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New attempts to decrease the variability of ovarian response to PMSG in cattle.

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Summary. The variability of ovarian response in cattle after superovulatory treatment with PMSG is mainly due to differences in the follicular population on the day of PMSG injection. We have tried to modify this follicular population either by destroying some of the follicles at the time of PMSG injection or by pretreatment at the beginning of the cycle. The electrocauterization of follicles on day 16 delayed luteolysis and did not reduce variability. The injection of 1 000 i.u. of PMSG on day 3 post-œstrus produced 35.7 p. 100 of twin ovulations when 600 i.u. were injected on day 16. Another explanation of response variability is the duration of PMSG action which can be modified by controlling the PMSG-œstrus interval with prostaglandin analogs; lenghtening that interval increased the ovulation rates from 1.75 to 4.60 and decreased the number of large follicles growing after ovulation from 5.13 to 0.60. Moreover, when PMSG prostaglandin treatment was associated with progestagen, PMSG efficiency increased. So after 500 i.u. of PMSG at the end of a progestagen-prostaglandin treatment we obtained à 34.1 p. 100 twinning rate. Finally, repeated treatments (2 000 i.u. PMSG-0.5 mg $PGF_{2\alpha}$ analog) every 6 weeks during one year produced the same variability in the response pattern : response was constant in 29.8 p. 100 of the heifers, 27.7 p. 100 showed a definite decrease after 3 to 5 treatments, and 36.2 p. 100 a decrease followed by an increase.

Many factors could explain the variability in animal response to a superovulatory treatment. Among these the most important is probably the population of antral follicles of the treated females (Saumande *et al.*, 1978). However, from the results presented by Schams *et al.* (1978) on the pattern of injected PMSG and from hormonal studies of non-superovulated animals (Saumande, 1978), it appears that the treatments themselves explain part of this variability. Furthermore, we have presented preliminary results (Saumande *et al.*, 1978) which show that variability appears not only with one treatment, but also in the response pattern to repeated stimulation. The present reports presents the results on each of these aspects.

I. Attempts to decrease response variability in the large-follicle population at the time of PMSG injection.

The number and size of antral follicles at the time of PMSG injection affects response to superovulatory treatment (Saumande *et al.*, 1978). In sheep this antral fol-

licle population is quantitatively correlated with the magnitude of the second FSH peak occurring after the previous œstrus (Cahill *et al.*, 1979).

Using these observations, were tried to control the large follicle population present on day 16 either (i) by destroying all or part of the apparent follicles on day 16, or (ii) by pretreating with PMSG on day 3 the follicles stimulated by the second FSH peak in order to protect them from atresia.

1. *Cauterization of follicles.* Twenty-four Friesian cows and 16 crossbred Normand × Charolais heifers were divided into 4 groups depending on the size of the follicles destroyed and on whether or not PMSG was injected.

Group 1 : on day 16 follicles larger than 8 mm in diameter were destroyed by electrocauterization using endoscopy approach (Locatelli, 1976) ;

Group 2 : same as group 1 but 1 600 i.u. of PMSG were injected simultaneously ;

Group 3 : same as group 1 but all the apparent follicles (larger than 1 mm) were destroyed ;

Group 4 : same as group 3 but 1 600 i.u. of PMSG were injected simultaneously.

Oestrus was detected twice daily using a vasectomized bull. The ovaries were observed 4 to 10 days after post-treatment œstrus in order to count the corpora lutea and the follicles.

The animals were observed to be in œstrus 7 to 8 days after the treatment (table 1); this interval was unaffected by PMSG injection. In non-stimulated animals one ovulation was observed in almost all cases ; the PMSG treatment induced the usual type of superovulation : ovulation rate according to dose of PMSG and type of animal, and also variability.

TABLE 1

Effect of follicle cauterization on day 16 on œstrous cycle length and ovulation rate

	Follicles larger than 8 mm destroyed		All apparent follicles destroyed	
	Dose of PMSG (IU)		Dose of PMSG (IU)	
	0	1 600	0	1 600
Injection-œstrus interval (days).....	7.71 * ± 1.70 (7)	7.90 ± 2.93 (10)	7.88 ** ± 2.81 (8)	6.30 ± 1.60 (10)
Ovulation rate	0.90 ± 0.32 (10)	4.10 ± 4.75 (10)	0.90 ± 0.32 (10)	3.30 ± 3.43 (10)

* No œstrus in 3 animals.
** No œstrus in 2 animals.
( ) Number of animals.

Whether PMSG was injected or not, and whatever the size of the follicles destroyed, cauterization lengthened the œstrous cycle by 3 to 4 days. The method of anesthesia and observation of the ovaries by endoscopy prolonged this cycle by 1.5 to

2 days ; thus cauterization of the follicles alone delayed the onset of œstrus by 2 days (table 2).

TABLE 2

Effect of anaesthesia, endoscopy and follicle cauterization on interval between D_{16} and œstrus (days)

Breed	PMSG (IU)	Control	Anesthesia	Anesthesia + endoscopy	Anesthesia + endoscopy + cauterization
Charolais cows	0	4.55 ± 1.18			
Charolais cows	1 600	3.74 ± 1.29		5.19 ± 1.90	
Normand cows	1 600	3.71 ± 2.29		6.72 ± 3.15	
FPN cows	0	4.36 ± 1.17	5.75 ± 1.19		8.56 ± 2.47
FPN cows	1 600	3.92 ± 0.83			7.92 ± 2.84

The increase in œstrous cycle length can be explained in two ways :

— delay for the differentiation of new follicles able to ovulate. The destruction of all apparent follicles or only those larger than 8 mm resulted in the same delay ;

— prolonged life span of the corpora lutea. The progesterone pattern (fig. 1) showed that this was the main factor, as the interval between decrease in progesterone concentration and œstrus was normal in all cases.

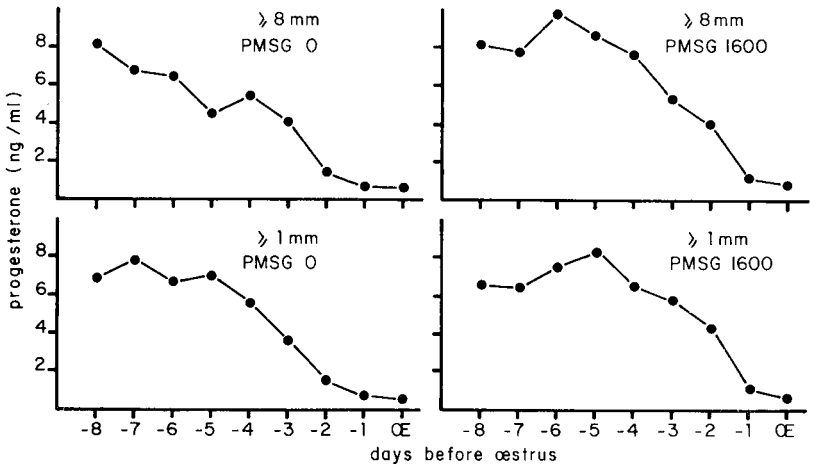

FIG. 1. — *Progesterone patterns following cauterization of follicles of different size, with ou without injection of PMSG.*

However, both are associated, as Karsch *et al.* (1970) and Ginther (1971) have demonstrated that at least in sheep the destruction of antral follicles increases the life span of the corpus luteum, and Caldwell *et al.* (1972) have shown that estradiol is necessary for the secretion of $PGF_{2\alpha}$.

The present experiment did not allow us to draw conclusions on the relationship between the antral follicle population and response variability to PMSG.

2. *Pretreatment with* PMSG *on day* 3. For this experiment, 37 Charolais nursing cows and 36 Normand milking cows were divided into three groups. The dose of PMSG was corrected according to breed sensitivity (Saumande *et al.*, 1978). About half the animals in each group received an injection of $PGF_{2\alpha}$ analog (Estrumate ICI Co.) on day 16.

The distribution of animals in the different treatment groups is given in table 3.

TABLE 3

Treatments and animals used to evaluate the efficiency of PMSG injection on day 3

	Breed	Dose of PMSG (IU) injected on :		Number of animals injected on day 16 with	
		day 3	day 16	0 µg estrumate	500 µg estrumate
Group 1	Charolais	0	1 600	5	7
	Normand	0	1 800	4	6
Group 2	Charolais	0	600	7	3
	Normand	0	800	7	6
Group 3	Charolais	1 000	600	8	7
	Normand	1 000	800	6	7

Oestrus was detected twice a day with a vasectomized bull. Ovulation was determined by endoscopy 4 to 10 days after œstrus.

The dose of PMSG was correctly adjusted so that in each treatment group the same ovulation rates were obtained for Charolais and Normand cows. Consequently, the results presented in table 4 are those obtained for both breeds.

TABLE 4

Mean ovulation rate after PMSG *injection on* D_{16} *: effect of* PMSG *injection on* D_3 *and* $PGF_{2\alpha}$ *analog injection on* D_{16} ($M \pm SD$)

Treatment group *	0 µg $PGF_{2\alpha}$ Analog on D_{16}	500 µg $PGF_{2\alpha}$ Analog on D_{16}
1	2.22 ± 3.67 (9) **	3.69 ± 3.75 (13)
2	1.14 ± 0.36 (14)	1.44 ± 1.33 (9)
3	1.14 ± 0.36 (14)	1.50 ± 0.85 (14)

* see table 3.
** ( ) Number of animals (both breeds)

The highest mean ovulation rate was obtained in animals treated with the highest dose of PMSG ; there were no differences between groups 2 and 3. In all groups, the mean ovulation rate was higher when Estrumate was injected with PMSG.

As previously emphasized (Saumande *et al.*, 1978) the mean ovulation rate is not informative. For this experiment, the same mean ovulation rate in groups 2 and 3 (animals treated with $PGF_{2\alpha}$ analog) was obtained with the different individual results : in group 2 one animals had five ovulations as compared with group 3 in which all superovulated animals had only two ovulations (table 5). These results showed that pretreatment with 1 000 i.u. PMSG on day 3 increased the response after a low dose of PMSG on day 16 without increasing the variability ; this could be observed only when Estrumate was injected simultaneously.

The efficiency of this treatment in decreasing response variability when a higher ovulation rate is needed remains to be tested.

TABLE 5

Ovulation rate after PMSG *injection on* D_{16} : *effect of* PMSG *injection on* D_3 *and* $PGF_{2\alpha}$ *analog injection on* D_{16}

Treatment group	Ovulation rates	0 µg $PGF_{2\alpha}$ analog on D_{16} (p. 100)	500 µg $PGF_{2\alpha}$ analog on D_{16} (p. 100)
1	0-1	88.9	53.8
	2-4	—	15.4
	> 4	11.1	30.8
2	0-1	85.7	88.9
	2-4	14.3	—
	> 4	—	11.1
3	0-1	85.7	64.3
	2-4	14.3	35.7
	> 4	—	—

II. Effect of treatment.

1. *Effect of day of* $PGF_{2\alpha}$ *analog injection with* PMSG *treatment on day* 10.

Adult Friesian milking cows received 1 600 i.u of PMSG on day 10 and 0.5 mg of $PGF_{2\alpha}$ analog either on day 10 (8 animals), day 11 (11 animals) or day 12(10 animals). Corpora lutea and follicles larger than 6 mm were observed by endoscopy 4 to 10 days after induced œstrus.

It appeared (table 6) that : — PMSG-œstrus interval increased when $PGF_{2\alpha}$ analog injection was delayed ; — the percentage of superovulated animals was higher when $PGF_{2\alpha}$ analog was injected 48 hrs after PMSG ; — the number of large follicles present in the ovaries 4 to 10 days after œstrus decreased when the PMSG-œstrus interval increased.

When PMSG was injected on day 16 and Estrumate on day 15, 16, 17 or 18 the same results were obtained in regard to the PMSG-œstrus interval. However, better superovulation results were obtained when PMSG and $PGF_{2\alpha}$ analog were injected

on the same day (Saumande *et al.*, 1978). This discrepancy could reflect differences in the follicular population during the luteal *vs* the follicular phase.

TABLE 6

Time of estrumate according to PMSG *injection and ovulation rate* (FPN dairy cows — 1 600 I. U. PMSG *on day* 10)

Day of PG injection	Number of animals	PMSG injection-œstrus interval (days)	Ovulation rate		Percentage of animals with			No. of follicles
			M	(range)	0-1	2-4	> 4	
					ovul.	ovul.	ovul.	
10	8	2.94	1.75	(1-4)	62.5	37.5	0	5.13
11	11	4.00	6.09	(1-22)	45.4	9.1	45.4	1.45
12	10	4.50	4.60	(1-14)	20.0	50.0	30.0	0.60

It is apparent that when ovulation takes place rapidly after PMSG injection the gonadotrophin level remains high enough to stimulate the growth of many antral follicles. This observation agrees with the hypothesis that the large follicles observed at endoscopy 4 to 10 days after œstrus have grown after ovulation (Saumande, 1978).

2. *Effect of day of* PMSG *injection at the end of estrogen-progestagen-prostaglandin treatment.* In two experiments, a new treatment for the control of the œstrous cycle (Chupin and Pelot, 1978) was used together with a low dose of PMSG. This treatment consists of progestagen implants (Norgestomet = SC 21009, Searle) for 9 days with the injection of 5 mg of œstradiol valerate at the beginning of the treatment and 0.5 mg of $PGF_{2\alpha}$ analog 2 days before implant removal.

In the first experiment 500 i.u. of PMSG were injected either 2 days before (61 Friesian milking cows) or at implant removal (62 Freisian milking cows). Pregnancy rate was high and about the same in the two treatments (67.2 and 67.7 p. 100, respec-

TABLE 7

Effect of day of PMSG *injection at the end of combined progestagen-prostaglandin treatment* (300 μg norgestomet implants-9 days 500 μg of $PGF_{2\alpha}$ analog injected 2 days before implant removal)

Day of injection of 500 IU PMSG	Number of cows	p. 100 pregnant (synchronized œstrus)	p. 100 twin gestations *
At implant removal	62	67,7	16,7
Two days before implant removal	61	67,2	34,1

* Among those pregnant after synchronized œstrus.

tively). The percentage of twin gestations was higher when PMSG was injected 2 days before implant removal (34.1 *vs* 16.7 p. 100) (table 7). This and the previous results on the effect of day of $PGF_{2\alpha}$ analog injection relative to PMSG treatment demonstrated that the efficiency of PMSG injection depended on the duration of the stimulation.

This treatment appeared to be the more efficient for hormonal induction of twinning ; up to now such a twinning percentage could only be obtained with treatments inducing more than 4 ovulations in some animals, resulting in low overall fertility of the treated group.

In the second experiment the combined treatment described above was compared with the PMSG-$PGF_{2\alpha}$ analog treatment in Normand suckling cows. In both cases 600 i.u. of PMSG and $PGF_{2\alpha}$ analog were injected together.

These experiments were conducted a year apart. Ovulation rates were determined by rectal palpation.

The results presented in table 8 show that the combined treatment induced a higher percentage of twin ovulations.

TABLE 8

Effect of synchronization treatments on the response to PMSG (600 IU)

Treatments	Ovulation rate *	Animal No.	p. 100
2 $PGF_{2\alpha}$ analogue injections	0-1	27	93,1
14 days apart **	2-4	2	6,9
	> 4	0	—
Progestagen implants and	0-1	20	66,7
prostaglandin two days	2-4	10	33,3
before implants removal **	> 4	0	—

* Measured by rectal palpation.
** PMSG and prostaglandin analogue were injected at the same time.

We cannot conclude whether this difference was due to the PMSG-œstrus interval or to the synchronizing treatments themselves. Furthermore, each treatment was tested at a different time.

More experiments are needed to compare the combined treatment with the previous ones and to determine the reasons for its efficiency.

III. Variability of response pattern after repeated superovulatory treatments.

Every 6 weeks during one year, 47 Friesian heifers received 2 000 i.u. of PMSG on day 8 and 0.5 mg of $PGF_{2\alpha}$ analog 48 h later. The ovulation rates were determined by endoscopy 4 to 10 days after induced œstrus.

The animals were classified into 6 groups according to their response pattern during 9 successive treatments (fig. 2) :

— in 30 heifers the ovulation rate decreased and then increased in 17 but not in the other 13 ;

— in 14 heifers the response remained constant at a high (more than 10 ovulations : 6 animals), medium (5 to 10 ovulations : 4 animals), or low (less than 5 ovulations : 4 animals) level ;

— in 3 heifers the response was erratic.

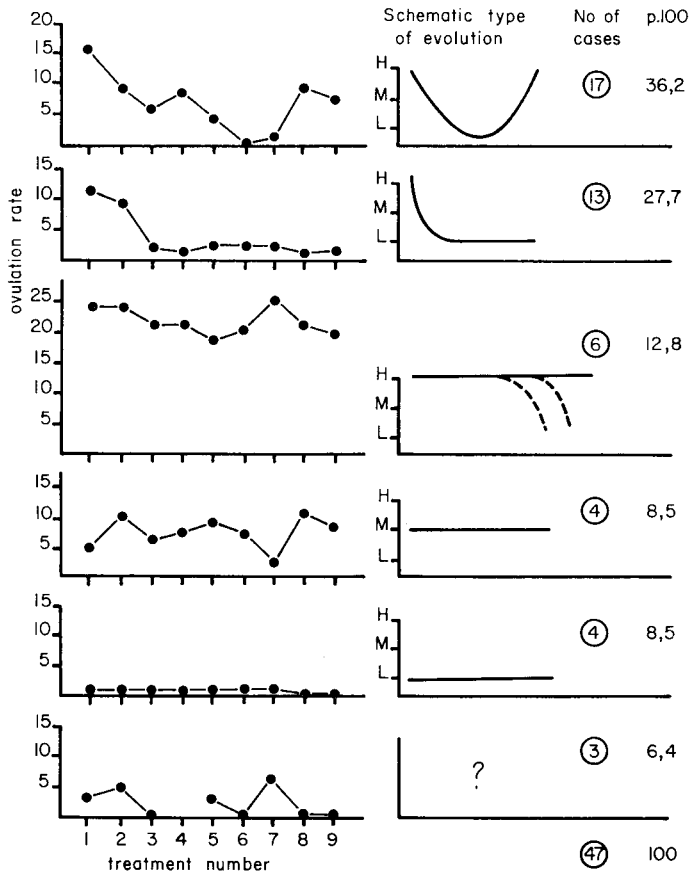

FIG. 2. — *Individual response to repeated superovulatory treatments.*

It has been shown by many authors (Willet *et al*, 1953 ; Hafez *et al.*, 1965 ; Jainudeen *et al.*, 1966 ; Mariana *et al.*, 1970 ; Laster *et al.*, 1971 ; Betteridge and Mitchell, 1974 ; Saumande and Chupin, 1977) that the ovulation rate decreases after repeated treatments. In the present study, this was observed in 63.9 p. 100 of the cases.

We reported previously (Saumande and Chupin, 1977) that after this decrease the mean ovulation rate increased after the 5th or 6th treatments. In our new experiment, this was true for only 36.2 p. 100 of the animals.

In 14 heifers (29.8 p. 100 of the treated animals) the ovulation rate remained constant. The commercial use of females as embryo donors requires that they produce a high number of ovulations regularly ; only 12. 8 p. 100 of our animals would have met this requirement.

Conclusions.

As far as reduction of ovulation rate variability after one stimulation is concerned two of the treatments tested seemed to be efficient, at least for hormonal induction of twinning : pretreatment with PMSG on day 3 and the synchronization of œstrus with the combined treatment. While the efficiency of the first method can be explained, that of the second remains to be discovered. Both treatments, however, need to be tested when a high ovulation rate is required.

The variability of response pattern during repeated superovulatory treatments was confirmed. As we could not determine the reasons for these differences, we are still unable to control them. This problem would limit the possibility of genetic improvement with embryo transfer techniques.

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Résumé. La variabilité des réponses ovariennes à une injection de PMSG est due principalement aux différences de nombres de follicules susceptibles de croître en réponse à cette injection. Nous avons cherché à modifier cette population folliculaire soit en détruisant une partie des follicules au moment de la stimulation, soit en la préstimulant par une injection en début de cycle. La destruction des follicules par électrocautérisation à J_{16} retarde la lutéolyse et ne modifie pas la variabilité. Par contre, l'injection de 1 000 u.i. de PMSG 3 jours après l'œstrus permet d'obtenir 35.7 p. 100 d'ovulations doubles après 600 u.i. de PMSG le 16^{e} jour du cycle.

Une autre cause de variabilité serait liée à la durée d'action de PMSG. On peut modifier celle-ci en contrôlant l'intervalle PMSG-œstrus grâce aux analogues de prostaglandines. L'allongement de cet intervalle permet une augmentation du nombre d'ovulations (de 1,75 à 4,60) et une diminution du nombre de follicules qui se développent après l'ovulation de 5,13 à 0,60. De plus, lorsque l'on associe PMSG-prostaglandine à un traitement prosgestagène on augmente l'efficacité (nombre d'ovulations) d'une faible dose de PMSG. Ainsi, 34,1 p. 100 de gestations obtenues après injection de 500 u.i. de PMSG en fin d'un traitement progestagène-prostaglandines étaient gémellaires.

Enfin, en répétant toutes les 6 semaines pendant un an le traitement 2 000 u.i. de PMSG-0,5 mg de $PGF_{2\alpha}$ analogue (Estrumate), nous avons pu montrer que la variabilité individuelle apparaissait sur la nature des courbes de réponses : 29,8 p. 100 des génisses maintiennent une réponse constante toute l'année, tandis que 27,7 p. 100 montrent une chute sans reprise et 36,2 p. 100 une chute suivie d'une reprise de la réponse.

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