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Effects of monoclonal antibody against PMSG administered shortly after the preovulatory LH surge on time and number of ovulations in PMSG/PG-treated cows

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Summary. Normally cyclic heifers received 2500 i.u. PMSG i.m. at Day 10 of the oestrous cycle and 15 mg prostaglandin (PG) i.m. 48 h later. From 30 h after PG the LH concentration in the peripheral blood was estimated every hour using a rapid RIA method which allowed the LH concentration to be known within 4 h. Monoclonal antibody against PMSG was injected in the jugular vein of 29 heifers at 4·8 h after the maximum of the preovulatory LH peak; 28 heifers were not treated with anti-PMSG (controls). Peripheral blood concentrations of PMSG, LH, progesterone and oestradiol were compared. Ovaries were collected by ovariectomy at fixed times, 22–30 h after the LH peak, and numbers were counted of small (2–10 mm), large (> 10 mm) and ovulated follicles, and of follicles with a stigma.

In anti-PMSG-treated cows, the PMSG concentration fell sharply to non-detectable levels within 2 h of the treatment, indicating that PMSG was neutralized in these cows at the onset of final follicular maturation. In all cows, the concentration of oestradiol showed a significant decrease at about 8 h after the LH peak. After anti-PMSG treatment ovulations took place from 24 until 30 h after the LH peak, whereas in control cows follicles had already ovulated at or before 22 h and ovulations continued until 30 h. At 30 h 90% of the follicles had ovulated in anti-PMSG-treated cows *vs* 72% in the controls, resulting in 15 and 8 ovulations per cow respectively ($P < 0{\cdot}05$). Also, administration of monoclonal antibody against PMSG synchronized final follicular maturation and shortened the period of multiple ovulations.

In conclusion, neutralization of PMSG shortly after the preovulatory LH peak suppresses adverse effects of PMSG on final follicular maturation, leading to an almost 2-fold increase of the ovulation rate.

Introduction

Enhancement of the number of offspring of valuable cows by using superovulation procedures whether it be with pregnant mare serum gonadotrophin (PMSG) or with follicle-stimulating hormone (FSH), still shows considerable variability of the number of viable embryos at transfer. The interval between start of superovulation and recovery of the embryos can be roughly divided into two periods, i.e. the period of selection, growth and maturation of preovulatory follicles and that of early embryonic development lasting about 5 and 7 days respectively. Reduced and variable yield of transferable embryos may be due to disorders occurring at any stage of these two periods.

Superovulation with PMSG induces a second wave of follicles after ovulation, causing high concentrations of oestradiol in the peripheral blood during early embryonic development (Bouters *et al.*, 1983) which appears to be due to the long half-life of PMSG (about 5 days: Schams *et al.*, 1978; M. M. Bevers & S. J. Dieleman, unpublished observations). Administration of an antiserum

against PMSG at 60 h after the injection of prostaglandin (PG) increased the number of transferable embryos, indicating that the highly oestrogenic environment negatively affects early embryonic development (Bouters *et al.*, 1983). However, these authors also reported an increase of the number of corpora lutea observed at Day 7 after treatment with the anti-PMSG serum. This suggests that PMSG interferes with follicular development of which three stages can be distinguished: (1) primary stimulation between administration of PMSG and of PG, (2) selection and growth after PG before the endogenous peak of luteinizing hormone (LH), and (3) final maturation after the LH peak until ovulation. This supposition is supported by the observation that aberrations do occur in oocyte maturation (cow: Callesen *et al.*, 1986; Hyttel *et al.*, 1986; sheep: Moor *et al.*, 1985) and follicular fluid concentrations of steroids (Dieleman & Kruip, 1980; Fortune & Hansel, 1985) after PMSG/PG-stimulated superovulation.

It appears that PMSG is necessary until oestrus since administration of anti-PMSG serum before oestrus (i.e. during the first two stages of follicular development) results in regression of the stimulated follicle population (Dhondt *et al.*, 1978). This may also be inferred from the marked reduction of the number of embryos and corpora lutea when anti-PMSG serum is administered before oestrus (Bouters *et al.*, 1983). PMSG may therefore cause disorders during final follicular and oocyte maturation which is known to proceed according to a chronologically fixed hormonal sequence in normally cyclic cows (Dieleman *et al.*, 1983a, b; Kruip *et al.*, 1983; Dieleman & Blankenstein, 1984, 1985).

Therefore, we investigated whether monoclonal antibody against PMSG affects the ovulation process in the cow, when administered at the onset of final maturation initiated by the preovulatory LH peak.

Materials and Methods

Animals. The study was carried out from September to January with 64 Dutch-Friesian and Meuse-Rhine-Yssel heifers which were distributed at random to the experimental groups. After at least three observed normal oestrous cycles on pasture, the heifers were housed indoors in groups of 8 animals under conditions which ensured normal ovarian cyclicity; this housing and detection of oestrus were as described previously (Dieleman *et al.*, 1983b). Experiments were started after at least one normal cycle indoors as determined by oestrous behaviour and pattern of the progesterone concentration in the peripheral blood. All animals received 2500 i.u. PMSG i.m. (Folligon: Intervet, Boxmeer, The Netherlands) on Day 10 at 00:00 h, and 15 mg prostaglandin (PG) i.m. (Prosolvin: Intervet) on Day 12 at 00:00 h; this dose of PG is used to induce luteolysis in normally cyclic cows. Blood samples were collected by jugular puncture as described previously (Dieleman *et al.*, 1983b) once a day from Day 0 until Day 10, then every 4 h until 24 h after injection of PG, and thereafter at hourly intervals until ovariectomy or at least until 60 h after PG.

Treatment with anti-PMSG. Monoclonal antibody against PMSG, generously provided by Dr J. Th. Gielen (5 ml Neutra-PMSG; Intervet International B.V.), was used in a dose sufficient to suppress PMSG-induced development of follicles in cows after ovulation as determined by the absence of the second surge of the oestradiol concentration in the peripheral blood (Bouters *et al.*, 1983; Moyaert *et al.*, 1985; Dieleman *et al.*, 1987). The antibody against PMSG was injected in the jugular vein to 29 cows at 8 h after the last blood sample with a basal LH value. The LH surge was considered to occur when the LH concentration increased in three successive samples as determined by a rapid radioimmunoassay (RIA) which was performed every hour from 30 h after injection of PG. Therefore, the antibody against PMSG was administered shortly after the LH surge, since the mean duration of the LH surge is $8{\cdot}0 \pm 0{\cdot}2$ (s.e.m.) h in normally cyclic cows (Dieleman *et al.*, 1986). The control group consisted of 28 cows not treated with anti-PMSG. The other 7 cows were excluded from the experiments because the LH surge was not detected until 60 h after PG; in normally cyclic heifers the interval between onset of regression of the corpus luteum and onset of the LH surge is 58 h (Dieleman *et al.*, 1986).

Collection of ovaries. The ovaries of 57 animals were recovered by ovariectomy (Dieleman *et al.*, 1983b) at set intervals after the maximum of the LH surge, around the expected time of ovulation, i.e. at 22, 24, 26, 28 and 30 h after the LH peak respectively. Care was taken to obtain a balanced distribution of cows to the respective experimental groups with regard to the interval between PG and the LH peak, since this interval varies markedly (Saumande, 1980; Callesen *et al.*, 1986). After weighing of the ovaries the numbers of small (diameter 2–10 mm) and large (>10 mm) follicles, of follicles with a stigma and of ovulations were determined; small and large follicles were considered to be non-ovulatory and of preovulatory size respectively. Follicular fluid and tissues were collected for other studies on steroid-synthesizing ability and gonadotrophin-binding capacity.

Radioimmunoassay of progesterone, oestradiol, LH and PMSG. Concentrations of progesterone were estimated by a direct solid-phase ^{125}I RIA method (Cóat-A-Count TKPG; Diagnostic Products Corporation, Los Angeles, CA, U.S.A.) in 100 µl samples in duplicate according to the manufacturer. The main cross-reactivities were 2·4, 2·0, 1·7 and 1·3% for deoxycortisol, 20α-hydroxypregn-4-ene-3-one, deoxycorticosterone and 5β-pregnane-3,20-dione, respectively, and <1% for other steroids tested, according to the manufacturer. The sensitivity was 0·15 nmol/l and the interassay coefficient of variation was 11% (n = 16). A correlation of r = 0·97 (P<0·001) was observed for the comparison of the progesterone concentrations throughout the oestrous cycle of 5 cows as estimated by this RIA method with those as estimated by the method described previously (Dieleman & Schoenmakers, 1979; Dieleman *et al.*, 1986).

Concentrations of oestradiol-17β were estimated by a solid-phase ^{125}I RIA method (Coat-A-Count TKE; Diagnostic Products Corporation) according to the manufacturer with slight modifications. Plasma samples of 1·0 ml were extracted once with 2 ml freshly opened diethyl ether (Analar quality; BDH, Poole, U.K.). After evaporation of the solvent under a nitrogen stream, the residue was dissolved in 250 µl 0·02 M-borate buffer (pH 8·5) in 0·9% (w/v) NaCl) by sonification during 5 min, and 100 µl in duplicate were assayed. The recovery was established by extracting 5 parallel samples to which 10 000 d.p.m. tritiated oestradiol had been added (mean recovery: 85·1 ± 2·2 (s.d.)% for 8 assays). A standard curve of 3·7–231 fmol was used. The main cross-reactivities were 1·1, 0·32 and 0·16% for oestrone, oestriol and oestradiol-17α respectively, and < 0·01 for other steroids tested according to the manufacturer. The sensitivity was 7·5 pmol/l and the interassay coefficient of variation was 8·9% (n = 8). A correlation coefficient of r = 0·98 (P < 0·001) was obtained for the comparison of the oestradiol concentrations (n = 50) as estimated by this RIA method with those estimated by the method described previously (Dieleman *et al.*, 1986).

Concentrations of LH were estimated in triplicate by a rapid solid-phase RIA method using the bovine preparation bLH-7981, for iodination and standard curve, and the antiserum of the standard (long) assay (Dieleman *et al.*, 1983a). Briefly, 800 µl 0·01 M-sodium phosphate-buffered saline (PBS; pH 7·0) containing 1% (w/v) bovine serum albumin (BSA; Sigma Chemical Company, St Louis, MO, U.S.A.) and 0·05% (w/v) sodium azide, and 100 µl iodinated bLH-7981 were added to 100 µl samples and standards in antiserum-coated tubes. After incubation for 3 h at 37°C in a closed water bath the liquid phase was thoroughly removed from the tubes which were then counted for 30 sec in a four-detector gamma counter (Multi-Prias; Packard Instruments Company, Downers Grove, IL, U.S.A.). The polystyrene tubes (LP/3; Luckham Ltd, Burgess Hill, Sussex, U.K.) were coated for at least 4 h at room temperature with 1 ml antiserum diluted 1:10 000 in 0·05 M-sodium bicarbonate buffer (pH 9·0), and washed twice with the 1% BSA-PBS buffer; the tubes were stored at −25°C in sealed plastic bags until RIA. This rapid RIA method allowed the LH concentration to be known within 4 h after taking a blood sample. The LH concentration of blood samples during the LH surge was also estimated afterwards by the standard assay for all animals used, to validate the precision of the determination of the time when the maximum of the LH surge occurred. Comparison of the intervals between the LH peak and the time of ovariectomy as determined by the rapid and the standard assay produced the regression equation $y = 0{\cdot}97x + 0{\cdot}75$ (r = 0·96; P < 0·001).

All concentrations of PMSG were estimated in duplicate in one assay with an homologous double-antibody RIA. Before RIA, samples of cows treated with anti-PMSG were incubated overnight at room temperature with donkey anti-mouse antibody-coated cellulose suspension (0·5 ml plasma with 0·2 ml Sac-Cel; Wellcome Diagnostics, Dartford, U.K.) and then centrifuged for 15 min at 4°C at 4800 ***g*** to remove monoclonal antibody against PMSG and its complexes with PMSG. For the RIA method antiserum against PMSG raised in sheep (generously provided by the late Professor Dr R. A. Bouters, Veterinary Faculty, University of Ghent, Belgium) was used in a final dilution of 1: 160 000. Highly purified PMSG (PMSG PM23-2P; a generous gift of Intervet International B.V.) was used as standard and for iodination. The cross-reactivity was <0·1% for bovine NIH-LH-B4 and the purified bFSH preparation of Cheng (1978). The lowest detectable amount of PMSG was 0·3 µg/l plasma; samples with a non-detectable concentration were arbitrarily assigned a value of 0·3 µg/l. The intra-assay coefficient of variation was 7·1% (n = 12) and the recovery was 97·1 ± 2·7% (n = 10).

Statistical analysis. Correlation was calculated according to the method of least squares regression (Snedecor & Cochran, 1980). Student's t test or when appropriate the paired t test was used to compare the means of two samples; the sign test was used when there was no quantitative scale of measurement (Snedecor & Cochran, 1980). Differences between the means of samples in grouped data were tested for significance by analysis of variance according to Scheffé (1959).

Results

Hormone concentrations in peripheral blood

The monoclonal antibody against PMSG was administered at 4·8 ± 0·2 (s.e.m.) h (n = 29) after the maximum of the preovulatory LH peak, i.e. at the time when the LH concentration had returned to basal values. The pattern of the PMSG concentration (Fig. 1) showed a marked decrease of about 85% within 1 h after administration of the anti-PMSG; at the time, in about half of the cows, the PMSG concentration was already less than or equal to the detection limit of the RIA method.

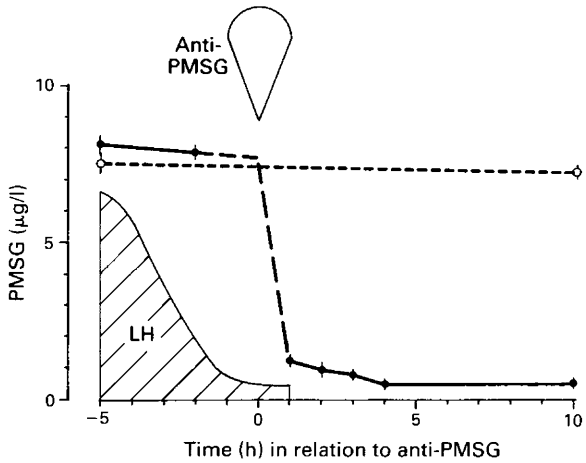

Fig. 1. PMSG concentrations in the peripheral blood of cows before and after injection i.v. of monoclonal antibody against PMSG (●; N = 29), and in control cows (○; N = 28) during PMSG/PG-induced superovulation.

Table 1. Progesterone concentrations (nmol/l) in peripheral blood of cows during PMSG/PG-induced superovulation with or without antibody against PMSG

	Time relative to injection of prostaglandin (h)										
	−65	−41	−29	−17	−5	+3	+11	+19	+31	+43	+55
Controls (N = 28)	14·2 ± 0·9	20·4 ± 0·9	21·5 ± 1·3	24·8 ± 1·1	25·9 ± 1·5	18·4 ± 1·5	7·66 ± 0·79	3·18 ± 0·25	1·78 ± 0·19	1·18 ± 0·10	1·02 ± 0·13
Anti-PMSG treated (N = 29)	15·5 ± 1·1	20·1 ± 0·9	23·4 ± 1·5	24·8 ± 1·1	25·9 ± 1·2	16·7 ± 0·8	7·03 ± 0·48	3·24 ± 0·25	2·03 ± 0·17	1·34 ± 0·13	1·08 ± 0·17

Values are mean ± s.e.m.

No differences were observed between the patterns of the progesterone concentration of control and anti-PMSG-treated cows (Table 1). The progesterone concentration showed an increase after PMSG injection, a decrease to a value $<3{\cdot}2$ nmol/l within 20 h after PG injection and it was $<1{\cdot}5$ nmol/l at the time of the LH peak. The pattern of the oestradiol concentration (Fig. 2) showed a maximum at the time of the LH peak and a significant ($P<0{\cdot}01$) decrease at 8 h after the LH peak; no differences were observed between the control and anti-PMSG-treated cows in this respect.

The mean maximum concentration of the LH peak was not significantly different for control and anti-PMSG-treated cows, $13{\cdot}7 \pm 1{\cdot}0$ ($n = 28$) and $15{\cdot}3 \pm 1{\cdot}0$ ($n = 29$) μg/l respectively. Also, no significant differences were observed for the mean interval between PG and the LH peak (Table 2). However, a significant ($P < 0{\cdot}05$) inverse correlation was found between this interval and the sum of the numbers of large follicles and ovulations (Fig. 3) for control and anti-PMSG-treated cows (regression equations: $y = -0{\cdot}43x + 34{\cdot}3$ ($r = 0{\cdot}40$) and $y = -0{\cdot}70x + 45{\cdot}5$ ($r = 0{\cdot}46$), respectively); the sum is considered to represent the number of preovulatory follicles before ovulations occurred.

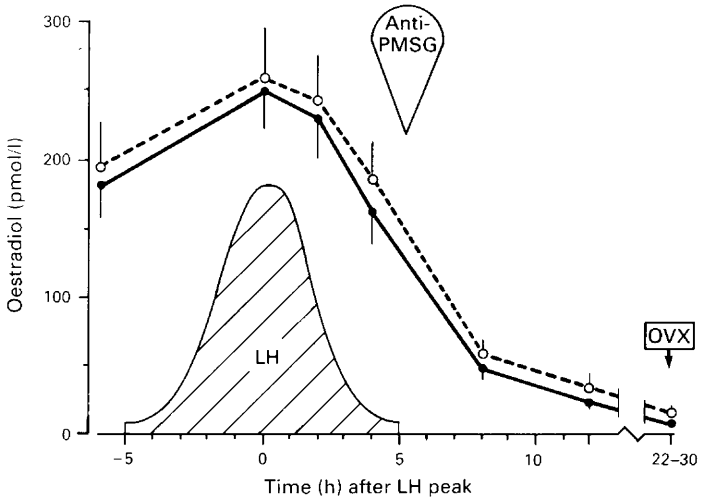

Fig. 2. Oestradiol concentrations in the peripheral blood of cows before and after the LH peak until ovariectomy (OVX) after PMSG/PG-induced superovulation with (●; N = 29) or without (○; N = 28) treatment with antibody against PMSG.

Table 2. Intervals between the injection of prostaglandin and the occurrence of the maximum of the LH surge in cows during PMSG/PG-induced superovulation with or without antibody against PMSG

	Time of ovariectomy after LH (h)				
	22	24	26	28	30
Controls	46·0 ± 7·8 (3)	45·0 ± 2·8 (7)	41·1 ± 1·3 (7)	45·2 ± 4·4 (6)	43·8 ± 3·4 (5)
Anti-PMSG treated	45·7 ± 3·9 (3)	45·1 ± 2·5 (8)	41·6 ± 1·6 (7)	42·0 ± 1·3 (6)	39·2 ± 2·1 (5)

Values are mean ± s.e.m. for the no. of cows in parentheses.

Ovarian morphology

The mean weights (g) of the left and right ovary were 13·9 ± 1·4 and 16·1 ± 1·5 for control cows (n = 28), and 13·9 ± 1·3 and 16·1 ± 1·5 for anti-PMSG-treated (n = 29) cows, respectively (differences non-significant). The regressed corpus luteum was found to be present significantly ($P < 0·025$) more frequently in the right ovary (for 36 out of 54 cows) than in the left; in 3 cows both ovaries had a regressed corpus luteum. Also, the mean number of small non-ovulatory follicles (60% of this number 2–5 mm and 40% 5–8 mm) was significantly ($P < 0·001$) higher in the right ovary than that in the left, i.e. 8·9 ± 1·1 and 1·8 ± 0·5 for control cows (N = 28), and 9·4 ± 1·1 and 1·9 ± 0·4 for anti-PMSG-treated cows (N = 29), respectively. This apparent preference for the right ovary, however, was not reflected in a difference between the number of preovulatory follicles before ovulation. The sum of the numbers of large follicles and ovulations was 8·0 ± 0·6 and 7·6 ± 0·6 (n = 57) for the right and left ovaries respectively. The sum of large follicles and ovulations in anti-PMSG-treated cows was not significantly different from that in control cows (15·7 ± 2·5) (N = 29) and 15·4 ± 1·6 (N = 28) respectively); its mean per experimental group (Table 3) also did not show significant differences.

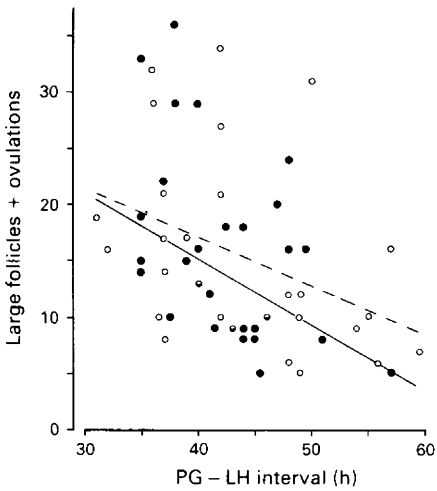

Fig. 3. Variation of the sum of the numbers of large follicles and ovulations with the interval between injection of PG and the maximum of the LH peak in cows after PMSG/PG-superovulation with (●, complete regression line; N = 29) or without (○, broken regression line; N = 28) treatment with antibody against PMSG.

Table 3. Sum of the numbers of large follicles and ovulations for the experimental groups after PMSG/PG-induced superovulation with or without antibody against PMSG

	Time of ovariectomy after LH (h)				
	22	24	26	28	30
Controls	22·0 ± 4·6 (3)	11·9 ± 1·8 (7)	20·1 ± 4·2 (7)	14·0 ± 3·3 (6)	11·6 ± 2·7 (5)
Anti-PMSG treated	20·0 ± 8·3 (3)	15·6 ± 3·4 (8)	13·6 ± 1·9 (7)	15·3 ± 3·1 (6)	16·8 ± 3·9 (5)

Values are mean ± s.e.m. for the no. of cows in parentheses.

At 22 h after the LH peak a proportion (12·2%) of the follicles in control cows showed signs of the ovulation process, such as stigmata and ovulations, whereas almost no such signs were visible for follicles in anti-PMSG-treated cows at that time (Fig. 4). At 2 h later 40·5% of the follicles in anti-PMSG-treated cows had formed a stigma but only a few had ovulated, and after a further 2 h, at 26 h after the LH peak, 47% of this follicle population showed a stigma and 37·1% had ovulated. In control cows at corresponding times, the proportion of follicles with a stigma was less but not significantly so, and the percentage of ovulations was similar to that in anti-PMSG-treated cows. Finally, at 30 h after the LH peak the ovulation process appeared to be completed and a significantly ($P < 0·05$) higher percentage of the follicles had ovulated after neutralization of the PMSG with antibody against PMSG (Fig. 4). In control cows, ovulations had already taken place at 22 h after the LH peak and the number of ovulations per cow (Fig. 5) reached a maximum of about 8 at 26 h to remain at the same level until 30 h. However, in anti-PMSG-treated cows some ovulations were observed at 24 h after the LH peak after which the number increased almost linearly during the following 2 h periods to reach a maximum of 15 ovulations per cow at 30 h after the LH peak (Fig. 5).

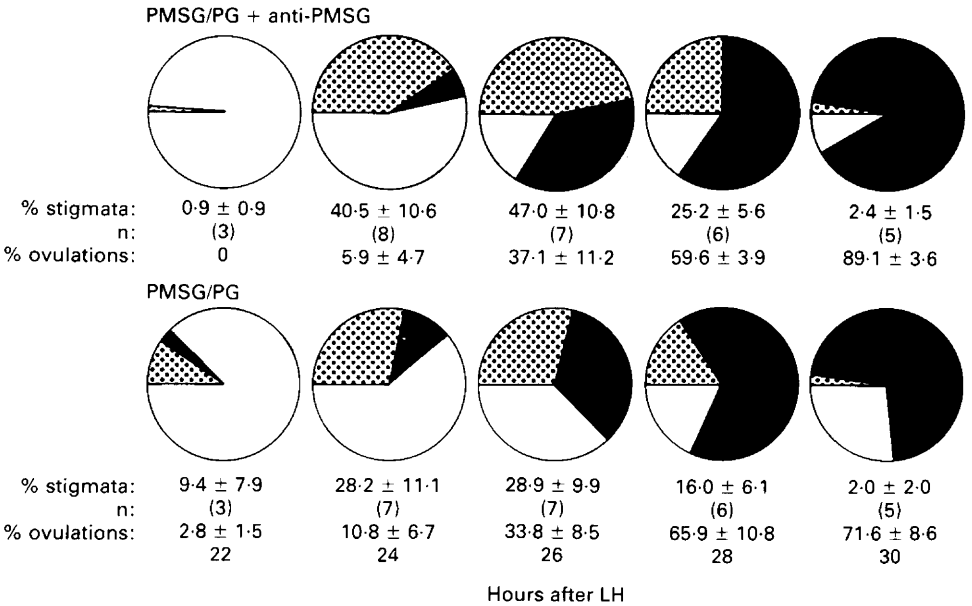

Fig. 4. Pie-charts of the mean numbers of large follicles without (blank segments) or with (stippled segments) a stigma and of ovulations (black segments) relative to the sum of the numbers of large follicles and ovulations in cows at 22–30 h after the LH peak during PMSG/PG-induced superovulation with (1st row) or without (2nd row) antibody against PMSG. Values are ± s.e.m. for the number of cows in parentheses.

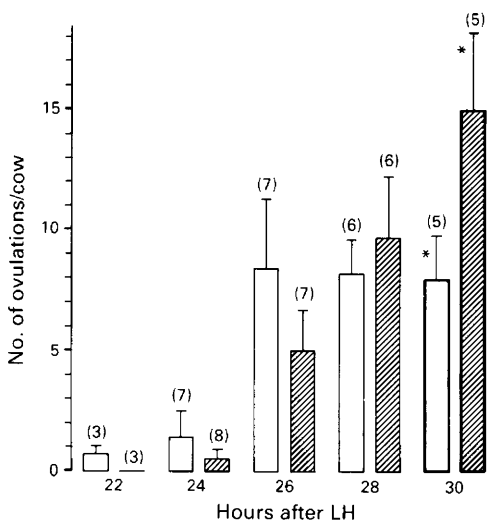

Fig. 5. The number of ovulations (± s.e.m. for the no. of cows in parentheses) per cow at 22–30 h after the preovulatory LH peak during PMSG/PG-induced superovulation without (blank columns) or with (hatched columns) monoclonal antibody against PMSG. $*P < 0{\cdot}05$.

Discussion

Injection of 5 ml monoclonal antibody against PMSG into the jugular vein neutralized circulating PMSG almost immediately in cows which had received 2500 i.u. PMSG and 15 mg PG about 96 and 48 h before, respectively. The concentration of PMSG decreased to 10% of its original value within 2 h after administration of the anti-PMSG and was non-detectable in two-thirds of the cows at the time. This strongly indicates that the monoclonal antibody against PMSG can be used to investigate adverse effects of PMSG on the final maturation stage of preovulatory follicles between the endogenous LH peak and ovulation. The use of a rapid LH RIA method allowed the neutralization of PMSG to be accomplished at an endocrinologically defined time, i.e. immediately after the preovulatory LH surge.

The patterns of the concentrations in the peripheral blood of progesterone, oestradiol and LH were similar for control and anti-PMSG-treated cows, and are generally in agreement with the patterns reported by Saumande (1980) for PMSG/PG-stimulated cows. The oestradiol concentration showed a decrease after the LH peak and conforms to that observed in normally cyclic cows (Dieleman *et al.*, 1986). This indicates that oestradiol production was inhibited in follicles stimulated by PMSG in a way similar to that in normal preovulatory follicles (Dieleman & Bevers, 1987). It may also be assumed that PMSG does not interfere with this aspect of final follicular maturation, since the oestradiol patterns were the same in control and anti-PMSG-treated cows.

The number of preovulatory follicles was significantly correlated with the duration of the interval between the injection of PG and the LH peak, indicating that the LH peak occurs earlier when a larger number of follicles has been induced by PMSG. The higher oestradiol concentration as a result of a larger number of follicles (M. M. Bevers & S. J. Dieleman, unpublished observations) possibly increases pituitary sensitivity to LH-releasing hormone (Kesner *et al.*, 1981) more rapidly. However, potential effects of administered antibody against PMSG cannot be attributed to accidentally introduced differences between control and treated cows, since the variation of the PG–LH interval was evenly distributed in the respective experimental groups. Although a marked preference for the right ovary was observed with regard to the presence of the regressed corpus luteum and non-ovulatory follicles, this obviously also did not influence the results. Moreover, the number of preovulatory follicles was similar for left and right ovaries, indicating that intraovarian regulatory mechanisms present in normally cyclic cows (Moor *et al.*, 1984) are suppressed after PMSG stimulation.

In general, PMSG-stimulated ovulation appeared to be delayed for some hours in comparison with ovulation in normally cyclic cows in which it takes place at about 24 h after the preovulatory LH peak (Dieleman *et al.*, 1983b). In the control and anti-PMSG-treated cows ovulations apparently were terminated at 30 h after the LH peak. At that time almost no more follicles with a stigma were present and about 75% of the remaining large follicles were atretic (unpublished data). Formation of stigmata probably preceded ovulation by about 2 h as indicated by the proportions of follicles with a stigma and those ovulated at 24, 26, 28 and 30 h after the LH peak in anti-PMSG-treated cows. For instance, at 24 h 40·5% of the follicles showed a stigma and only 5·9% had ovulated, 2 h later 37·1% of the follicles had ovulated and 47·0% were with stigma; from 26 h the sum of the proportions of follicles with a stigma and those ovulated remained constant until 30 h after the LH peak. This pattern was less clear in control cows which suggests that the presence of PMSG during final maturation causes disorder in the timing of ovulation. Moreover, the times of ovulation were more scattered in control cows in which it can be assumed that ovulation had already occurred by 22 h after the LH peak, whereas ovulations started at 24 h in anti-PMSG-treated cows.

The proportion of follicles which had ovulated concurred with the almost linear increase of the number of ovulations per cow when PMSG had been neutralized. Contrary to this, in control cows the continuous increase of the proportion of ovulated follicles was not reflected in the pattern of the number of ovulations per cow at the respective times after the LH peak, since this number reached

a plateau at 26 h. The PMSG probably causes disorders during final follicular maturation, which prevent follicles from acquiring characteristics necessary for ovulation. This concept is supported by the occurrence of a significantly higher proportion of ovulated follicles and number of ovulations per cow when PMSG had been neutralized, while the numbers of preovulatory follicles were not significantly different for the respective experimental groups.

Effects on the number per cow of corpora lutea and/or transferable embryos at Day 7 have been investigated previously using polyclonal anti-PMSG sera raised in turkeys (Dhondt *et al.*, 1978), goats (Kummer *et al.*, 1980) and sheep (e.g. Bouters *et al.*, 1983). These reports can only be broadly compared with the results presented here, since the anti-PMSG sera have been administered at fixed times after PG or signs of oestrus regardless of the time of occurrence of the LH peak; the interval between PG and the LH peak is known to vary considerably (Saumande, 1980; this study: 31–59 h) and the LH peak is absent in about 15% of PMSG-superovulated cows (Saumande, 1980; M. M. Bevers & S. J. Dieleman, unpublished observations). The observed increase of the number of ovulated follicles from 8 to 15 per cow upon treatment with the monoclonal antibody against PMSG is consistent with similar increases of the number of corpora lutea counted upon slaughter at Day 7 as reported by Dhondt *et al.* (1978), Bouters *et al.* (1983) and Wang *et al.* (1987); a slight non-significant increase of the number of corpora lutea was observed by Kummer *et al.* (1980). Saumande *et al.* (1984) found no increase as established by rectal palpation at Day 7 or 8; the anti-PMSG serum, however, was injected at 12 or 24 h after the onset of oestrus which may have been at a time when final follicular maturation could no longer be affected, since the LH peak, followed by ovulation 24 h later, occurs shortly after onset of oestrus in normally cyclic cows (Dieleman *et al.*, 1986). Kim *et al.* (1987) observed 12 corpora lutea per cow after anti-PMSG treatment which was higher than that found after superovulation stimulated by FSH.

It is concluded that neutralization of PMSG shortly after the preovulatory LH peak synchronizes final follicular maturation and shortens the period in which multiple ovulations take place. Thus, adverse effects of still circulating PMSG after PMSG/PG-stimulated superovulation in the cow may be suppressed, resulting in a 2-fold increase of the ovulation rate.

With this paper we remember the late Professor Dr R. A. Bouters who with his colleagues initially developed antisera against PMSG to enhance embryo production. Folligon and Prosolvin were generously supplied by Intervet International B.V., Boxmeer, The Netherlands; bovine pituitary hormones by the pituitary hormone distribution program of the NIAMDD, Bethesda, U.S.A. We thank Dr P. Fontijne and Dr G. C. van der Weyden and assistants for the surgery; Mrs D. M. Blankenstein, Mrs H. T. M. van Tol and Mr A. V. P. van de Poll for round-the-clock technical assistance; Mr S. H. J. Mook and co-workers for tending the animals; and Mr W. Bes for drawing the figures. This study was supported by a grant from Intervet International B.V.

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