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Superovulation in cattle using PMSG followed by PMSG-monoclonal antibodies

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ABSTRACT

The advantages of using pregnant mares' serum gonadotrophin (PMSG) to stimulate increased preovulatory follicular development are that it is available in large quantities at low cost, and can be administered as a single dose because of its long half-life. The long half-life, however, can have disadvantages since it may cause over and/or prolonged stimulation, leading to a second wave of follicular development after ovulation and a secondary rise in oestradiol-17β. The latter may interfere with embryo quality.

PMSG antiserum[1] has been extensively tested in an effort to selectively remove PMSG from the peripheral blood of PMSG stimulated cattle after the initial phase of follicular stimulation but before the secondary post-ovulatory phase of stimulation. This neutralisation of PMSG with PMSG antiserum prevents the secondary development of ovarian follicles and the accompanying rise in oestradiol-17β; however, the evidence regarding the effect on the number of usable embryos recovered is conflicting. The best results, in this respect, have been obtained when PMSG antiserum was administered 5–6 h after the preovulatory LH peak but this event is difficult to assess in practice. Administration of PMSG antiserum at a fixed time in relation to the use of either PMSG or a synchronising injection of a prostaglandin analogue, or in relation to the onset of behavioural oestrus is unsatisfactory because of the variability of the timing of the LH peak in relation to these events. It may, however, be possible to use the preovulatory peak of oestradiol-17β as a marker for the administration of PMSG antiserum.

INTRODUCTION

Cattle, in common with all mammals, produce thousands of oocytes which are never utilised in the normal reproductive process (Erickson, 1966). Methods have been developed to collect and fertilise such oocytes which offer ways of increasing both commercial production and genetic potential (Seidel, 1981). One approach to the collection of oocytes has exploited the use of superovulation induction in pre- or post-pubertal heifers and cows by either the direct use of exogenous gonadotrophins or the indirect stimulation of en-

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[1]The term PMSG antiserum as used throughout this paper includes antisera from cattle, goat or turkey and commercially produced monoclonal antibodies.

dogenous gonadotrophins by means of gonadotrophin-releasing hormone, immunisation with gonadal steroids or inhibin, or other means (Sreenan, 1984; Price and Webb, 1988). When gonadotrophin therapy has been used, a preparation with high follicle stimulating hormone (FSH) activity has normally been administered, in the mid-luteal phase of either natural or synchronised oestrous cycles, to stimulate increased preovulatory follicular development. Ovulation has generally been achieved without the administration of an ovulatory luteinizing hormone (LH) via the endogenous oestrogen positive feedback/ovulatory gonadotrophin surge mechanism (Kesner et al., 1981; Karsch, 1987).

The FSH preparations used include: extracts of domestic animal pituitaries, particularly those of the pig (pFSH), of various degrees of purity and FSH/LH ratio; gonadotrophins of pituitary origin extracted from human postmenopausal urine (human menopausal gonadotrophin, hMG); recombinant FSH preparations; preparations of equine chorionic gonadotrophin (eCG) derived from the serum of pregnant mares (usually called pregnant mares' serum gonadotrophin, PMSG) (McGowan et al., 1985). An inherent problem in the use of gonadotrophin preparations for superovulation is the high variability of response, both within and between individuals. In some cases, when direct comparisons have been made, the use of pFSH has resulted in more satisfactory and predictable responses than the use of PMSG. The advantages of PMSG are, however, that it is available in large quantities at a

TABLE 1

Mean ovulation rate (corpora lutea, CL), total number of ova/embryos and number of usable embryos from synchronised heifers treated with different doses of PMSG

Dose of PMSG (IU)	No. of CL observed	Total no. of ova/ embryos recovered	No. of usable embryos recovered
1000	3.6	2.1	2.1
2000	9.4	7.1	6.3
3000	12.7	6.6	4.8
4000	9.3	5.6	3.2

TABLE 2

Mean number of large follicles (greater than 10 mm in diameter) observed in the ovaries of synchronised heifers treated with different doses of PMSG

Dose of PMSG (IU)	No. of large follicles
1000	0.3
2000	0.9
3000	3.4
4000	5.1

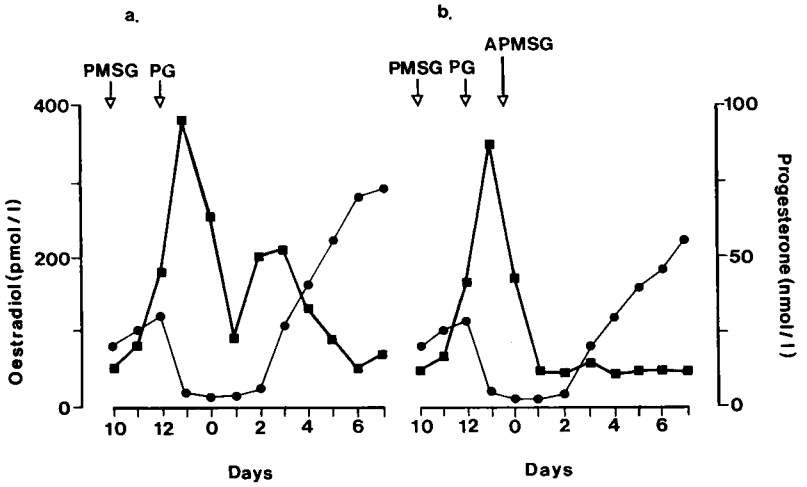

Fig. 1. Oestradiol-17β (■—■) and progesterone (●—●) profiles from cattle treated with 2500 IU of PMSG (i.m.) on Day 10 of the oestrous cycle (oestrus designated Day 0), followed by injection (i.v.) 84 h later of either (a) a placebo of equivalent volume or (b) an equivalent dose of PMSG antibody (APMSG).

TABLE 3

Mean number of large follicles (greater than 10 mm in diameter) at Day 7 after oestrus and mean concentration of oestradiol-17β (OE2) during the post-ovulatory rise in synchronised cows treated either with PMSG alone or PMSG followed by PMSG-monoclonal antibody (APMSG) 4 days later ($n=18$)

Treatment	No. of large follicles	Post-ovulatory OE2 rise ($pmol\,l^{-1}$)
PMSG	1.8	200
PMSG+APMSG	0.2	40

low cost and can be used in countries into which the importation of certain porcine products is banned. Also, PMSG can be administered as a single dose compared with the multiple injections required when using pituitary preparations. This is because PMSG has a much longer biological half-life than pituitary FSH owing to its higher sialic acid content (Schams and Papkoff, 1971). Measurable amounts of PMSG have been detected in cattle up to 10 days after injection (Schams et al., 1978). The long half-life of PMSG may, however, be a disadvantage in some cases, causing problems of either overstimulation and/or prolonged stimulation. Early studies of cattle superovulation using PMSG showed that there was an upper limit to the dose used for an optimal ovulatory response (Folley and Malpress, 1944; Hafez et al., 1963). The use of doses higher than this optimum may increase the ovulation rate in

TABLE 4

Superovulatory responses in cattle treated with PMSG and PMSG antiserum (APMSG)

PMSG (IU)	Time[1]	Treatment	Animals		Mean no. of CL	Mean no. of ova/embryos		Reference
			Type	No.		Total	Usable	
1000	108–144 h	APMSG[2]	Beef heifers	8	2.8	1.5	1.5	Alfuraiji et al., unpub. data, 1991
		Control		8	4.5	2.6	2.6	
1500	108 h	APMSG[2]	Beef cows	10	11.2	5.3	2.4	Wang et al., 1988
		Control		12	9.0	3.4	0.3	
2000	108–144 h	APMSG[2]	Beef heifers	8	10.3	7.7	7.4	Alfuraiji et al., unpub. data, 1991
		Control		8	8.5	6.5	5.5	
2250	108 h	APMSG[2]	Mixed breed cattle	49	–	7.1	4.5	Greve et al., 1988
		Control		52		6.7	4.0	
2500	12 h after OE	APMSG	Cross-bred heifers	6	16.2	–	–	Saumande and Chupin, 1986
	24 h after OE	APMSG		7	18.3	–	–	
2500	4.8 h after LH peak	APMSG[2]	Mixed breed heifers	29	15.0	–	–	Dieleman et al., 1987
		Control		28	8.0	–	–	
2500	96 h	APMSG	Cull cows	8	13.9	6.0	4.0	Wang et al., 1987
	108 h	APMSG		8	17.0	9.7	5.3	
		Control		8	7.8	4.5	2.1	
2500	108 h	APMSG[2]	Holstein heifers	15	12.5	9.8	4.9	Chupin et al., 1988
2500	125–142 h	APMSG[2]	Dairy heifers	38	20.0	9.0	3.0	Callesen et al., 1989
		Control		43	16.0	9.0	4.0	
2500	84 h	APMSG[2]	Beef cows	18	10.6	8.5	7.0	Alfuraiji et al., unpub. data, 1991
	96 h	APMSG[2]		18	9.7	7.4	3.5	
	108 h	APMSG[2]		18	11.1	8.4	6.3	
		Control		18	14.3	10.8	7.3	
3000	At OE	APMSG	Dairy cows	12	19.0	6.7	5.2	Dhondt et al., 1978
		Control		102	9.4	2.9	1.8	

3000	96 h	APMSG	Belgium cattle	24	6.2	–	–	Bouters et al., 1983
	108 h	APMSG		24	15.5	–	–	
	120 h	APMSG		14	14.4	–	–	
		Control		20	8.4	–	–	
3000	12 h after OE	APMSG	Friesian cows	11	9.5	7.7	5.6	Saumande et al., 1984
	24 h after OE	APMSG		12	9.8	7.8	5.3	
		Control		11	10.8	7.2	3.5	
3000	At OE	APMSG[2]	Dairy cattle	15	9.5	4.4	2.7	Moyaert et al., 1985
3000	At 1st AI	APMSG	Cross-bred beef cows	10	11.9	7.3	5.1	Kim et al., 1987
		Control[3]		10	4.4	3.9	3.6	
3000	108 h	APMSG[2]	Beef cows	11	16.3	9.2	2.9	Wang et al., 1988
		Control		10	10.5	2.0	0.3	
3000	5.8 h after LH peak	APMSG[2]	Friesian cows	16	18.9	15.6	9.1	Dieleman et al., 1989
		Control		16	14.0	11.9	5.3	
3000	108–144 h	APMSG[2]	Beef heifers	8	15.3	8.4	5.7	Alfuraiji et al.,
		Control		8	10.1	4.9	4.1	unpub. data, 1991
4000	108–144 h	APMSG[2]	Beef heifers	8	7.6	2.5	2.0	Alfuraiji et al.,
		Control		8	11.0	8.6	4.5	unpub. data, 1991
4500	108 h	APMSG[2]	Beef cows	11	16.3	5.1	0.6	Wang et al., 1988
		Control		10	11.8	5.1	0.6	
5000	5 h after OE	APMSG	Mixed breed heifers	8	22.1	17.8	12.5	Kummer et al., 1980
		Control		8	18.0	6.9	2.9	
5000	12 h after OE	APMSG	Cross-bred heifers	6	3.2	–	–	Saumande and Chupin, 1986
	24 h after OE	APMSG		6	8.5	–	–	
7500	12 h after OE	APMSG	Cross-bred heifers	5	1.4	–	–	Saumande and Chupin, 1986
	24 h after OE	APMSG		6	2.2	–	–	

[1]The time of administering PMSG antiserum is relative to PMSG injection unless otherwise indicated.
[2]Monoclonal preparation.
[3]These authors used 36 mg of FSH as a control with PMSG antiserum treatment.
OE, oestrus; AI, artificial insemination; CL, corpora lutea.

TABLE 5

Weighted mean numbers[1] of corpora lutea (CL), total ova/embryos and usable embryos in cattle treated either with PMSG alone or PMSG followed by PMSG antiserum (APMSG)

Treatment	No. of CL observed	Total no. of ova/embryos recovered	No. of usable embryos recovered
PMSG	10.7	5.8	3.1
PMSG+APMSG	13.0	8.0	4.7

[1]Based on data given in table 4.

TABLE 6

Mean (±SEM) numbers of total embryos and usable embryos recovered from beef cattle superovulated with PMSG alone or followed by an equivalent dose of PMSG antiserum (APMSG) given approximately 8 h after the preovulatory oestradiol-17β peak

Treatment	No. of cattle	Mean (±SEM) oestradiol-17β peak (pmol l^{-1})	Embryos recovered	
			Total	Usable
PMSG	7	560±99	10.6±1.2	4.4±1.4
PMSG+APMSG	7	590±64	12.6±1.8	12.3±1.8

some cases, but the quality of the resulting embryos is impaired, and hence the number of transferable embryos will be reduced (Sreenan and Beehan, 1976; Greve et al., 1979; Table 1).

It has been suggested that even at the optimum dose there may be a loss of embryo quality owing to ovarian stimulation after ovulation as a consequence of the prolonged action of PMSG. This may lead to the development of an unfavourable steroid environment for early embryo oviductal transport and development (Saumande, 1978; Boland et al., 1978). PMSG induced superovulation is followed by a second wave of ovarian follicular development (Booth et al., 1975; Betteridge, 1977; Dhondt and Bouters, 1976–1977; Renard et al., 1978; Mauleon et al., 1980; Dieleman and Bevers, 1987; Saeed et al., 1989), which is observed more frequently with higher doses of PMSG (Table 2) and is accompanied by high concentrations of oestradiol-17β in peripheral blood (e.g. see Bouters et al., 1983; Alfuraiji et al., 1990; Fig. 1(a)).

THE USE OF ANTI-PMSG

It became clear, therefore, that selective removal of PMSG from the peripheral blood of PMSG-stimulated cattle after the initial phase of multiple follicular stimulation but before the phase of post-ovulatory stimulation of

secondary follicles and the secondary rise of oestradiol-17β, may lead to an improved recovery of usable embryos. The availability of large quantities of well characterised PMSG-monoclonal antibody has led to a large number of experiments to test this possibility (see Table 4 for references). Neutralisation of PMSG with PMSG antiserum prevents the development of ovarian follicles and the secondary rise in oestradiol-17β in peripheral blood (Dieleman and Bevers, 1987; Alfuraiji et al., 1989, 1990; See also Table 3 and Fig. 1(b)).

Despite these findings, evidence that PMSG antiserum administration improves the number of usable embryos recoverable has been conflicting (Table 4). The weighted means for numbers of corpora lutea, total numbers of ova/embryos and the number of usable embryos from all the studies summarised in Table 4 are given in Table 5, indicating a clear balance of benefit in favour of PMSG antiserum treatment following the use of PMSG for superovulation.

The data in Table 4 indicate, however, a wide range of response from experiments with varying protocols, ages and types of cattle, doses of PMSG and potencies of PMSG antisera, which are usually not stated or checked for their ability to fully neutralise the PMSG administered to the animal. The critical difference in protocol, however, between trials has probably been the different timing, and means of timing, of the PMSG antisera injections. The best results, in terms of increased numbers of usable embryos, have been achieved by administering the PMSG antiserum 5–6 h after the preovulatory LH peak (Dieleman and Bevers, 1987; Dieleman et al., 1989). The evidence suggests that fixed chronological times between administration of the PMSG antiserum and the PMSG or synchronising prostaglandin analogue, may be unsatisfactory because of variability in the timing of its physiological effect. The interval between the prostaglandin injection and the LH surge, for example, varies from 36 to 72 h (Saumande, 1980). If PMSG antiserum is administered relative to behavioural oestrus, variations still occur because ovulation may be spread over 72 h from the onset of oestrus in superovulated cattle (Moncada Angel, 1980). Finally, some preparations of PMSG antiserum may cross-react with hypophysial gonadotrophins, and hence if given too early will interfere with the surge of ovulatory gonadotrophin (Saumande and Chupin, 1986). In superovulated cattle the oestradiol-17β preovulatory peak coincides with the LH peak (Lemon et al., 1975; Dieleman and Bevers, 1987). In our experiments on beef cows and heifers (Alfuraiji et al., 1990) we used doses of PMSG between 1000 and 4000 IU followed by equivalent doses of PMSG antiserum at different times following the prostaglandin analogue synchronisation injection. There were no apparent effects on embryo quality despite the elimination of secondary follicular development and the secondary oestradiol-17β rise. A retrospective recalculation of the data to examine the effect of antiserum when given at known times after the preovulatory oestradiol-17β peak shows that administration of PMSG antiserum ap-

proximately 8 h after the preovulatory oestradiol-17β peak not only eliminated secondary follicular development and the secondary oestradiol-17β rise, but also markedly increased the mean number of usable embryos from 4.4 to 12.3 (Table 6).

IMPLICATIONS AND CONCLUSIONS

The practical implications of an increase of this magnitude in yield of usable embryos are that embryos can be produced at a substantially lower cost and the variability of response has less practical importance. Increasing the average response means that it is easier to maintain the minimum desirable family size in genetic improvement schemes involving multiple ovulation and embryo transfer (MOET), such as those discussed by Smith (1988), even though the variability may remain high.

The data reviewed in Table 4 and summarised in Table 5 show a weighted mean increase of usable embryos of 51.6% following the use of PMSG antiserum which would reduce the cost per embryo by approximately one-third. The reductions in cost would be substantially greater if the yield of embryos was increased by the proportion achieved by Dieleman et al. (1989), as in our recalculated sub-set of data, provided the number of pregnancies increased by a similar amount. An econometric study by Slenning and Wheeler (1989) showed that a PMSG-induced superovulation was more cost-effective than an FSH-induced superovulation, but did not consider the effect of PMSG plus PMSG antiserum on costs. The major factors affecting the cost-effectiveness of the different superovulation treatments are the cost of the superovulatory drugs, the labour involved in their administration and the number of pregnancies achieved per superovulation. Superovulatory drugs cost approximately one-sixth (PMSG-induced) or one-third (PMSG plus PMSG antiserum-induced superovulation) that of an FSH-induced superovulation at UK prices. The labour involved in their administration is one-eighth and one-quarter, respectively, that required for FSH. The literature reviewed by Slenning and Wheeler (1989) showed that PMSG-induced superovulations achieved an average of 4.4 pregnancies compared with 3.9 pregnancies for FSH-induced superovulations.

These figures give some idea of the cost constraints within which the correct timing of administering PMSG antiserum must be achieved by detection of the LH or oestradiol-17β peak, which may involve blood sampling at 4 h intervals over a period of up to 36 h, or other means. When used in association with an economic and effective means of timing administration, PMSG-monoclonal antibody in conjunction with PMSG treatment could be a very reliable superovulatory treatment for obtaining large numbers of usable cattle embryos.

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