.5	8.3 $\pm$ 1.5	44.1 $\pm$ 4.5

Figure 1 : *Corpora lutea* distribution on the ovulating females of experiment 2.

PMSG induce ( $P < 0.001$ ) a normal distribution of the number of *corpora lutea* in these females, further, one third (4 out of 12) of the ovulating does had only one to three *corpora lutea*. These results could be due to the too high dose employed, resulting in an excessive oestradiol production and in alterations on the pituitary response to LHRHa. All this could be considered as an anomalous response to a PMSG dose, already this dose produces superovulation in adult females (ILLERA *et al.*, 1988), on 17 weeks old does seems to exist two types of animals in relation to the reproductive tract development (response to PMSG).

Besides, a negative effect of high PMSG doses on the quality of growing follicles cannot be discarded. Atresia rate could have been increased as a consequence of oestradiol mediated depression of endogenous secretion of GnRH and gonadotropins. This hypothesis cannot be confirmed from this work, because no observation was made of the ovaries at the moment of LHRHa injection.

In conclusion, in young females lower doses of PMSG should be employed. PMSG between 25 and 50 IU have proved to be effective in the ovarian stimulation of females aged 18 weeks (GARCIA and

VICENTE, 1990) and in adult rabbit females (REBOLLAR, 1993).

We had not found differences between left and right ovaries according to the general reproductive knowledge and with previous observation in nulliparous and multiparous does (GOSALVEZ *et al.*, 1987).

From our results it can be concluded that beginning of female rabbit reproduction can be advanced to 17 weeks of age, provided previous ovarian stimulation by nutritional flushing. Nevertheless more work is needed in order to confirm our results with more animals and to assess the proper PMSG dose to enhance ovarian response in young females.

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