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THE RELATIONSHIP BETWEEN PLASMA PROGESTERONE CONCENTRATION AND EMBRYO PRODUCTION IN DAIRY COWS SUPEROVULATED WITH FSH OR PMSG

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INTRODUCTION

Reliable superovulation remains an important limitation to embryo transfer in cattle. The problems incurred in embryo transfer are now biological rather than technical, and embryo yield, is still highly variable and unpredictable **(Callesen et al., 1988)**. Pregnant mare serum gonadotrophin (PMSG) and Follicle stimulating hormone (FSH) are the most widely used agents for superovulation in cattle. Results of ovarian response and embryo yield in cows treated with these hormones are controversial. Some authors **(Elsden et al., 1978; Monniaux et al., 1983 and Almedia, 1987)** found better response on superovulation with FSH while other investigators **(Parmigiani et al., 1984 and Yadav et al., 1986)** reported higher results with PMSG treatment.

Non-responding animals to exogenous gonadotrophin treatment constitute the main obstacle in developing embryo transfer technique. They can be defined as animals that failed to superovulate, animals from which no embryo can be recovered or animals that do not produce any transferable embryos **(Saumande et al., 1985)**. Therefore, the availability of reliable screening method prior to induction of superovulation could reduce the high percentage of non-responding donors and would thereby contribute to substantial savings of financial and time resources **(Herrier et al., 1990)**. Since a relationship between plasma progesterone concentration at the time of superovulatory induction and subsequent ovarian response and embryo yield was demonstrated by many investigators **(Elsaesser et al., 1981; Yadav et al., 1986; Goto et al. 1987; Callesen et al., 1988; Goto et al., 1988 and Herrier et al., 1990)**, determination of progesterone levels at initiation of superovulation could be of considerable significance for screening of potential donor cows.

The present work, therefore, was conducted to (1) compare between the superovulatory effect of FSH and PMSG in dairy cows. (2) illustrate the progesterone profile before and after superovulation in both groups and (3) ascertain whether a relationship existed between plasma concentration and ovulation rate, number of recovered embryos and number of transferable embryos in dairy cows treated with FSH or PMSG.

MATERIAL AND METHODS

This study was conducted on lactating Holstein-Friesian cows in a military farm nearby Cairo during April through June, 1992. Animals had been kept under the same feeding and managemen conditions, were late in their first lactation (3.5 to 4.5 years old) and were milked twice daily.

Based on rectal examination, 14 cows having normal genitalia with palpable corpora lutea were chosen. These cows were synchronized using a single injection of 25 mg prostaglandin $F_{2\alpha}$ (Lutalyse, Upjon Co., Kalamazo, MI), thenceafter they were randomly assigned into two groups. In the midluteal phase (day 10), animals were treated either with FSH-P (group I, 6 cows) or PMSG (group II, 8 cows). Superovulatory regimen in the first group included treatment with a total dose of 32 mg FSH-P (Schering Corporation, USA) administered as two i.m. injections per day at 12-hours intervals in decreasing doses (6,6,5,5,3,3,2 and 2 mg) over 4 consecutive days. Animals in the second group received a single i.m. injection of 3000 iu PMSG (Intervet International B.V., Holland). Two days after the beginning of the superovulatory treatment, luteolysis was achieved by applying two doses of prostaglandin $F_{2\alpha}$ (one in the morning and the other in the evening). At estrus, each cow was mated two times, at least, by two fertile bulls. Embryo recovery was carried out nonsurgically at day 7 of the cycle using the method described by **Newcomb et al. (1978)**. Estimation of the number of corpora lutea and unovulated follicles were performed through rectal palpation just before embryo recovery.

About 20 ml of blood was collected in heparinized tubes by jugular venipuncture early in the morning at the first day of gonadotrophin treatment (day-4), day of prostaglandin treatment (day-2) day of estrus (day 0), first day of superovulatory cycle and every other day till the day of recovery (day + 7). Plasma samples were stored at -20°C pending hormonal analysis.

Plasma progesterone was measured by RIA technique using I^{125} as tracer (coat-A-count progesterone kits of Diagnostic Products

Corporation, USA) as the method employed by **Abraham (1981) and Sheehan (1982).** Gamma counter (Berthold) was used for counting and the produced number converted by the way of calibration curve for measuring of progesterone in the unknown samples. The sensitivity of the assay, defined as the smallest concentration significantly ($P < 0.05$) distinguishable from zero, was 0.1 ng/ml plasma . The average intra-and inter assay coefficients of variation were 7.6% and 8.4%, respectively.

Differences in plasma progesterone concentrations, ovarian response and embryo production between FSH and PMSG-treated groups were tested by Student's t-test. Correlation coefficients were calculated between progesterone levels and each of these parameters (**Snedecor and Cochran, 1976**). Moreover, hormone concentrations in plasma taken during the 7 sampling periods were analysed by analysis of variance for repeated measurements (Winer, 1971).

RESULTS

Ovarian response and embryo production in cows superovulated with FSH-P or PMSG are shown in Table 1.

Treatment with FSH-P resulted in a significantly higher ($P < 0.05$) ovulation rate than treatment with PMSG (11.33 ± 1.85 vs. 6.63 ± 1.14 CL). Ovulation rates were variable in both groups ranging from 4 to 8 and 0 to 11 in FSH-P and PMSG-treated cows, respectively. On the contrary to the pattern of ovulation rate, a significantly more ($P < 0.01$) large follicles (> 10 mm) were palpated in cows su-

Table (1) : Effect of FSH and PMSG treatment on the ovarian response and embryo yield (Mean ± SME) .

Treatment	FSH	PMSG
Number of treated cows	6	8
Number of cows responded to superovulation (%)	6 (100%)	7 (87.5%)
Ovulation rate	11.33±1.85*	6.63±1.14
Unovulated follicles > 10mm.	0.33±0.19**	2.00±0.40
Percentage of ovulation	96.30±2.14**	80.54±3.98
Recovered ova/embryos	7.17±1.75*	3.43±0.65
Fertelized ova	5.17±1.2*	2.44±0.45
Transferable embryos	4.16±1.01*	1.86±0.42

* P< 0.05 ** P< 0.01

perovulated with PMSG (2.00±0.40) than those superovulated with FSH-P (0.33±0.19).

The mean number of recovered ova/embryos (Table 1) was significatntly higher ($P < 0.05$) in the FSH-P (7.17 ± 1.75) than in the PMSG (3.43 ± 0.65) treated cows. The number of collected ova/embryos varied from 0 to 14 and 0 to 6 in both groups, respectively. Although fertilization rates were almost similar in FSH-P and PMSG-treated cows (72.11 vs. 71.14%), a significantly higher ($P < 0.05$) number of fertilized ova were collected from FSH-P (5.17 ± 1.20) than from PMSG (2.44 ± 0.45) groups. In the same aspect, more transferable embryos ($P < 0.05$) were identified in the cows that received FSH-P (4.16 ± 1.01) as compared to those treated with PMSG (1.86 ± 0.42).

In the FSH-treated gorup, progesterone levels at this time were significantly correlated ($P < 0.05$) with the subsequent ovulation rates (0.83), number of embyos recovered (0.90) and number of transferable embryos (0.81), whereas in the PMSG-treated animals the respective correlations were not significant (Table 2). When the data were pooled across both superovulated gorups, these relationships were still highly significant (0.74, 0.69 and 0.67; $P < 0.01$).

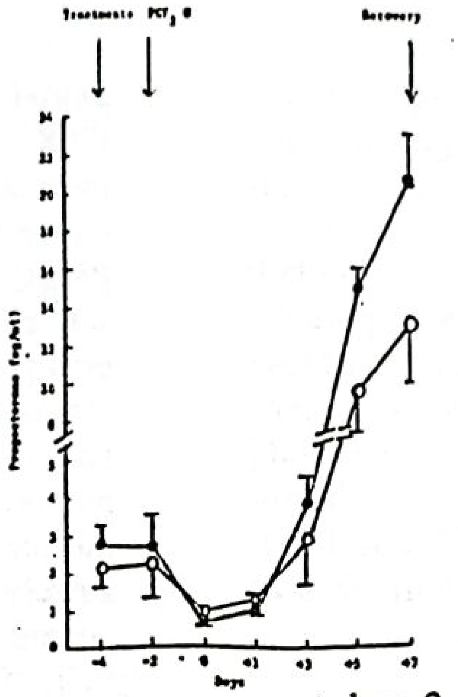
Plasma progesterone concentrations in both superovulated gorups (Fig. 1) at the day of prostaglandin $F_{2\alpha}$ treatment remained close to those estimated at the initial tret-

Table (2) : Coefficients of correlation between ovulation rate, number of recovered embryos and number of transferable embryos and plasma progesterone concentrations at the different days of treatment.

		Days of treatment						
		-4	-2	0	+1	+3	+5	+7
Ovulation Rate	FSH	0.83*	0.12	0.25	0.17	0.19	0.51	0.94**
	PMSG	0.64	0.10	0.22	0.19	0.20	0.45	0.82*
	Overall	0.74**	0.11	0.23	0.18	0.20	0.48	0.92**
Number of embryos recovered	FSH	0.90*	0.15	0.22	0.16	0.14	0.55	0.93**
	PMSG	0.39	0.20	0.24	0.22	0.26	0.38	0.41
	Overall	0.69**	0.18	0.22	0.19	0.21	0.47	0.88**
Number of Transferable embryos	FSH	0.81	0.19	0.18	0.21	0.24	0.42	0.93**
	PMSG	0.61	0.14	0.16	0.09	0.12	0.35	0.18
	Overall	0.67**	0.15	0.17	0.14	0.16	0.36	0.86**

* $P < 0.05$ ** $P < 0.01$

Plasma progesterone



ment. At day of estrus (day 0), progesterone concentration dropped below 1 ng/ml and still low on day + 1 in both groups. A marked ($P < 0.01$) rise in progesterone concentrations was noticed on day + 3 post-estrus in FSH-P (3.8 ng/ml) and PMSG (2.9 ng/ml) treated groups. These concentrations shot up to 14.9 and 9.4 ng/ml on day + 5 post- estrus in the two gorups, respectively. Maximum concentrations (20.67 and 12.97ng/ml) were measured in both groups on the day of embryo recovery (day + 7). No differences were noted in progesterone levels between treatments up to day + 3 post-estrus, whereas significant variations ($P < 0.05$) were estimated at days + 5 and + 7 post-estrus.

As depicted in Table 2, no significant correlations could be obtained between plasma progesterone concentrations and any of the studied parameters in both groups at days-2, 0,+1,+ 3 and +5 post-estrus. At the day of embryo recovery, progesterone concentrations were highly correlated ($P < 0.01$) with the ovulation rate (0.94), number of embryos recovered (0.93) and number of viable embryos (0.93) in FSH-treated cows (Table 2). In PMSG- treated cows, the respective correlations were 0.82, 0.41 and 0.18. Across all superovulated gorups, thes correlations were estimated to be 0.92, 0.88 and 0.86 ($P< 0.01$).

DISCUSSION

The results of this investigation revealed that FSH was more effective in inducing superovulation than PMSG. Higher ovulation rate was achieved in cows treated with FSH as compared to those received PMSG. Similar results were reported by **Elsden et al., (1978), Monniaux et al. (1983) and Almedia (1987).** It was emphasized that the difference between these hormones was due to biological potency rather than source of gonadotrophins **(Bellows and Short, 1972)**. In contrast, **Parmigiani et al. (1984) and Yadav et al. (1986)** reported a higher ovulation rate in PMSG than in FSH-treated cows. Moreover, some authors **(Crister et al., 1980 and Becker and Pinheiro, 1986)** found no significant differences between both hormones as superovulatory agents.

In accordance with the findings of **Sreenan et al. (1978), Monniaux et al. (1984) and Yadav et al. (1986)**, our results showed higher number of unovulated follicles (> 10 mm) in PMSG than in FSH-treated groups. These follicles were presumably stimulated to grow due to the long half-life of PMSG but failed to ovulate directly because of the rapid increase in progesterone soon after the LH surge **(Yadav et al., 1986)** and/or indirectly owing to the secondary post-ovulatory rise in concentration of estradiol and estrone **(Echternkamp, 1978 and Moyaert et al., 1985)**. This increase in the circulating estrogen was described to be responsible for hormonal imbalance which interfered with the mechanism of ovulation **(Kummer et al., 1980)** and might have a supressive effect on the ovulation and sex behavioural centers in bovine brain **(Bouters et al., 1983)**.

In agreement with the results of **Seidel et al. (1977), Greve et a (1983) and Caral et al. (198** more embryos per donor were r covered from FSH than fro PMSG-treated cows. This findin was closely related to the high embryo recovery rate in FSH grou (55 vs 45%). On the other hand, i PMSG-treated cows, the excessiv number of unovulated follicles i the presence of corpora lutea ad versely influenced the embryo re covery rate because of an unfa vourable estrogen/progesteron ratio which allegedly affected gam ete and embryo transport **(Dros 1986)**. Additionally, this disturbe hormonal ratio has a supressive e fect on the quality of recovere embryos **(Becker and Pinheiro 1986)**. The latter hypothesis wa supported herein by the decrease o transferable embryos recovered from PMSG than those collected from FSH-treated cows.

Plasma progesterone concentration before and after superovulation in FSH and PMSG-treated groups showed a typical pattern; decreasing from the first day of treatment to reach baseline values at the day of estrus, then increased linearly to peak levels at the day of recovery. Similar pattern of progesterone profile was reported by **Jensen et al. (1982) and Goto et al. (1987)**. The higher levels of progesterone in FSH than in PMSG-treated cows during the different sampling days in this experiment could be due to the higher ovulation rate in the former gorup.

In the current study, coefficients of correlation between progesterone concentrations at initiation of superovulation and each of the ovulation rate, number of embryos recovered and number of transferable embryos were higher in FSH than in PMSG-treated gorups. Moreover, across all superovulated animals in both gorups, the respective correlations were still highly significant. These strong relationships indicated that the ovarian function at the time of treatment was an important factor for reliable superovulation in dairy cows. Recently, **Kweon et al. (1985) and Goto et al. (1987)** reported similar results. The latter authors suggested that cows with progesterone levels of 3 ng/ml and more at the first day of gonadotrophin treatment showed good ovarian response to superovulation. In the same aspect, **Callesen et al. (1988)** informed that higher levels of progesterone at start of superovulation tended to supress the basal LH discharge from initial injection of gonadotrophin to injection of prostaglandin; this allowed for greater storage of LH and subsequently produced a broader LH surge with a higher peak level. Such an LH discharge pattern has been found to be favourable in terms of embryo quality **(Jensen et al., 1982 and Donaldson, 1985)**. On the contrary, animals with low progesterone concentrations at the initial injection of gonadotrophin produced low numbers of embryos of inferior quality **(Elsaesser et al. 1981 and Boland et al., 1985)**, although no eggs were usually recovered **(Greve et al., 1983 and Lindsell et al., 1986)**. These animals either had not formed functional corpora lutea **(Greve et al., 1983 and Smith, 1986)** or they had experienced premature regression of corpora lutea **(Greve, 1982 and Jensen et al., 1992)**.

Saumande et al. (1985) reported significant relationships between ovulation rate and plasma progesterone concentration as early as the second day of the superovulatory cycle up to the day of recovery. In the present study such relationships existed as late as the seventh day post- estrus (day of recovery). The strong relationships between plasma progesterone concentraiton at the day of recovery and each of the ovulation rate, number of recovered embryos and number of transferable embroys were recorded by **Jensen et al. (1982), Greve et al. (1983) and Saumande et al. (1985)**. It was found that low levels of progesterone at the day of recovery indicated premature regression of corpora lutea which was associated with poor quality embryos **(Jensen et al., 1982 and Lindsell et al., 1986)**.

In conclusion, this experiment demonstrates that FSH is more effective in inducing superovulation and yielding more viable embryos than PMSG. Also, the ovarian function on the first day of superovulatory treatment, as indicated by plasma progesterone concentration, is and important factor for reliable superovulation with FSH or PMSG. Moreover, high progesterone level two days before recovery and at the day of recovery indicates the possibility of collecting higher number and more viable embryos from cows superovulated with FSH or PMSG.

SUMMARY

Fourteen primiparous lactating cows, 3.5 to 4.5 years old in a military dairy farm nearby Cairo (Egypt) were used in this study. Following synchronization, the animals were randomly assigned into two groups; the fist group (6 cows) was superovulated with a total dose of 32 mg FSH-P whereas the second group (8 cows) was treated with 3000 iu PMSG. Embryos were collected non-surgically on day 7 (day of estrus = day 0). Plasma samples were taken from cows of both groups on day-4 (first day of gonadotrophin treatment), day-2 (day of $PGF_{2\alpha}$ injection), day 0 (day of estrus) days 1,3,5 post-estrus and day 7 (day of recovery) for progesterone assay.

The results revealed that FSH was more effective as a superovulatory agent than PMSG. Higher ($P < 0.05$) ovulation rate (11.33 ± 1.85 vs 6.63 ± 1.14), number of embryos recovered per donor (5.17 ± 1.20 vs 2.44 ± 0.45) as well as number of transferable embryos (4.16±1.01 vs 1.86±0.42) were obtained from FSH than from PMSG-treated cows. However, more follicles (> 10 mm) were palpated in the PMSG-treated group (2.00±0.40 vs 0.33 ± 0.19; $P < 0.01$). Coefficients of correlation between progesterone concentration at initiation of superovulation and each of the ovulation rate, number of embryos recovered and number of transferable embryos were higher in FSH than in PMSG-treated cows. When the data of both groups were pooled, the respective correlations (0.74, 0.69, 0.67) were still highly ($P < 0.01$) significant. Moreover, significant correlations were estimated between progesterone concentration and the mentioned parameters in FSH group at the day of recovery.

This study revealed that measuremen[t] of plasma progesterone concentration ca[n] serve as a prognostic tool to predict th[e] yield of fertile eggs and quality of em[-] bryos.

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