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PLASMA PROGESTERONE ASSAYS IN SUPEROVULATED CATTLE

By

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SOLTI, L., T. GREVE and H. H. KOEFOED-JOHNSEN: *Plasma progesterone assays in superovulated cattle.* Acta vet. scand. 1978, *19*, 298—309. — Plasma progesterone was measured in 14 normally cycling heifers and cows subjected to non-surgical recoveries of embryos. A radioimmunoassay (RIA) method was used for progesterone determination. The average progesterone concentration increased from 7.5 to 11.6 ng/ml in 8 of the animals following treatment with PMSG on day 8—12. Six animals had a decrease from 5.0 ± 2.1 to 3.9 ± 2.5 ng/ml. The overall increase was from 6.4 ± 2.7 ng/ml to 8.3 ± 4.8 ng/ml. Prostaglandin $F_{2\alpha}$-analogue (cloprostenol) treatment resulted in a sharp decrease in plasma progesterone followed by a rapid increase to an average of 46.8 ng/ml on day 16. A high degree of variability in this peak value was observed, and it was not correlated with the number of corpora lutea. The superovulatory cycle was generally prolonged. The heat following the superovulatory treatment was silent, and a typical ovarian resting period was observed during which the progesterone concentration remained low and the ovaries small.

superovulation; plasma progesterone; cattle.

One of the most important steps in the embryo transplantation procedure is the superovulation of the donor animal. Several methods have been described recently (*Hill et al.* 1973, 1976, *Elsden et al.* 1974, *Moore* 1975, *Newcomb et al.* 1975, *Sreenan & Beehan* 1975 and others), and due to its relatively low price and good effect pregnant mare serum gonadotropin (PMSG) has been the most commonly used agent for induction of multiple ovulations in cows. It is administered in doses from 1500 to 3000 i.u. i.m., and the superovulatory treatment is initiated either on day 15—16 of the oestrus cycle followed by injection of human

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chorion gonadotropin (HCG) on day 19—20, or on day 8—12 of the cycle followed by administration 48 hrs. later of a luteolytic agent (Prostaglandin $F_{2\alpha}$ or an analogue). In terms of number of ovulations the latter (PMSG/$PGF_{2\alpha}$) regimen has appeared to be superior to the day 15—16 administration (*Elsden et al., Philippo & Rowson* 1975), conceivably because the use of a luteolytic agent can control the PMSG-oestrous interval in a more exact way. This interval is supposed to be important for the superovulatory response and must be 4 or 5 days (*Newcomb* 1976). The superovulatory response to a given PMSG stimulation is extremely variable, and the individual constitution of the animal supercedes most other causes of variation, e.g. season, breed, nutritional, lactational or hormonal status, age, and batch of the drug used (*Greve* 1976, 1977, *Betteridge* 1977).

According to the literature the plasma steroid levels will be altered following the superovulatory treatment (*Lemon & Saumande* 1972, *Henricks & Lamond* 1972, *Henricks et al.* 1973, *Spilman et al.* 1973, *Booth et al.* 1975, *Nancarrow & Miller* 1975, *Agthe et al.* 1976, *Fournier et al.* 1976, *Rajamahendran et al.* 1976), and progesterone concentration subsequent to induced multiple ovulations often reaches extremely high levels. Some authors could correlate the level of progesterone with the number of corpora lutea (*Agthe et al.*), while others did not find such clear relationship (*Lamond & Gaddy* 1972, *Rajamahendran et al., Pedersen* 1977). The extremely high progesterone levels were found to prolong the oestrus cycle in certain cows (*Spilman et al., Booth et al.*) and could be terminated by administration of $PGF_{2\alpha}$ or a synthetic $PGF_{2\alpha}$-analogue.

It was the aim in this experiment to evaluate the changes in plasma progesterone in animals used as donors in non-surgical embryo tranplantation work.

MATERIALS AND METHODS

Fourteen normally cycling cows and heifers of various breeds (Holstein-Friesian, Red Danish Milk Breed and Jersey) were examined from a total of 55 animals used as donors during the last 2 years. The donor animals were given 2000—3000 i.u. PMSG* i.m. on day 8—12 (day 0) of their oestrus cycle followed 48 hrs. later by a single injection of $PGF_{2\alpha}$-analogue (500 µg

* Antex® — Leo Pharmaceuticals, Denmark.

cloprostenol*). Heat occurred 48 hrs. later and the animals were inseminated twice, 18—24 and 36—48 hrs. after the onset of standing heat. Six to 8 days later non-surgical collection of embryos was performed according to the method described by *Greve et al.* (1977).

Blood samples from the experimental animals were collected from the jugular vein into heparinized tubes before PMSG administration, on the day of prostaglandin administration, at the time of heat and then daily or every alternate day. The plasma samples were stored at —20°C until assayed. The concentration of plasma progesterone was measured by radioimmunoassay (RIA) using a method described by *Hoffmann et al.* (1973) and slightly modified by *Koefoed-Johnsen* (1976) and *Pedersen* (1976). The procedure consisted of the following steps: 50—500 µl plasma was pipetted into glass scintillation vials fitted with screw lids, and 6 ml petroleum ether (Mallinckrodt, Nanograde) was added. Extraction was carried out by mechanical shaking at room temperature for 20 min. The aqueous layer was then frozen out and the supernatant petroleum ether decanted into glass reaction tubes. The extraction procedure was repeated and the pooled supernatants were evaporated overnight in a vacuum drying oven at approx. 40°C. Progesterone standards were prepared and evaporated, and standards as well as samples were dissolved in 0.1 ml phosphate buffer (0.1 M, pH 7.2) containing 0.005 M sodium azide. To each tube was added 0.1 ml labelled progesterone dissolved in the same buffer and containing 23 nCi and 70 pg ^{3}H-progesterone (1,2,6,7-^{3}H-progesterone, New England Nuclear, spec. act. 104 Ci/mmol) and 0.5 ml antiserum diluted 1:10,000 in phosphate buffer with 0.1 % bovine serum albumin. The antiserum** was raised in rabbits against 11-OH-progesterone-hemisuccinate-bovine serum albumin. After mixing and incubation at 37°C for 15 min. interrupted by a further mixing, the samples were placed in an ice-water bath for at least 1 hr. Then 0.5 ml ice-cold constantly stirred charcoal-dextran suspension (0.2 % Norit A, Serva and 0.02 % Dextran T 70, Pharmacia Fine Chemicals) was pipetted into the tubes which were shaken en bloc by hand for exactly 1 min., replaced in the ice-water for

* Estrumate® — ICI. Kindly supplied by Mr. Jørgen Frederiksen, ICI-Pharma, Copenhagen, Denmark.

** Kindly supplied by Dr. B. Hoffmann, Technische Universität, München, BRD.

another 5 min. and finally centrifuged for 20 min. at 4000 r.p.m. in a refrigerated centrifuge at 2°C. From the supernatant (bound fraction) 0.8 ml aliquots were transferred to counting vials; 6 ml scintillation medium (Ready Solve VI HP, Beckman) was added, and after mixing the samples were counted in a Beckman LS-3150T liquid scintillation spectrometer to a preset error of 2 % or for 5 min., whichever of the 2 was first reached.

The accuracy or reliability of the RIA method was tested by *Pedersen* (1976). By adding crystalline progesterone to plasma of an ovariectomized heifer he found 83 % recovery of this progesterone. The repeatability or precision at replicate measurements was tested in the same study for 3 plasma pools and was found acceptable. Approx. 0.1 ng/ml plasma was found to be a reasonable detection rate. Hence the variation in progesterone concentration between animals does not originate from methodological errors.

Previous data from this laboratory concerning the progesterone pattern of normally cycling milking cows and heifers *(Pedersen* 1977) were in good agreement with those described in the literature (*Stabenfeldt et al.* 1969, *Edqvist et al.* 1970, *Hoffmann & Karg* 1970, *Hoffmann et al.* and others), and it was not considered necessary to set up a control group in order to establish a "typical progesterone profile" in this study.

RESULTS AND DISCUSSION

The progesterone profile from a non-superovulated, normally cycling heifer is shown in Fig. 1. The progesterone patterns of the superovulated animals were substantially different. The progesterone level rose after PMSG administration from 6.4 ± 2.7 ng/ml to 8.3 ± 4.8 ng/ml (Table 1). In 8 out of 14 animals the progesterone level increased from an average of 7.5 ± 2.8 ng/ml to an average of 11.6 ± 3.1 ng/ml. The remaining 6 animals had a decrease from 5.0 ± 2.1 to 3.9 ± 2.5 ng/ml. Formation of luteal tissue in the follicles and enhanced adrenal secretion of progesterone may be responsible for the observed cases of increase which have been shown to occur within 8—14 hrs. in rats (*Sashida & Johnson* 1976). The reason why the remaining 6 animals failed to exhibit a similar change in progesterone profiles is uncertain; however, biological variation among animals in the pattern of response of the target organs following gonadotropin administration may exist.

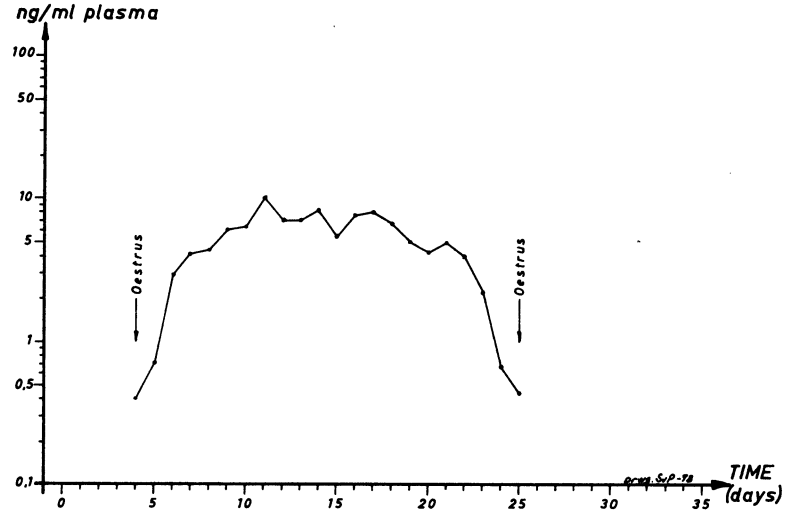

Figure 1. Plasma progesterone in a normally cycling heifer. (Semilog. scale).

Table 1. Peripheral plasma progesterone concentrations following PMSG administration.

Animal No.	Plasma progesterone concentration	
	before PMSG administration (ng/ml)	before prostaglandin administration (ng/ml)
27	6.4	5.8
45	2.6	1.0
101	3.5	2.0
102	6.0	10.4
103	11.2	13.5
104	7.4	15.0
105	11.8	14.2
106	6.4	11.8
108	7.0	12.7
123	6.3	10.0
127	3.7	5.1
192	5.1	4.1
193	8.3	7.7
200	4.3	2.8
Mean ± s	6.4 ± 2.7	8.3 ± 4.8

The cloprostenol injection 2 days after the PMSG treatment caused a sharp reduction in the plasma progesterone level (Figs. 2—5), and the animals showed clinical signs of heat within 2—3 days as described by *Booth et al.* (1975).

The heifers and cows that superovulated successfully had a rapid increase in plasma progesterone (Figs. 2, 3, 5) and an

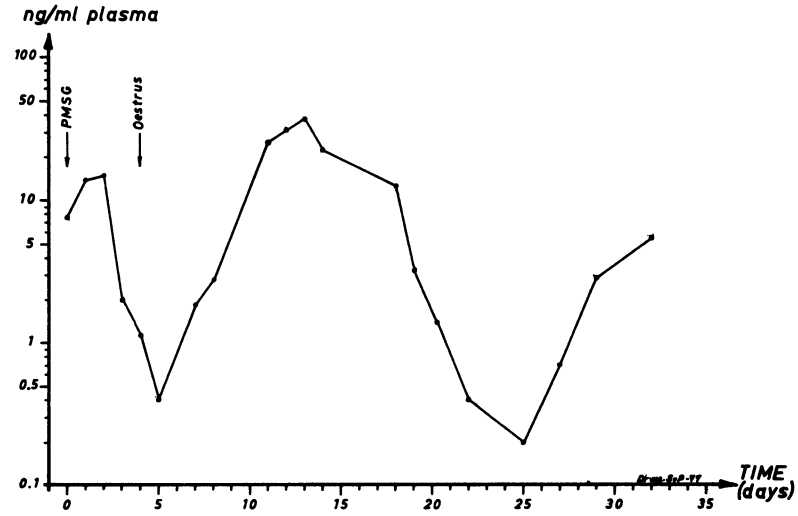

Figure 2. Plasma progesterone in a superovulated heifer that experienced an increase following PMSG administration. The length of the cycle was normal. (Semilog. scale).

average peak value of 46.8 ng/ml. The highest peak measured in these animals was 120 ng/ml at day 16 of the cycle (Fig. 3, A). This heifer, however, developed only 9 corpora lutea, while another animal with 13 corpora lutea had a plasma progesterone peak of only 8.6 ng/ml. From the present study it cannot be concluded that there was a direct correlation between the number of corpora lutea and the actual progesterone level. However, the elevated progesterone levels might be due to corpora lutea having been formed both by ovulated and non-ovulated luteinized follicles (*Booth et al.*), and obviously there must be some relationship between the high level of hormone and the enhanced luteal function after superovulatory treatment (*Lamond & Gaddy* 1972, *Spilman et al.* 1973, *Agthe et al.* 1976). It was also anticipated that there might be a correlation between the plasma progesterone level at the time of the PMSG administration and

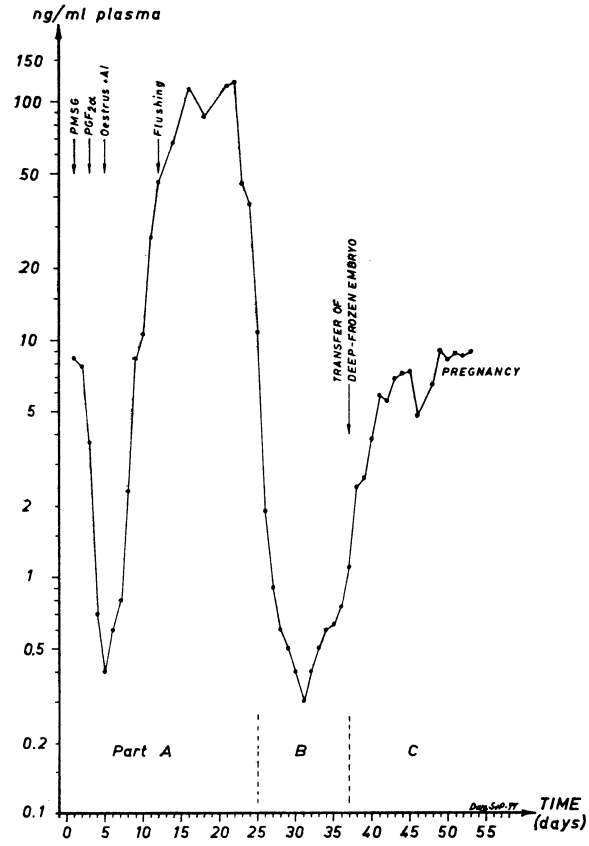

Figure 3. Plasma progesterone in a heifer that assumed very high values and had a prolonged oestrus cycle. Transfer of a deep-frozen egg resulted in normal pregnancy. (Semilog. scale).

the subsequent number of corpora lutea, but this could not be substantiated.

The increased luteal activity decreased spontaneously, and most of the animals had progesterone values below 1 ng/ml within 17—24 days after heat (Figs. 2, 3,A+B, 5), although the majority did not exhibit external signs of heat. Low progesterone profiles continued in some animals as long as 8—16 days during which period neither active corpora lutea nor Graafian follicles could be palpated by rectal examination. This anoestrus syndrome after superovulatory treatment indicating an ovarian "resting period" was succeeded by resumption of normal ovarian

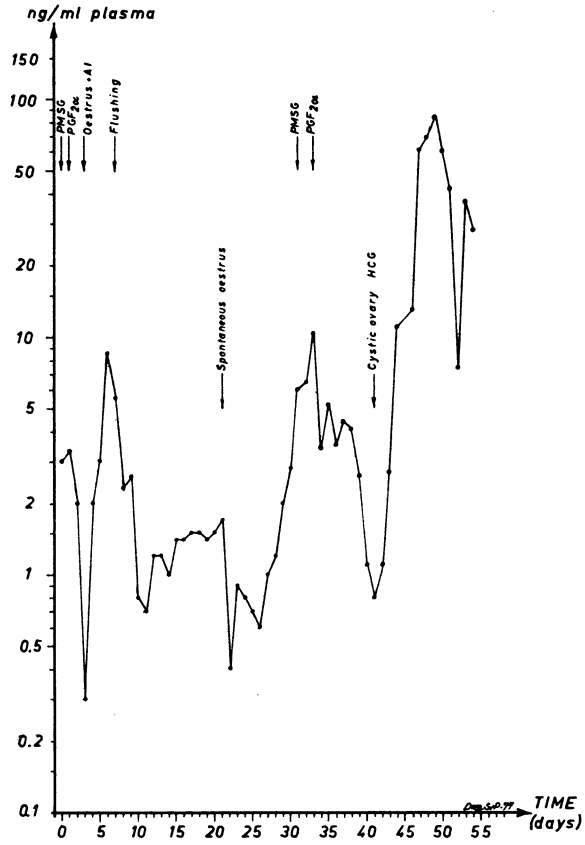

Figure 4. Plasma progesterone in a heifer that did not experience high peak values subsequent to the first superovulation. A second stimulation lead to formation of several anovulatory follicles (cysts) in both ovaries. (Semilog. scale).

and luteal activity (Figs. 3B, 4). The reason for this exhaustion is not known, but may be due to a temporary inhibition or blocking of progesterone receptors, and it certainly would discourage renewed superovulation in the subsequent cycle.

Having used 1 of these heifers as recipient she became pregnant following the surgical transfer of a deep-frozen fertilized egg (*Lehn-Jensen & Greve* 1977). Progesterone pattern of this animal is shown in Fig. 3C.

Only 3 animals developed cysts after superovulation. In 1 heifer, however, a second PMSG/$PGF_{2\alpha}$ treatment resulted in

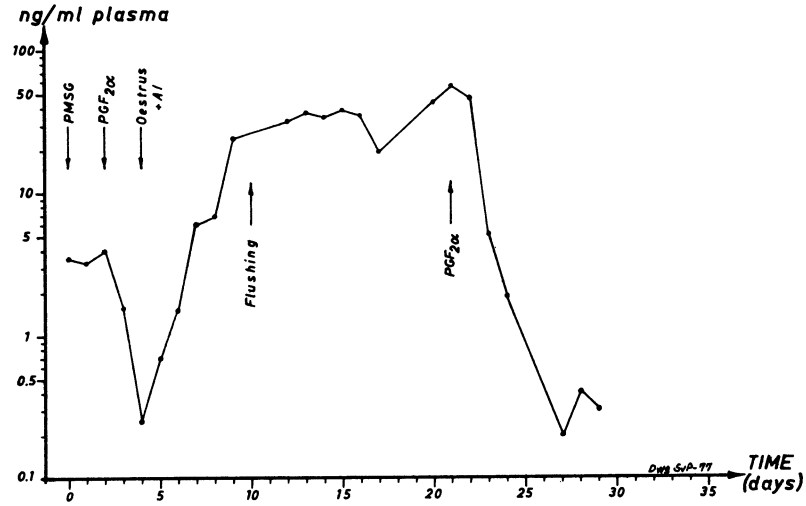

Figure 5. Plasma progesterone in a superovulated heifer with a prolonged oestrus cycle. $PGF_{2\alpha}$ treatment resulted in a slow decrease of plasma progesterone (number of days from treatment to heat = 6). (Semilog. scale).

formation of several anovulatory follicles as diagnosed by rectal palpation. Intravenous administration of 3000 i.u. HCG terminated this condition and resulted in development of 12—15 corpora lutea (Fig. 4). In this case the second superovulatory treatment (36 days after the first PMSG administration) may have lead to an endocrine disturbance resulting in the development of a higher number of ovarian cysts. The long ovarian "resting period" may have contributed to this condition. Two of the experimental animals maintained a prolonged luteal function after PMSG/$PGF_{2\alpha}$ treatment which was regulated by repeated $PGF_{2\alpha}$ injection (Fig. 5).

According to our experiences injection of a $PGF_{2\alpha}$-analogue in superovulated animals will result in a sharp decline in plasma progesterone within 2—3 days, but in a few cases a certain delay in luteolytic response was observed.

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SAMMENDRAG

Plasmaprogesteronbestemmelser ved superovulation af kvæg.

Plasmaprogesteron blev målt på 14 normale kvier og køer, der anvendtes som donorer ved ikke-kirurgiske fostertransplantationer. Progesteronbestemmelserne udførtes med en radioimmunoassay (RIA) metode. Progesteronværdierne steg efter behandling med PMSG fra gns. 6,4 ± 2,7 ng/ml til 8,3 ± 4,8 ng/ml. Hos 8 af dyrene forekom der en stigning fra gns. 7,5 ± 2,8 til 11,6 ± 3,1 ng/ml. Hos de øvrige 6 dyr konstateredes et fald fra gns. 5,0 ± 2,1 til 3,9 ± 2,5 ng/ml. Cloprostenolbehandling 2 dage senere resulterede i et markant fald efterfulgt af en hurtig stigning op til gennemsnitligt 46,8 ng/ml på dag 16. Der var en betydelig individuel variation i maksimum værdierne, og der observeredes ingen sammenhæng mellem disse og antallet af corpora lutea. I visse tilfælde var den superovulerede brunstcyklus af forlænget varighed. Den brunst, som optrådte efter superovulationen, efterfulgtes af en længere ovarial hvileperiode med meget lave plasmaprogesteronværdier ($\leqq$ 1 ng/ml) og ingen følelig ovarial aktivitet.

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