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**Development and Evaluation of a Method of
Hormonal Treatment to Increase Fertility in
Dairy Cows**

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Abstract

Development and Evaluation of a Method of Hormonal Treatment to Increase Fertility in Dairy Cattle. J. H. Britt¹, M. Rosenberg², S. P. Washburn¹, and M. Kaim².

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The objectives were: 1) to develop a practical method for improving conception rates of dairy cows by administering progesterone during the critical period of the luteal phase prior to first insemination, 2) to determine which cows respond best to such a progesterone treatment, and 3) to determine physiological changes that are associated with this treatment. Experiment 1, conducted in the US and Israel, tested three treatment protocols. Holstein cows: 1) served as controls, 2) received two injections of prostaglandin F₂ α (PGF) 14 days apart, or 3) received two PGF injections and were inserted with a progesterone releasing intravaginal device (PRID) for 6 days beginning 9 days after the first injection of PGF. Fertility was positively related to plasma progesterone concentrations prior to the second PGF injection, but PRID treatment did not improve fertility in cows with low progesterone. Cows in synchronized estrus 2-8 days after the second injection of PGF had higher fertility and better reproductive performance than non-synchronized cows. Experiment 3, conducted in Israel, involved three studies that assessed effect of PRID treatment on: 1) progesterone concentrations in ovarian tissue and uterine tissue and fluids, 2) changes in systemic LH and estradiol, and 3) development and function of dominant follicles. Treatment with PRID increased progesterone in uterine tissue and fluid exclusive of effects on systemic concentrations. Low progesterone resulted in persistent follicles that exceeded normal size and produced excessive estrogen. Experiment 4, conducted in the US, evaluated influence of endogenous progesterone on embryo development. Cows were given two injections of PGF 14 days apart and single embryos were recovered 7 days after estrus. Among cyclic cows, there was a linear relationship between progesterone concentration and percentage of cows from which an embryo was recovered. Overall these experiments show that systemic progesterone concentrations affect fertility in high-producing dairy cows through effects on follicular development and embryo survival.

Objectives of the Original Research Proposal

The objectives of the original proposal were:

- 1) *To develop a practical method for improving conception rates of dairy cows by administering progesterone during the critical period of the luteal phase prior to first insemination,*
- 2) *To determine which cows respond best to such a progesterone treatment, and*
- 3) *To determine physiological changes that are associated with this treatment.*

In order to accomplish these objectives, the following experiments were conducted:

Experiment 1: U.S. and Israel

Results from our groups and from others indicated that conception rates were positively related to plasma progesterone levels during the luteal phase preceding A.I. However, the frequency of cows with low progesterone concentrations may vary under different environmental and management conditions, so it was important to study the effect of PG treatment with and without PRIDs on a large number of animals on multiple farms over an extended period of time.

Therefore, the **first aim** of the experiment was to compare the conception rate of untreated control cows with that of cows treated with two PG injections, with or without an exogenous progesterone source supplied by PRIDs. The **second aim** was to refine the treatment procedure, based on rapid measurement of endogenous progesterone levels, so that progesterone treatment would be given selectively only to “low progesterone” cows to increase conception rate. The **third aim** was to determine whether change in body condition during the first 5 wk postpartum was correlated with the response to treatment with progesterone.

Experiment 2

This experiment was eliminated in response to reviewer’s comments and as part of the original budget reduction.

Experiment 3: Israel

Our previous research indicated that many cows which had low plasma progesterone levels following PRID insertion attained conception rates exceeding 60%. We postulated, therefore, that

PRID exerted at least part of its effect directly on the proximal reproductive organs, namely the uterus, oviduct and ovary.

The **first aim** of this experiment was to investigate whether treatment with PRIDs affected the concentration of progesterone in the uterine endometrium. The **second aim** was to study follicle function in vivo and in vitro following progesterone treatment.

Experiment 4: U.S.

It was unclear whether plasma progesterone levels preceding A.I. affected processes determining the viability of the young embryo or other phenomena that are expressed during the period after fertilization. For example, progesterone level before A.I. might influence oviduct and uterine function so that the reproductive tract of "low progesterone" cows is not capable of supporting the development of a normal embryo or the embryo may develop asynchronously with the uterus. Differences in rates of recovery between cows with different concentrations of progesterone or differences in embryonic development would be taken as evidence that effects of progesterone concentration on embryo survival are exerted before day 6 or 7 after A.I.

The **first aim** was to assess early embryonic development in cows that differed in progesterone status before first AI induced by two injections of PG given 14 days apart. If no differences were found in part 1 of this experiment, the **second aim** was to assess whether progesterone status of the recipient affected embryonic development beyond day 7. [Note: The experiment conducted to test the first aim accounted for effects of progesterone, therefore that experiment was expanded and the experiment planned for the second aim was eliminated.]

Relevant Data, Methodology, Results and Discussion

Experiment 1. This experiment was conducted in the US and Israel. It was a replicated study involving three treatment protocols used in one herd in the US and two herds in Israel. Cows that calved in 3-week clusters served as controls, received two injections of prostaglandin F2 α (PGF) given 14 days apart beginning on days 43 to 63 days postpartum, or received the two PGF injections 14 days apart and were inserted with a progesterone releasing intravaginal device (PRID) for 6 days beginning 9 days after the first injection of PGF.

Experiment 3. This experiment was conducted in Israel. The experiment involved three studies focused on effect of PRID treatment on: 1) progesterone concentrations in ovarian tissue and uterine tissue and fluids, 2) follicular development and associated changes in LH and estradiol, and 3) development and function of follicles.

Experiment 4. This experiment was conducted in the US. Cows that calved in 3-week clusters were given two injections of PGF 14 days apart beginning on days 43 to 63 postpartum and milk samples were collected thrice weekly for determination of concentrations of progesterone. The uterus of each cow was flushed to recover the embryo 7 days after estrus, and embryo recovery and development were evaluated in relation to the progesterone profiles of the luteal phase preceding estrus and AI.

Experiment 1 (Israel and US) Influence of progesterone supplementation on cows with high or low endogenous progesterone concentrations during the luteal phase of the estrous cycle.

The rationale for this experiment was based on our previous observations that conception rate in high-producing dairy cattle is related positively to the concentration of progesterone in blood during the 2-week period before insemination. Also, in previous experiments we found that insertion of a progesterone releasing intravaginal device (PRID) during the luteal phase preceding artificial insemination increased conception rate in cows with low plasma progesterone concentration, but it somewhat lowered conception rate of cows with high progesterone concentrations. We postulated that high conception should be obtained when, following a single prostaglandin F (PGF) injection "low progesterone cows" receive exogenous progesterone followed by a second injection of PGF and "high progesterone cows" be given a second PGF only. The aim of the present experiment was to test this hypothesis, in both Israel and the US in high producing Holstein cows. In addition, we aimed to test the hypothesis that excessive loss in body condition predisposes cows to lower fertility and that this could be alleviated by insertion of a PRID.

For this replicated trial, we compared the reproductive performance of untreated control cows with that of cows treated with two PGF injections with or without a PRID.

Methods

The effect of plasma progesterone concentrations and of postpartum changes in body condition on conception were determined during 2 years for 603 high-yielding dairy cows (>10,500 kg annually) on two farms in Israel (Herds IL1 and IL2) and 212 high-yielding cows (10,300 kg annually) on one farm in the US (Herd US). The same experimental procedures, including the same system for clustering cows in 3-week groups, the same blood sampling protocol and the same progesterone assay procedures were used in Israel and the US. In general, the day-to-day management procedures among herds and the experimental routines were conducted

as similarly as possible, given the differences in climate and facilities between countries and among herds.

Cows were examined by a veterinarian 1 to 2 weeks after calving and again 2 (US) or 3 (Israel) weeks later. Reproductive and other disorders were treated and only healthy or fully recovered cows were assigned to treatments. Body condition was scored on a 1 to 5 scale at calving and again during week 5 postpartum. In Israel, weekly blood samples for progesterone determination were collected from all cows beginning at calving to assess onset and regularity of estrous cyclicity. In the US, milk samples were collected thrice weekly to assess cyclicity, and during the period of treatment blood samples were also collected for comparison with Israeli data. In both countries, assessment of "high" or "low" progesterone for assignment to treatment groups was based on plasma progesterone collected 8 days after the first PGF injection.

Forty-three to 63 days after calving, cows were allocated according to parity, medical history and change in body condition score during the first five weeks after calving, into an untreated group (Control, n=321; Table 1.1) and two hormonally-treated groups (PGF only, n=214 and PRID, n=280).

All hormonally treated cows were given one PGF injection between 43 and 63 days postpartum (Figure 1.1). Eight days after the first PGF injection, blood was sampled and assayed for progesterone concentrations. Cows that had progesterone concentrations ≤ 2.0 ng/ml ("Low P4") were allotted into subgroups according to their postpartum change in body condition. In Herds IL1 and US, half of the "Low P4" cows were inserted 9 days after the PGF injection with a PRID for 6 days. One day before PRID removal the cows received a second PGF injection. The other half were given the second PGF only. Treated cows that had high plasma progesterone ("High P4", ≥ 2.1 ng/ml) 8 days after the first PGF injection were also allotted into similar subgroups.

In Herd IL2 a somewhat different experimental design was applied. In the first year of the experiment cows in Herd IL2 were allocated, as described above into an untreated control group and one hormonally treated group that received the PRID treatment. In the second year

Figure 1.1. Protocol for treatment of cows in Experiment 1.

hormonally-treated cows in Herd IL2 that had "Low P4" 8 days after the first PGF were inserted with a PRID whereas cows with "High P4" were given the second PGF only.

In both countries, cows of the treated groups were inseminated following the detection of post-treatment estrus, whereas control cows were inseminated at the first spontaneous estrus observed beyond 60 days postpartum. Cows of all groups that had not been detected in estrus by 100 days after calving were rectally examined and clinically treated, if necessary. Cows that were detected in estrus following the first postpartum A.I. were reinseminated without further treatment.

Data from the three herds were combined into a single data set for statistical analyses. In general, data were subjected to least-squares analysis of variance using the PROC GLM of SAS. Classification data were analyzed Chi Square procedures within PROC CATMOD. Statistical models usually included treatment, herd and parity (1 vs ≥ 1) and the two-way interactions.

Results

The number of cows assigned to each of the treatment groups in the three herds is presented in Table 1.1. Due to the modified experimental design in Herd IL2, the number of cows assigned to the PGF treatment in this herd was less than the number of cows in the other two treatment groups.

Acyclic cows. The proportion of cows classified as acyclic, based on plasma or milk progesterone before being assigned to the three treatment groups, is presented in Table 1.2. Overall, 12.8% of cows were acyclic, with more acyclic cows in the US herd than in Herds IL1 or IL2. In the US, 50% of the acyclic cows were primiparous compared with 30% in Herds IL1 and 35% in IL2.

Body condition. The proportion of cows with low, medium or high body condition scores at calving and 5 weeks postpartum is presented in Table 1.3. In the US herd, a higher proportion of cows were classified as having a higher body condition score compared to the two Israeli herds. However, when the loss in body condition between calving and week 5 postpartum was compared, there was no difference between the Herd IL1 and Herd US (Table 1.4).

Table 1.1. Number of cows assigned to treatment groups in Israel and US.

Treatment group	Israel		US	Total
	IL1	IL2		
Control	149	101	71	321
PGF	122	25	67	214
PRID	125	81	74	280
Total	396	207	212	815

Table 1.2. Proportion of cows classified as cyclic or acyclic before treatment.

Herd	Cyclic	Acyclic	Total	% Acyclic
IL1	350	46	396	11.4
IL2	187	20	207	9.7
US	174	38	212	17.9
Combined	711	104	815	12.8

Table 1.3. Proportion of cows with various body condition scores at calving and 5 weeks post partum.

Body condition score:	Low (≤ 2.0)			Medium (2.25-2.75)			High (≥ 3.0)			
	Herd:	IL1	IL2	US	IL1	IL2	US	IL1	IL2	US
<u>Primiparous</u>										
Week 0 (calving)		7.8	-	1.5	68.1	-	0	24.1	-	98.5
Week 5		65.5	51.6	3.1	33.6	45.3	24.6	0.9	3.1	72.3
<u>Multiparous</u>										
Week 0 (calving)		11.5	-	1.1	58.6	-	1.1	29.9	-	97.9
Week 5		68.9	66.7	4.3	29.1	30.8	38.3	2.0	2.6	57.5

Reproductive performance. Reproductive performance of cyclic and acyclic cows is presented in Tables 1.5 and 1.6. Among cyclic cows (Table 1.5), a PROC GLM analysis (SAS) of the interval from parturition to first service and of the conception rate at first service revealed no significant differences among herds or treatments. In all treatment groups the interval from calving to conception (open days) was shorter in the US herd than in Herds IL1 or IL2 ($P < 0.02$).

In acyclic cows (Table 1.6) there was a significant effect of treatment on conception rate ($P < 0.05$). In Herd IL1, the PGF-treated acyclic cows had the highest conception rate, whereas in the US herd there was practically no difference in conception rate between treatments. The number of acyclic cows in the hormonally treated groups in Herd IL2 was too small to evaluate the effect of treatment on conception rate.

The reproductive performance of all cows that conceived during the experiment (pregnant cows) for each herd x treatment subclass is presented in Table 1.7, and a summary of reproductive performance by treatment class is in Table 1.7a. Results in Table 1.7 reveal there were no significant differences in the interval from parturition to first service and in conception rate among the three herds. Number of open days in multiparous cows in the US herd was significantly lower than in Herds IL1 and IL2 ($P < 0.02$). The analysis of the combined data from all three herds (Table 1.7a) revealed that in both hormonally-treated groups the mean interval from parturition to first insemination was reduced by 3 days ($P < 0.02$). In primiparous cows conception to first service in both hormonally-treated groups combined was 62.7% and tended to be higher than in the control group (Chi square = 1.943, 1 df, $P = 0.163$).

Distribution of interval to first service. The frequency distribution for days from parturition to first service (DFS) among herd x treatment subclasses is presented in Table 1.8 and a summary by treatment class is in Table 1.8a. In multiparous cows of Herds IL1 and US only 73% and 71%, respectively, of the cows in the control group were inseminated by 90 days postpartum compared to 83% and 86% in cows of the PRID treatment. When the data for all cows were combined only 74.5% of the control cows had less than 90 days to first service compared to 82.7% and 85.7% of the cows in the PGF and PRID treatment groups, respectively ($P < 0.005$).

Table 1.4. Proportion of cows with various degrees of loss in body condition from calving to week 5 postpartum.

Loss in condition	Parity: Herd:	Primiparous cows		Multiparous cows	
		IL1	US	IL1	US
≤0.25		32.8	26.1	27.9	20.2
0.50		36.2	53.9	32.0	38.3
≥0.75		31.0	20.0	40.1	41.5

Table 1.5. Reproductive performance of cyclic cows.

Trait	Treatment: Herd:	Control			PGF			PRID		
		IL1	IL2	US	IL1	IL2	US	IL1	IL2	US
No. of cows		130	93	54	108	23	47	110	81	57
No. of cows culled		10	17	---	6	2	---	10	11	---
Days to 1st AI, d		82	81	87	81	78	81	79	78	76
Conception rate 1st AI, %		43.9	45.2	51.9	46.3	52.2	48.9	52.7	40.7	47.4
Days open (pregnant cows) ^a		113	108	106	110	109	100	106	121	94
No. of A.I./conception		1.94	1.93	---	1.95	2.05	---	1.80	2.39	---

^aSignificant effect of herd, $P < 0.02$.

Table 1.6. Reproductive performance of acyclic cows.

Trait	Treatment: Herd:	Control			PGF			PRID		
		IL1	IL2	US	IL1	IL2	US	IL1	IL2	US
No. of cows		18	12	7	14	2	14	13	6	12
No. of cows culled		3	1	---	4	0	---	0	0	---
Days to 1st AI		87	83	100	89	64	68	89	86	90
Conception rate 1st AI, % ^a		38.9	41.7	57.1	64.3	50.0	50.0	30.8	71.4	50.0
Days open (pregnant cows)		117	114	100	97	120	80	131	92	107
No. of A.I./conception		2.1	1.9	---	1.3	2.5	---	2.3	1.3	---

^aSignificant effect of treatment $P < 0.05$

Table 1.7. Reproductive performance of pregnant cows by herd, treatment group and parity

Herd:		IL1			IL2			US		
Trait	Treatment:	Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID
Primiparous cows										
No. of cows		45	35	36	31	13	20	19	23	23
Milk yield (305 d), kg		10,635	10,165	10,611	10,942	10,695	11,468	9,946	8,947	9,330
Days to 1st AI		84	84	83	83	78	82	89	79	78
Concep. 1st AI%		46.7	51.4	63.9	58.0	76.9	60.0	63.2	65.2	69.6
Days open		114	112	107	110	87	107	110	93	88
Multiparous cows										
No. of cows		90	77	77	55	10	52	31	27	36
Milk yield (305 d), kg		10,906	12,473	10,569	11,005	11,115	10,897	11,036	10,902	11,438
Days to 1st AI		82	80	79	80	75	78	88	79	79
Conception 1st AI, %		46.7	53.2	50.7	50.9	30.0	44.2	64.5	55.6	47.2
Days open ^a		113	108	110	108	139	123	103	99	101

^aSignificant effect of herd $P < 0.02$.

Table 1.7a. Summary of reproductive performance of pregnant cows by parity and treatment group.

Parity Treatment	Primiparous cows			Multiparous cows			All cows		
	Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID
No. of cows	95	71	79	176	114	165	271	185	244
Days to 1st AI ^a	84	81	81	83	79	79	83	80	80
Conception 1st AI, %	53.7	60.6	64.6	51.1	51.8	47.9	52.0	55.1	53.3
Days open	112	101	101	110	108	112	111	106	108

^aSignificant effect of treatment in combined data of all cows, $P < 0.02$.

Table 1.8. Frequency distribution (%) of the interval from parturition to first AI

Trait	Treatment:	Control			PGF			PRID		
	Herd:	IL1	IL2	US	IL1	IL2	US	IL1	IL2	US
<u>Primiparous cows</u>										
No. of cows		45	31	19	35	13	23	36	20	23
Postpartum interval, d:										
≤90		80.0	74.2	52.6	85.7	92.3	82.6	83.3	95.0	87.0
91-100		8.9	16.1	21.1	5.7	7.7	4.4	8.3	0	4.4
≥101		11.1	9.7	26.3	8.6	0	13.0	8.3	5.0	8.7
<u>Multiparous cows</u>										
No. of cows		90	55	31	77	10	27	77	52	36
Postpartum interval, d:										
≤90 ^a		73.3	81.8	71.0	81.8	80.0	77.8	83.1	86.5	86.1
91-100		10.0	3.6	9.7	5.2	20.0	7.4	2.6	5.8	2.8
≥101		16.7	14.6	19.4	13.0	0	14.8	14.3	7.7	11.1

^aSignificant effect of treatment; Chi square=7.29, 2 df, P<0.03 (CATMOD Procedure)

Table 1.8a. Summary of frequency distribution (%) of the interval from parturition to first insemination.

Trait	Parity: Treatment:	Primiparous cows			Multiparous cows			All cows		
		Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID
No. of cows		95	71	79	176	114	165	271	185	244
Postpartum interval, d:										
≤90 ^a		72.6	85.9	87.3	75.6	80.7	84.9	74.5	82.7	85.7
91-100		13.7	5.6	5.1	8.0	7.0	3.6	10.0	6.5	4.1
≥101		13.7	8.5	7.6	16.5	12.3	11.5	15.5	10.8	10.3

^aSignificant effect of treatment, Chi square=10.66, 2 df, P<0.005 (CATMOD Procedure)

Distribution of open days. The frequency distribution for the interval from parturition to conception (days open) for herd x treatment subclasses is presented in Table 1.9, and a summary by treatment class is in Table 1.9a. In Herd IL1 the proportion of cows with less than 90 open days was similar in the control and the PGF treatment subgroups, but the PRID treatment increased proportion of cows that were pregnant by 90 days postpartum in primiparous and multiparous cows (Chi square = 3.973, 1 df, $P < 0.05$). In Herds IL2 and US treatment with a PRID increased the proportion of cows with less than 90 open days among primiparous cows but not among multiparous cows.

Blood progesterone concentrations. The changes in plasma progesterone concentrations in the hormonally treated cows during the treatment period are illustrated in Figure 1.2. Progesterone profiles in Herds IL1 and US were almost identical. In both herds insertion of a PRID increased the mean progesterone concentrations during the period of insertion by 0.6 to 1.0 ng/ml. Figure 1.3 illustrates the progesterone concentrations of the IL1 and US herds, classified as having "Low P4" (≤ 2.0 ng/ml) or "High P4" (≥ 2.1 ng/ml) on the day before PRID insertion. Insertion of a PRID in both groups increased the concentration of plasma progesterone during the period of insertion, but the actual concentrations in the "Low P4" cows that were treated with PRID were still lower than those of the "High P4" cows in the PGF treatment group.

Conception at first service in "Low P4" and "High P4" cows. Conception rates at first service for cows with "Low P4" (≤ 2.0 ng/ml) and "High P4" (≥ 2.1 ng/ml) 8 days after the first PGF, are presented in Table 1.10. The overall conception rate of the "High P4" cows was 55.8%, which was significantly greater than the 44.4% conception rate of the "Low P4" group ($P = 0.018$). Within the PGF treatment group the conception rate among "High P4" cows was significantly greater than among "Low P4" cows (57.1% vs 43.5%; $P < 0.05$). Within the PRID treatment group the difference in conception rate between "High P4" and "Low P4" cows was smaller and not significant statistically (Chi square = 1.79, 1 df, $P = 0.181$). In Herds IL1 and US the conception rate within the "High P4" group was similar for the PRID-treated and the PGF-treated

Table 1.9. Proportion of cyclic cows open for various postpartum intervals.

Trait	Treatment:	Control			PGF			PRID		
	Herd:	IL1	IL2	US	IL1	IL2	US	IL1	IL2	US
<u>Primiparous cows</u>										
No. of cows		39	27	18	32	13	18	31	17	17
Postpartum interval, d:										
≤90		33.3	48.2	33.3	37.5	76.9	55.6	58.1	58.8	70.6
91-100		7.7	7.4	27.8	18.8	0	11.1	9.7	0	11.8
101-130		35.9	22.2	11.1	25.0	15.4	11.1	19.3	11.8	11.8
≥131		23.1	22.2	27.8	18.8	7.7	22.2	12.9	29.4	5.9
<u>Multiparous cows</u>										
No. of cows		81	48	28	70	8	23	69	49	32
Postpartum interval, d:										
≤90 ^a		40.7	45.8	46.4	44.3	12.5	39.1	49.3	42.9	46.9
91-100		11.1	10.4	10.7	10.0	25.0	17.4	10.1	10.2	9.4
101-130		21.0	27.1	25.0	25.7	25.0	26.1	20.3	10.2	37.5
≥131		27.2	16.7	17.9	20.0	37.5	17.4	20.3	36.7	6.3

^aSignificant effect of treatment, Chi square=8.01, 2 df, P<0.02 (CATMOD Procedure)

Table 1.9a. Summary of proportion of cyclic cows open for various postpartum intervals.

Treatment:	Parity:	Primiparous cows			Multiparous cows			All cows		
		Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID
No. of cows		84	63	65	157	101	150	241	164	215
Postpartum interval:										
≤90		38.1	50.8	61.5	43.3	40.6	46.7	41.5	44.5	51.2
91-100		11.9	12.7	7.7	10.8	12.9	10.0	11.2	12.8	9.3
101-130		26.2	19.1	15.4	23.6	25.7	20.7	24.5	23.2	19.1
≥131		23.8	17.5	15.4	22.3	20.8	22.7	22.8	19.5	20.5

subgroups. In Herd IL2 conception rate of the PRID-treated cows (0 of 8) was lower than that of the PGF-treated group ($P < 0.02$).

Figure 1.2. Progesterone in control and PRID-treated cows.

Figure 1.3. Progesterone in cows with low or high progesterone with or without treatment with PRID.

Table 1.10. Conception rate at 1st AI among cows with low or high progesterone 8 days after PGF.

Herd	"Low P4" ($P4 \leq 2.0$ ng/ml)			"High P4" ($P4 \geq 2.1$ ng/ml)		
	PGF	PRID	Total	PGF	PRID	Total
IL 1	41.7 (72)*	42.3 (78)	42.0 (150)	58.0 (50)	61.7 (47)	59.8 (97)
IL 2	66.7 (3)	48.0 (73)	48.7 (76)	50.0 (22)	0 ^a (8)	36.7 (30)
US	45.0 (40)	44.7 (47)	44.8 (87)	63.2 (19)	57.9 (19)	60.5 (38)
All farms	43.5 (115)	45.0 (198)	44.4 (313)	57.1 ^b (91)	54.1 (74)	55.8 ^c (165)

*No. of inseminated cows in parentheses.

^aSignificantly lower than "High P4" cows treated with PGF. Chi square=6.32, 1 df, $P < 0.02$.

^bSignificantly higher than "low P4" cows in PGF treatment group, Chi square=3.79, 1 df, $P = 0.05$.

^cSignificantly higher than all "low P4" cows, Chi square=5.572, 1 df, $P = 0.018$.

Changes in body condition score and conception. The effect of change in body condition between calving and week 5 after calving on the proportion of acyclic cows and on conception at first AI, in Herds IL1 and US is in Table 1.11. It may be seen that in multiparous cows in Herd IL1, the proportion of acyclic cows tended to increase in cows that had the highest loss in body condition score. It may also be seen that among primiparous cows in the US the highest conception rate was attained by cows that suffered the slightest loss in body condition (≤ 0.25). Overall, however, no statistically significant correlation was found between change in body condition score and conception.

Reproductive performance by month of treatment (season). The experimental procedure that involved clustering cows in 3-week groups that were treated simultaneously, enabled us to study the effect of the month of treatment on reproductive performance (Table 1.12). In the control group, there was a significant effect of season on conception rate ($P < 0.08$) and open days ($P < 0.02$). In the PGF treatment group there was a significant effect of season on the proportion of cows classified as acyclic prior to treatment ($P < 0.03$) and in the PRID-treated group the effect of season on the interval to first service approached significance ($P < 0.08$).

Reproductive performance of synchronized and non-synchronized cows. An analysis was done for the reproductive performance of cows that were classified as synchronized or non-synchronized, based on the occurrence of estrus in the target window 2 to 8 days following the second PGF injection (Table 1.13). The proportion of cows that were synchronized was affected by treatment group and attained 17.3%, 64.9%, and 73.0% in the control, PGF and PRID groups, respectively ($P < 0.0001$).

In all treatment groups among all three herds, the reproductive performance of the synchronized cows was superior to the non-synchronized ones. Overall, the mean interval to first service in the synchronized cows ($N=345$) was 74 ± 0.4 days compared to 88 ± 1.0 days in the non-synchronized cows ($N=355$). The number of open days in the synchronized cows was 99.3 ± 2.2 days compared to 117.5 ± 2.5 days in non-synchronized cows. The conception rate at first service

Table 1.11. Proportion of acyclic cows and conception at 1st A.I (CR) among cows with various degrees of loss in body condition from calving to week 5 postpartum.

Trait: Herd:	% acyclic cows		CR 1st AI (%)	
	IL1	US	IL1	US
Loss in body condition:	<u>Primiparous cows</u>			
≤0.25	10.5	29.4	44.7	82.4
0.5	11.9	8.6	64.3	60.0
≥0.75	13.9	30.8	50.0	61.5
Loss in body condition:	<u>Multiparous cows</u>			
≤0.25	2.9	21.0	60.3	47.4
0.5	9.0	8.3	41.0	58.3
≥0.75	15.3	10.3	50.0	56.4

Table 1.12. Reproductive performance of cows by month of treatment.

Month of treatment ^a :		Oct.-Nov.			Dec.-Jan.			Feb.-Mar.			Apr.-Jun.		
Treatment:		Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID	Con	PGF	PRID
No. of cows		66	50	57	81	60	70	81	47	67	93	57	86
Noncycling cows, %		9	4	9	9	13	10	11	15	15	18	25	14
Days to 1st AI		85	78	78	83	80	85	86	82	80	83	85	78
Conception rate 1st AI, %		34	39	44	53	61	50	41	44	46	51	50	48
Days open		124	105	107	101	96	100	107	100	110	104	100	103

^aCalendar month in which cows were allocated to treatment groups (43 to 63 days postpartum).

Table 1.13. Reproductive performance of synchronized and nonsynchronized pregnant cows in 3 herds.

Trait	Control		PGF		PRID	
	Synch.	Non-synch.	Synch.	Non-synch.	Synch.	Non-synch.
<u>Herd IL1</u>						
No. of cows	19	116	69	43	85	28
Days to 1st AI	75	84	76	91	75	98
Conception rate 1st AI, %	42.1	47.4	50.7	55.8	54.1	57.1
Days open ^a	105	115	105	115	102	115
<u>Herd IL2</u>						
No. of cows	21	65	17	6	53	19
Days to 1st AI	73	84	73	86	74	94
Conception rate 1st AI, %	57.1	52.3	70.6	16.7	52.8	36.8
Days open ^a	97	113	95	151	106	153
<u>Herd US</u>						
No. of cows	7	43	34	16	40	19
Days to 1st AI	73	91	70	99	71	95
Conception rate 1st AI, %	71.4	62.8	64.7	50.0	52.5	63.2
Days open ^b	89	108	85	120	88	111

^{a,b}Synchronized cows had significantly fewer open days than non-synchronized cows, ^aP<0.005, ^bP<0.0001.

in the synchronized cows was 54.8% and very similar to that of non-synchronized cows, that had a conception rate of 51.8%.

Discussion

The reproductive performance of the cows in the three herds was remarkably similar. The only significant difference among herds was in the interval from calving to conception (open days) for multiparous cows (Table 1.7). Multiparous cows in the US herd had fewer open days than cows in Herds IL1 and IL2.

Conception rate to first service in the control group was relatively high in all herds and was not affected by the hormonal treatments. The main effect of the hormonal treatments was on the proportion of cows that were inseminated, and on the proportion that conceived within 90 days postpartum (Tables 1.8a and 1.9a). In the PRID-treated group, the proportion of cows that had less than 90 open days was 10 percentage points greater than in the control group. The improved performance of the PRID-treated group is probably due to the higher proportion of synchronized cows in this treatment group (Table 1.13).

The results of the present experiment confirmed our previous observation (Folman et al., 1973; Fonseca et al., 1983; Folman et al., 1990) that conception rate is positively related to the concentration of progesterone in blood during the luteal phase of the cycle preceding insemination—the conception rate of the "Low P4" cows was lower than the conception rate of "High P4" cows (Table 1.10). On the other hand, treatment with a PRID did not improve the conception of "Low P4" cows, compared to "Low P4" cows that were treated with PGF alone.

Another problem that was tested in this experiment, was the relationship between loss in body condition score and conception. The results showed no relation between reproductive performance and loss in body condition between calving and week 5 postpartum. It is interesting to note that the absolute values of the body condition score were much higher in the US herd than in the Israeli herds, but the loss in body condition during the 5 weeks postpartum was similar in the two countries (Tables 3 and 4). The difference between Israel and the US might have been due to subjective differences in scoring between the technicians in the two countries. On the other

hand, the shorter interval from calving to conception in the US herd might perhaps be associated with the better body condition of the cows in the US herd. Our previous work had indicated that greater losses in body condition during the first 5 weeks postpartum were associated with lower fertility during the normal breeding period (Britt, 1992). The lack of such a relationship in the current studies may be associated with lower absolute losses in condition because cows calved at slightly lower body condition scores, or feeding practices may have differed.

The better reproductive performance among synchronized than non-synchronized cows was primarily associated with earlier service. Among all nine herd x treatment subclasses, synchronized cows had shorter intervals to first AI than non-synchronized cows (Table 1.13), and all nine groups had fewer open days for synchronized cows. About 70% of hormonally-treated cows were synchronized; therefore, if procedures could be developed to increase the synchronization rate, it would have a great impact on overall reproductive performance. Conception rates at first AI were greater among synchronized cows in only 5 of 9 groups.

Reproductive performance among cows treated with two injections of PGF given 14 days apart was equivalent to or better than that for cows inserted with a PRID between the two PGF injections. The cost and convenience of using this PGF scheme for reproductive management of clusters of cows makes it an attractive procedure for adoption by dairy farmers.

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Experiment 3 (Israel)

The aim of the experiment was to study putative mechanisms by which endogenous and exogenous progesterone may affect conception. This experiment included 3 studies which investigated effects of progesterone on: (a) the reproductive tract, (b) estradiol and LH concentrations in peripheral blood and (c) development and function of the ovulatory follicle.

Study A. The effect of PRID-treatment on progesterone concentrations and on DNA content in the uterus and in the ovaries

Results of our previous studies suggested that PRID exerted its effect not only by increasing peripheral plasma P4 concentrations, but possibly via a direct action on the reproductive tract. The aim of the experiment was to investigate the effect of PRID insertion on P4 concentrations and on DNA content in different regions of the reproductive tract.

Methods

Trial 1. Twenty-three cows received (i.m.) 500 µg of the prostaglandin F2α (PGF) analog cloprostenol (Estrumate). Fifteen cows that responded to the PGF injection with a decrease in plasma P4 concentrations were allotted to two groups according to parity and interval from calving: (a) control - received no further treatment; and (b) PRID-treated - PRID was inserted 8 days after the PGF injection. Cows were slaughtered 2 days after PRID insertion (i.e., 10 days after PGF injection).

Trial 2. Fifteen cows were treated in the same manner as in Trial 1, and 11 that responded to the PGF injection were slaughtered 4 days after PRID insertion (i.e., 12 days after PGF injection).

General procedures. Blood samples were collected from the jugular vein into heparinized vacutainers at the time of PGF injection, 2 days after PGF, at the time of PRID insertion, and on the day of slaughter. Immediately after slaughter, tissue samples from the uterine body (UB), uterine horns (proximal and distal to UB), corpus luteum (CL) and ovarian stroma were dissected. The CL was separated completely from the surrounding tissues and care was taken that it was free

of ovarian stromal tissue. The tissue samples were stored at -18°C . Progesterone concentrations in tissues were determined by the method of Batra and Bengtsson (1976).

In Trial 2, DNA was determined in tissue samples from the uterine body, and in duplicate samples from the proximal and the distal regions of the uterine horns. The uterine samples were separated into the myometrium and endometrium layers. DNA content was determined by the method of Giles and Myers (1965).

Results

Peripheral plasma progesterone concentrations. In Trial 1, the mean peripheral plasma P4 concentration was significantly higher on the day of PRID insertion in the PRID-treated group than in the control group (Table 3.1). Blood samples were collected immediately before the PRID was inserted; therefore, the P4 concentration on the day reflects only the endogenous P4 and values did not differ between groups. Peripheral P4 concentration was greater in PRID-treated than control cows on the day slaughter (Table 3.1). The increase in P4 concentrations during the days of treatment was greater in the PRID-treated group (Table 3.1).

In Trial 2, no significant difference in P4 concentration was found between the control and PRID-treated groups either on the day of PRID insertion or 4 days later (Table 3.1). In this trial, the increase in P4 concentrations during the days of treatment did not differ between control and PRID-treated groups (Table 3.1).

Progesterone concentrations in the reproductive tissues.

Uterine body and horns. In cows treated with a PRID for 2 days, P4 concentrations in the uterine body (UB) and in the adjacent sections of the uterine horns were significantly higher than in cows of the control group (Table 3.2). Four days after PRID insertion, P4 concentrations in the proximal part of the uterine horn was still higher than in the control group, whereas in the UB the P4 concentration in the treated group was similar to that found in the control group (Table 3.2). In both the control and the PRID-treated groups, no significant correlation was found between P4 concentrations in peripheral plasma and in the tissues (day 2: control, $r=-0.02$ and PRID, $r=0.18$; day 4: control, $r=0.24$ and PRID $r=0.37$).

Table 3.1. Mean progesterone concentrations (ng/mlSE) in the plasma of control and of PRID-treated dairy cows.

Treatment	Control	PRID
Cows slaughtered 2 days after PRID insertion (Trial 1)		
No. of cows	7	8
Day of PRID insertion	1.3±0.3*	2.1±0.2*
Day of slaughter	2.9±0.5**	4.7±0.5**
Plasma P4 increase during treatment	1.3±0.1*	2.5±0.4*
Cows slaughtered 4 days after PRID insertion (Trial 2)		
No. of cows	5	6
Day of PRID insertion	1.5±0.3 ^a	1.3±0.3 ^a
Day of slaughter	5.4±0.4 ^b	6.3±0.3 ^b
Plasma P4 increase during treatment	3.9±0.9	4.9±0.4

*,** means differ significantly (* P<0.05, ** P<0.01)

a,b - values with different superscripts differ significantly (P<0.05)

Table 3.2. Mean progesterone concentrations (ng/g wet tissue[±]SE) in the reproductive tract of control and PRID-treated dairy cows slaughtered 2 and 4 days after PRID insertion

Treatment	Control	PRID
Cows slaughtered 2 days after PRID insertion (Trial 1)		
No. of cows	7	8
Uterine body (UB)	26.2±1.8**	43.2±2.9**
Uterine horns		
proximal to UB	16.4±1.5*	26.3±3.9*
distal to UB	19.5±2.0	26.7±5.1
Ovarian stroma		
CL-bearing ovary	821±253+	912±298+
Ovary contralateral to CL	63±33	46±7.5
Corpus luteum (CL)	3718±1576	2713±484
Cows slaughtered 4 days after PRID insertion (Trial 2)		
No. of cows	5	6
Uterine body (UB)	20.6±1.8	24.8±2.6
Uterine horns		
proximal to UB	17.9±1.5*	28.3±3.4*
distal to UB	21.2±5.4	23.9±5.0
Ovarian stroma		
CL-bearing ovary	483±99++	772±130++
Ovary contralateral to CL	84±23.5	114±27
Corpus luteum (CL) ^a	7,655±1,548	8,005±1,493

*, **means of the control and PRID-treated groups differ significantly: * P<0.05, **P<0.001

+, ++significantly higher than in the contralateral ovary: + P<0.05, ++ P<0.001

^a significantly higher than in Trial (P<0.001)

Ovaries. In both the control and the PRID-treated cows in both experiments, the P4 concentration in the stroma of the CL-bearing ovary was significantly greater than in the stroma of the contralateral ovary. Treatment with PRID had no effect on P4 concentrations in the CL or in the ovarian stroma (Table 3.2). P4 concentrations in the CL of control and the PRID-treated groups slaughtered on day 4 were significantly higher ($p < 0.001$) than on day 2 (Table 3.2).

DNA in the reproductive tract. DNA content in the endometrium of the uterine horns (both proximal and distal to the UB) of cows treated with a PRID for 4 days was significantly greater than that of the control cows (Table 3.3). No such difference was found in the myometrium of the uterine horns. In PRID-treated cows, the DNA concentrations in the endometrium of the uterine horns were higher than in the myometrium, whereas in the control group both layers had similar DNA concentrations. In the control cows, the DNA content of the distal section of the uterine horns was significantly higher than that of the proximal sections (Table 3.3). No such difference was observed in the PRID-treated cows.

When tissue P4 concentrations were expressed in relation to DNA content, no significant differences were found between the control and the PRID-treated groups, in any of the sections that were examined (Table 3.4).

Discussion

Progesterone concentrations in the uterine body of cows 2 days after PRID insertion treated higher in the PRID-treated group than in the control group. In contrast, after 4 days of treatment no difference was found between the PRID-treated and control group. The higher P4 concentration in the UB of PRID-treated cows may have resulted from the increase in the peripheral plasma P4 concentrations following PRID insertion; however, this seems unlikely because there was no increase in the UB progesterone concentrations in cows that were treated with PRID for 4 days and these cows had higher peripheral P4 concentrations than cows treated for 2 days. It may rather be postulated that during the first days of treatment, large amounts of progesterone were released from the PRID into the vaginal fluids and were then transferred locally into the adjacent tissues.

Table 3.3. DNA content (mg/g wet tissue $\pm$ SE) in the reproductive tract of control and PRID-treated dairy cows slaughtered 4 days after PRID insertion (Exp. 2)

		Control	PRID
No. of cows		5	6
Uterine body (UB)	myometrium	3.6 $\pm$ 2.0	5.5 $\pm$ 1.8
	endometrium	4.0 $\pm$ 1.7	5.1 $\pm$ 1.7
Uterine horns			
proximal to UB	myometrium	3.2 $\pm$ 1.5	5.6 $\pm$ 1.5
	endometrium	1.8 $\pm$ 0.3*	8.8 $\pm$ 2.1*
distal to UB	myometrium	8.8 $\pm$ 1.1 ⁺⁺	7.9 $\pm$ 1.2 ^a
	endometrium	8.8 $\pm$ 0.9* ⁺	13.3 $\pm$ 1.9* ^b
Ovarian stroma			
Corpus luteum		3.5 $\pm$ 2.1	3.7 $\pm$ 1.3
(CL)-bearing ovary			
contralateral ovary		5.4 $\pm$ 1.8	6.5 $\pm$ 1.7
CL		4.6 $\pm$ 1.5	4.8 $\pm$ 1.2

* means of the control and PRID-treated groups differ significantly: $P < 0.05$

+, ++ significantly higher than the proximal section: + $P < 0.05$; ++ $P < 0.01$

a, b values with different superscripts differ significantly: $P < 0.05$

Table 3.4. Tissue progesterone concentration relative to weight and to DNA content (mean $\pm$ SE) in the reproductive tract of control and PRID-treated dairy cows slaughtered 4 days after PRID insertion (Exp. 2)

		P4 (ng/g wet tissue)		P4 (ng/mg DNA)	
		Control	PRID	Control	PRID
No. of cows		5	6	5	6
Uterine body (UB)	myometrium	20.2 $\pm$ 2.4	26.3 $\pm$ 4.6	8.1 $\pm$ 5.0	7.3.3
	endometrium	21.0 $\pm$ 2.9	19.2 $\pm$ 3.2	9.6 $\pm$ 2.9	6.0 $\pm$ 2.4
Uterine horns:					
proximal to UB	myometrium	16.2 $\pm$ 1.8*	31.4 $\pm$ 4.3*	8.8 $\pm$ 3.3 ⁺	7.7 $\pm$ 3.2 ⁺
	endometrium	19.5 $\pm$ 1.5	25.7 $\pm$ 3.6	10.2 $\pm$ 2.7 ⁺⁺	6.4 $\pm$ 2.6 ⁺⁺
distal to UB	myometrium	18.9 $\pm$ 4.7	23.8 $\pm$ 4.7	3.0 $\pm$ 0.9	3.6 $\pm$ 0.9
	endometrium	23.4 $\pm$ 6.7	24.1 $\pm$ 5.7	3.2 $\pm$ 0.7	2.3 $\pm$ 0.7

* means differ significantly: * $P < 0.01$

+, ++ Significantly higher than the distal section of the uterine horns: + $P < 0.05$, ++ $P < 0.01$

In cows that were treated with PRIDs for 2 or 4 days, the P4 concentrations in the sections of the uterine horns that are adjacent to the uterine body were significantly increased compared values for control cows. No such difference between the PRID-treated and control groups was found in the sections of the uterine horns distal to the uterine body. These results, together with the fact that no correlation was found between P4 concentrations in plasma and in tissues, support the hypothesis that P4 released from the PRID is locally transported from the vagina to the adjacent sections of the uterus. Local transport of P4 from the CL to adjacent sections of the uterus has been reported by several authors, who found that P4 concentrations in the tissues and fluids of the uterine horns ipsilateral to the CL-bearing ovary were higher than in the contralateral horn (Pope et al., 1982; Weems et al., 1988; Wittkowski et al., 1989). Local transport of P4 from the PRID into the reproductive tract may have a beneficial effect on conception as was found in a previous study, in which treatment with a PRID increased the conception rates of cows that had low peripheral P4 concentrations (Folman et al., 1990).

After 4 days of treatment, the DNA content in the endometrium of the uterine horns of PRID-treated cows was significantly higher than in the control cows. This suggests that treatment with PRIDs stimulated the proliferation of uterine endometrial cells. A stimulatory effect of progestins on cell proliferation has also been observed in long-term cell cultures of human endometrial stromal cells (Chen et al., 1989; Zue et al., 1990). Since the ratio between P4 concentrations and DNA content in the uterine body and the uterine horns of the PRID-treated cows was smaller than in the control group, it is postulated that the increase in the uterine P4 in the treated cows was due to an increase in the number of progesterone-binding cells, rather than a result of a higher P4 concentration per cell.

In conclusion, we suggest that P4 from PRIDs is delivered to the adjacent sections of the uterus by local transport via uterine fluids or by way of the lymphatic system. The greater P4 concentrations in the uterine tissues of the PRID-treated animals affect the rate of cell proliferation in the endometrium. Higher tissue P4 concentrations may promote the development of an adequate uterine environment for embryo implantation and subsequent development.

Study B. Influence of PRID treatment on follicular development and associated endocrine changes

The experiment was undertaken to assess the changes in reproductive hormones, particularly LH and estradiol, during treatment with a PRID. It is now clear that growth, development and persistence of dominant perovulatory follicle and follicular secretion of estradiol is regulated by pulsatile secretion of LH, which in turn is influenced mainly by the concentration of progesterone. So it was of interest to determine how PRID treatment influenced endocrine traits associated with follicular development.

Methods

Cows were allotted into two groups, a PGF group (n=33) and a PRID group (n=30). Both groups were given two injections of PGF 14 days apart and the PRID group was inserted with a PRID 8 days after the first PGF. The PRID was removed on the day of the second PGF (so that luteolysis could be evaluated in the absence of exogenous P4). Cows were between 40 and 60 days postpartum at the time of the first PGF, and blood was collected on alternate days for determination of progesterone and estradiol. From the second PGF injection until 24 hours after estrus, blood samples were collected 3 x daily for estrogen and LH. Additionally, blood samples were collected every 15 minutes for 6 hours on day 10, 2 days after insertion of the PRID.

Results and Discussion

Plasma progesterone concentrations for the two treatment groups are shown in Figure 3.1. Progesterone increased following PRID insertion and remained higher than in the PGF-treated cows up to the time of the second PGF injection. Estradiol concentrations in the two treatment groups are shown in Figure 3.2. Insertion of the PRID suppressed estradiol concentrations during the 3 days preceding estrus.

Characteristics of LH secretion during the 2nd day following PRID insertion are presented in Table 3.5 for cows with "Low P4" or "High P4" immediately prior to the PRID insertion. The PRID treatment did not influence the characteristics of LH secretion, indicating that the difference

in endogenous levels of progesterone probably were already regulating the pattern of secretion. If cows had been sampled later, perhaps on day 12, 4 days after PRID insertion, we might have detected differences in LH.

Figure 3.1. Progesterone in PGF- and PRID-treated cows (Study B).

Figure 3.2. Estradiol in PGF- and PRID-treated cows (Study B).

Table 3.5. Secretion of LH 10 days after the 1st PGF in cows
with low or high progesterone, Israel

Trait	Progesterone < 2.0 ng/ml			Progesterone > 2.1 ng/ml		
	PGF	PRID	Combined	PGF	PRID	Combined
No. cows	5	4	9	6	5	11
Plasma progesterone (ng/ml)	1.2	1.5	1.3	3.6	6.1	4.7
Basal LH (ng/ml)	6.0	5.2	5.7	4.4	3.9	4.2
LH pulse frequency (pulses/6h)	1.0	1.0	1.0	2.8	2.0	2.4
LH pulse amplitude (ng/ml)	1.8	1.4	1.6	1.3	0.6	1.0

Interval from the 2nd PGF to onset of estrus was affected by progesterone concentrations. The interval to estrus was 72.0 and 65.8 h for the PGF and PRID cows, respectively, within the "High P4" category compared with 77.8 and 89.3 within the "Low P4" category. This difference would be consistent with a different pattern of growth of the dominant follicle among "Low P4" and "high P4" cows, respectively, because cows with "High P4" were probably more likely to have a healthy pre-ovulatory follicle at the time of the 2nd PGF, whereas the "Low P4" group might have had to recruit a new pre-ovulatory follicle.

Study C. Effect of peripheral P4 concentrations on the development and function of the ovulatory follicle

The aim of the experiment was to study the effects of progesterone on the development and function of the ovulatory follicle. We developed an experimental protocol that enabled us to control peripheral plasma progesterone concentrations, and we examined effects of exposure to "Low P4" (<2.5 ng/ml) and "High P4" (>4.0 ng/ml) on the size of follicles, on the steroid content of the follicular fluid, and on the viability and steroidogenic capacity of the follicular cells.

Methods

Cows received (i.m.) 500 µg of cloprostenol and those detected in estrus following the PGF injection were allotted into four treatment groups:

HP4d9 - Cows in this group were treated for 3 days with progesterone-impregnated controlled release devices with the aim of obtaining high peripheral P4 concentrations. Two CIDRs (Eazibreed, Hamilton, New-Zealand, 1.9 g progesterone) were inserted into the vagina on day 6 and removed on day 9 of the estrous cycle.

LP4d9 - Cows in this group were treated to obtain low peripheral P4 concentrations. The cows were injected with PGF on days 6 and 7 of the estrous cycle to regress the corpus luteum, and two CIDRs were inserted on day 6 and removed on day 9 of the estrous cycle.

HP4d15 - Cows in this group were treated for 9 days with a progesterone-impregnated controlled release devices with the aim of obtaining high peripheral P4 concentrations. Two CIDRs

were inserted into the vagina on day 6 and replaced every 3 days until day 15, when they were removed.

LP4d15 - Cows in this group were treated to obtain low peripheral P4 concentrations for 9 days. The cows were injected with PGF on days 6 and 7 of the estrous cycle to regress the corpus luteum, and two CIDRs were inserted on day 6 and replaced every 3 days until day 15, when they were removed.

Cows from Groups HP4d9 and LP4d9 were slaughtered on day 9 of the estrous cycle and those from groups HP4d15 and LP4d15 were slaughtered on day 15. Within 15 to 20 minutes after slaughter the ovaries were removed and maintained on ice until dissection. The largest 1 to 3 follicles from each pair of ovaries were collected, each follicular diameter was measured and the follicular fluid was aspirated and frozen until determinations for progesterone, androstendione and estradiol-17 β (McNatty et al, 1981). The granulosa and theca cells of the largest follicles were separated from the follicular wall and their number and viability were determined (Meidan et al., 1990). Follicles with more than 10^6 viable granulosa cells which had a viability of >40% were considered to be healthy dominant follicles. Atretic follicles were defined as follicles with low viability of granulosa cells (<40%).

The steroidogenic capacity of the granulosa and theca cells of the dominant follicles was determined in an in vitro culture system (Meidan et al., 1990). Granulosa cells (100,000 cells/well) were incubated for 18 hours either with medium alone, or with medium supplemented with testosterone (300 ng/ml) as a substrate for aromatase activity. Theca cells were incubated with medium without and with forskolin (10 μ M), given to activate the adenylate cyclase system. After 18 h of incubation, spent media were collected and frozen until determinations for androstendione and estradiol-17 β were performed.

Results

Peripheral plasma progesterone concentrations. During the treatment period, plasma P4 concentrations in the "High P4" groups (HP4d9 and HP4d15) were in the normal range of luteal phase concentrations, whereas in the "Low P4" groups (LP4d9 and LP4d15) a constant low level

near 2 ng/ml was maintained throughout treatment (Figure 3.3). The P4 concentrations beyond day 6 in the "High P4" groups were greater than in the "Low P4" groups (treatment x day $P < 0.0001$).

Follicular characteristics. In all cows that were slaughtered on day 9 of the estrous cycle (groups HP4d9 and LP4d9), only one large follicle was found on each pair of ovaries. Cows from the "High P4" group that were slaughtered on day 15 (HP4d15) had at least two large follicles on their ovaries, but cows from LP4d15 group only one large dominant follicle on each pair of ovaries and no medium sized follicles were observed (Table 3.6). The diameter of the dominant follicle in the "Low P4" groups (LP4d9 and LP4d15) was significantly greater than in the "High P4" groups (Table 3.6).

Steroids in follicular fluid and in follicular cell culture media. In both "Low P4" groups (LP4d9 and LP4d15), the estradiol-17 β concentration in the follicular fluid was increased ten-fold compared to that found in follicles from the "High P4" groups (Table 3.7). Androstendione concentration in the "Low P4" groups tended to be higher than in the "High P4" groups, but the difference was not statistically significant.

Figure 3.3. Peripheral plasma concentrations during treatment period in cows with high (High P4) and low (Low P4) progesterone concentrations (Study C).

Table 3.6. Follicular characteristics in cows with high and low peripheral progesterone concentrations

Treatment	Low P4	High P4
<u>Day 9</u>		
No. of cows	4	3
No. of dominant follicles	4	4
No. of atretic follicles	0	0
Follicle diameter (mm)	20.0±0.0**	12.8±1.7
Follicular fluid volume (ml)	2.6±0.2*	1.1±0.4
<u>Day 15</u>		
No. of cows	5	5
No. of dominant follicles	5	6
No. of atretic follicles	0	6
Follicle diameter (mm)	21.6±1.8**	10.9±0.9
Follicular fluid volume (ml)	3.5±0.6**	0.7±0.1

*,** Means of experimental groups differ significantly: * p<0.01, ** p<0.001

Table 3.7. Steroids concentrations (ng/ml±S.E.) in follicular fluid of follicle from cows with high and low peripheral P4 concentrations

Treatment	Low P4	High P4
<u>Day 9</u>		
No. of follicles	4	4
Estradiol 17β	645±112**	50.6±23.6
Progesterone	34.3±10.6	38.8±3.0
Androstendione	8.0±2.8	5.5±2.3
<u>Day 15</u>		
No. of follicles	5	6
Estradiol-17β	36.3±111*	44.8±7.1
Progesterone	20.4±3.5	19.2±2.8
Androstendione	4.8±0.9	3.0±0.7

*,** Means of experimental groups differ significantly: * p<0.05, ** p<0.001

Granulosa cells that were incubated in vitro for 18 hours from the "Low P4" groups produced significantly more estradiol than cells from the "High P4" groups (Table 3.8). When testosterone was added to the incubation medium, the estradiol production by granulosa cells did not differ between LP4d9 and the HP4d9 groups. In contrast, granulosa cells from the LP4d15 group that were incubated in the presence of testosterone produced twice as much estradiol as cells from the HP4d15 group ($P < 0.05$; Table 3.8).

Theca cells from follicles of the LP4d9 group produced significantly more androstendione than theca cells from the HP4d9 group ($P < 0.05$; Table 3.8). No difference in androstendione production was found between the groups that were slaughtered on day 15 (LP4d15 and HP4d15).

Discussion

In untreated cyclic cows, the largest follicle that exists on the ovary on day 9 of the estrous cycle is a dominant follicle from the first follicular wave (Ginther et al., 1989; Fortune et al., 1991). In Study 3, cows with low peripheral P4 concentrations (LP4d9) as well as cows with normal P4 levels (HP4D9) had only one large follicle on their ovaries on day 9 of the cycle. The large follicle was dominant morphologically and functionally—it was the largest follicle on the ovary, it suppressed the recruitment of other follicles, and the estradiol/progesterone ratio in the follicular fluid was > 1 . Although the follicles from LP4d9 and HP4D9 groups originated from the first follicular wave and were of similar age, they differed considerably in size and function. Follicles that developed in the ovaries of cows with low peripheral P4 concentrations were larger, had higher estradiol concentrations in their follicular fluid and their theca cells produced more androstendione than follicles that developed in cows with high peripheral P4 concentrations.

In untreated cows, the largest follicle present on the ovary on day 15 of the estrous cycle is a dominant follicle from the second wave of follicular development (Lucy et al., 1992; Fortune et al., 1993). In the "High P4" group (HP4D15) at least two large follicles—one healthy and one atretic—were present on the ovaries. This situation indicates that a turnover of the first wave dominant follicle by a second wave follicle had taken place. In contrast, in the "Low P4" group (LP4D15) only one large follicle was present, and no medium-sized follicles were observed. It is

Table 3.8. Steroid production during 18h incubation by granulosa and theca cells from cows with high and low peripheral P4

	Day Low P4	9 High P4	Day Low P4	15 High P4
<u>Theca (ng androgen / 10⁵ cells)</u>				
Medium	0.7±0.05*	0.4±0.07	0.5±0.1	0.2±0.05
Medium+FRS ⁺	3.1±0.4*	0.7±0.2	1.4±0.6	0.7±0.3
<u>Granulosa (ng estradiol / 10⁵ cells)</u>				
Medium	1.6±0.1**	0.1±0.05	0.5±0.07*	0.05±0.009
Medium+T ⁺⁺	7.1±2.7	7.3±2.7	17.4±1.9*	8.7±1.5

*,**Means between high and low P4 groups differ significantly: * p<0.05, ** <0.001

⁺,⁺⁺ FRS - Forskulin, T - Testosterone

most probable that this large follicle originated from the first wave of follicles, since it has been found in an ultrasonographic study that treatment with low levels of P4 on days 6 to 13 of the estrous cycle prevented the turnover of the first wave with the second wave dominant follicle (Ahmad et al., 1995). In Study 3 we found that the persistent first-wave follicles differed from the follicles of the second wave—they were larger, had higher estradiol concentrations in the follicular fluid, and their granulosa cells possessed a higher aromatizing capacity (Table 3.8).

The first wave dominant follicles that developed in cows with low peripheral P4 concentrations reached their maximal size and their maximal estradiol concentration after 3 days of treatment. Longer development of follicles in a low P4 environment did not result in any further increase in size or steroid content. The results of the incubation procedures suggest that the high estradiol concentrations in the follicular fluid of young first wave follicles (LP4D9) resulted from higher androstendione production by their theca cells, whereas in the persistent first wave follicles (LP4D15) it resulted from high estradiol production by the granulosa cells. The persistent first-wave follicles in the LP4D15 group had some characteristics which are typical of follicular cysts—a large diameter, an extend period of development, and the ability to produce estradiol like normal follicles (Kesler and Garverick, 1982).

In Study 3 no difference were observed between follicles from the first or second follicular wave that developed in cows with high peripheral progesterone concentrations (HP4D9 and HP4D15). This is not surprising because in both "High P4" treatment groups the follicles that were collected were young follicles that had developed in a normal hormonal environment for a similar period.

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Experiment 4 (US) Influence of progesterone on embryo development.

The aim was to assess early embryonic development in cows that differed in progesterone concentrations before first AI that occurred at an estrus induced by two injections of PGF given 14 days apart. Differences in rates of recovery between cows that differed in progesterone concentrations or differences in fertilization rates or embryonic development would be taken as evidence that effects of progesterone level on embryo survival are exerted before day 7 after A.I.

Methods

Holstein cows in two herds (North Carolina Agricultural Research Service Dairy Field Laboratory, NCARS, and North Carolina Department of Agriculture Umstead Unit, NCDA) were assigned to clusters based upon calving dates—cows within a cluster calved within a 3-week period in the same herd (Figure 4.1). The two herds were of the same genetic base, were fed maize-silage based total mixed rations to support maximum milk yield, were milked twice daily and were housed in freestall barns with controlled access to dirt exercise lots for detection of estrus. Cows were subjected to experimental procedures during October through early June of two consecutive years to minimize direct effects of heat stress on fertility.

Commencing 3 weeks before the first of two injections of PGF, milk samples were collected each Monday, Wednesday and Friday for determination of concentrations of progesterone (Figure 4.1). Milk samples were stored at 4° C for up to 24 hours before being centrifuged to remove fat. Progesterone was determined on skim-milk samples by use of a solid-phase commercial radioimmunoassay (Morbeck et al., 1991).

The first injection of PGF was given on a Monday when cows within a cluster were 44 to 64 days postpartum, and the second injection of PGF was given 14 days (Figure 4.1). After the second injection of PGF, cows were inseminated at estrus with frozen-thawed semen from a single ejaculate of a Holstein bull with known fertility. Seven days after estrus, the uterus of each cow was flushed by standard non-surgical embryo recovery procedures (Shaw et al., 1995). One scientist (DWS) conducted 85% of the embryo recovery flushing procedures. Embryos were examined through the use of a stereomicroscope and classified (Farin et al., 1995) according to

stage (morulae, compact morulae, early blastocyst, blastocyst, expanded blastocyst, or hatched blastocyst) and grade (excellent, some defects, major defects or degenerate). Subsequently, each embryo was mounted on a microscope slide and stained for direct microscopic count of the number of cells.

Cumulative milk yield data were obtained from Dairy Herd Improvement (DHI) records for each cow for the period ending at the first test that occurred subsequent to embryo collection. Non-parametric data were analyzed by chi square analyses (SAS, 1988). Data regarding intervals and milk yield were subjected to least squares analysis of variance using models that examined effects of herd, parity, estrus status, embryo recovery status, and their potential interactions (SAS, 1988). Blood progesterone data were averaged for samples collected 11 and 14 days after the first PGF injection, and then subjected to frequency analysis. Embryo recovery percentage was compared by chi-square analysis for cows in each blood progesterone quartile. Milk progesterone data were subjected to a split-plot analysis for repeated measures with period (3-day sampling interval), day, herd and embryo recovery status as effects in the model. Main effects that were not significant in an analysis were eliminated from consideration in subsequent analyses of the same trait.

Results

Overall summary. A summary of results of Experiment 4 is in Table 4.1. Of 119 cows that were assigned to the study, 89 were in the NCARS herd and 30 from the NCDA herd. Cows were distributed among three parity groups (1, 2 $\geq$ 3), and three-fourths of the cows were experiencing estrous cycles before the first PGF injection based on profiles of milk progesterone. Among 63 cows detected in estrus after the second injection of PGF, an embryo was recovered from 41.

Cows were in estrus at 3.4 ± 0.2 and 5.2 ± 0.5 days after the second injection of PGF in the NCARS and NCDA herds, respectively (Table 4.2; $P < .05$), but the reason for this difference was unapparent. There were no differences in interval to estrus among parity groups or between embryo recovery groups (Table 4.2).

Table 4.1. Summary of data from Experiment 4.

	All Cows	Cows in Estrus
No. cows	119 (100%)	63 (100%)
Herd		
NCARS	89 (75%)	49 (78%)
NCDA	30 (25%)	14 (22%)
Parity		
1	47 (40%)	29 (46%)
2	34 (28%)	18 (29%)
≥ 3	38 (32%)	16 (25%)
Cyclic status before 1st PG		
Cyclic	88 (74%)	51 (81%)
Anestrus	31 (26%)	12 (19%)
Estrus after 2nd PG		
Yes	63 (53%)	63 (100%)
No	56 (47%)	0 (0%)
Embryo recovered		
Yes	41 (34.5%)	41 (65.1%)
No	78 (65.5%)	22 (34.9%)

Days from calving to embryo recovery did not differ among herds, parity groups or embryo recovery groups (Table 4.3), and there was little variation in the interval from calving to recovery because of the experimental design.

Milk yield did not differ between herds, but multiparous cows produced more milk in the first 90 days of lactation than primiparous ones (Table 4.4). There was no difference in milk yield between cows from which an embryo was recovered and from which none was recovered.

Cyclic status. Cyclic status before the first injection of PGF was not influenced by herd and proportions classified as anestrus were similar between all cows and cows detected in estrus after the second injection of PGF (Table 4.5). Among all cows, primiparous cows had a greater incidence of anestrus than multiparous cows (Table 4.6), but this was not true for cows detected in estrus at the target breeding window (Table 4.6).

Estrus status. Cows that were cyclic before the first injection of PGF were detected in estrus after the second injection of PGF at a greater rate than anestrus cows (Table 4.7). There were no differences between herds (Table 4.8) or among parities (Table 4.9) in proportion detected in estrus.

Embryo recovered status. There was no difference between cows classified as anestrus or cyclic in proportion from which an embryo was recovered, regardless of whether all cows were considered or only those that were detected in estrus were evaluated (Table 4.10). When all cows were considered, success of embryo recovery did not differ between herds (Table 4.11), but among cows detected in estrus, recovery success was greater in the NCDA herd than in the NCARS herd (Table 4.11). Parity had a major influence on success of embryo recovery, both among all cows (Table 4.12) and among those in estrus (Table 4.12). Among primiparous cows detected in estrus, success of embryo recovery was more than double the success rate in Parity 2 cows and was nearly 40% greater than success rate in older cows (Table 4.12).

Influence of progesterone. Among the 63 cows in estrus after the second injection of PGF, 56 were exhibiting regular estrous cycles as determined by thrice-weekly milk progesterone concentrations. The average milk progesterone concentration for samples collected thrice weekly

Table 4.2. Days from 2nd PGF to estrus for Experiment 4.

Classification	Mean $\pm$ SEM
Herd*	
NCARS	3.4 $\pm$ 0.2
NCDA	5.2 $\pm$ 0.5
Parity	
1	4.6 $\pm$ 0.3
2	3.9 $\pm$ 0.4
≥ 3	4.4 $\pm$ 0.5
Embryo recovered	
No	4.5 $\pm$ 0.4
Yes	4.1 $\pm$ 0.3

*P<.05

Table 4.3. Days from calving to embryo recovery for Experiment 4.

Classification	Mean $\pm$ SEM
Herd	
NCARS	76.7 $\pm$ 1.0
NCDA	77.6 $\pm$ 2.2
Parity	
1	78.5 $\pm$ 1.5
2	75.6 $\pm$ 1.9
≥ 3	77.4 $\pm$ 2.1
Embryo recovered	
No	78.1 $\pm$ 1.8
Yes	76.2 $\pm$ 1.3

Table 4.4 Milk yield (kg/d) until 90 days postpartum for Experiment 4.

Classification	Mean $\pm$ SEM
Herd	
NCARS	35.2 $\pm$ 0.9
NCDA	33.6 $\pm$ 2.4
Parity	
1	28.0 $\pm$ 1.3
≥ 2	40.8 $\pm$ 2.2
Embryo recovered	
No	34.5 $\pm$ 1.8
Yes	34.4 $\pm$ 1.4

Table 4.5. Herd x cyclic status for Experiment 4.

Herd	Cyclic Status	
	Cyclic	Anestrus
All Cows ^a		
NCARS	63 (71%)	26 (29%)
NCDA	25 (83%)	5 (17%)
Cows in Estrus ^b		
NCARS	39 (80%)	10 (20%)
NCDA	12 (86%)	2 (14%)

^aChi square 1.834 1 df P<.176

^bChi square .265 1 df P<.607

Table 4.6. Parity x cyclic status for Experiment 4.

Parity	Cyclic Status	
	Cyclic	Anestrus
All Cows ^a		
1	29 (62%)	18 (38%)
2	26 (76%)	8 (24%)
≥3	33 (87%)	5 (13%)
Cows in Estrus ^b		
1	23 (79%)	6 (21%)
2	14 (78%)	4 (22%)
≥3	14 (88%)	2 (12%)
^a Chi square 7.051 2 df P<.029		
^b Chi square .613 2 df P<.736		

Table 4.7. Cyclic status x estrus after 2nd PGF for Experiment 4 (All Cows).

Cyclic Status*	Estrus	
	No	Yes
Cyclic	37 (42%)	51 (58%)
Anestrus	19 (61%)	12 (39%)
*Chi square 3.408 1 df P<.065		

Table 4.8. Herd x estrus after 2nd PGF for Experiment 4 (All Cows).

Herd*	Estrus	
	No	Yes
NCARS	40 (45%)	49 (55%)
NCDA	16 (53%)	14 (47%)
*Chi square .634 1 df P<.426		

Table 4.9. Parity x estrus after 2nd PGF for Experiment 4 (All Cows).

Parity*	Estrus	
	No	Yes
1	18 (38%)	29 (62%)
2	16 (47%)	18 (53%)
≥3	22 (58%)	16 (42%)
*Chi square 3.239 2 df P<.198		

Table 4.10. Cyclic status x embryo recovered status for Experiment 4.

Cyclic Status	Embryo Recovered	
	No	Yes
All Cows ^a		
Cyclic	56 (64%)	32 (36%)
Anestrus	22 (71%)	9 (29%)
Cows in Estrus ^b		
Cyclic	19 (37%)	32 (63%)
Anestrus	3 (25%)	9 (75%)
^a Chi square .546 1 df P<.460		
^b Chi square .642 1 df P<.423		

Table 4.11. Herd x embryo recovered status for Experiment 4.

Herd	Embryo Recovered	
	No	Yes
All Cows ^a		
NCARS	61 (69%)	28 (31%)
NCDA	17 (57%)	13 (43%)
Cows in Heat ^b		
NCARS	21 (43%)	28 (57%)
NCDA	1 (7%)	13 (93%)
^a Chi square 1.400 1 df P<.237		
^b Chi square 6.111 1 df P<.013		

Table 4.12. Parity x embryo recovered status for Experiment 4.

Parity	Embryo Recovered	
	No	Yes
All Cows ^a		
1	22 (47%)	25 (53%)
2	28 (82%)	6 (18%)
≥3	28 (74%)	10 (26%)
Cows in Estrus ^b		
1	4 (14%)	25 (86%)
2	12 (67%)	6 (33%)
≥3	6 (38%)	10 (62%)
^a Chi square 12.674 2 df P<.002		
^b Chi square 13.725 2 df P<.001		

for 16 days prior to estrus in these cows was 1.57 ng/ml, and their average blood concentration of progesterone for days 11 and 14 after the first PGF was >1.0 ng/ml.

Embryos were recovered from 35 of 56 (62.5%) cows that were cyclic during 2-3 weeks prior to the 2nd PGF injection. Blood progesterone data (average of samples collected on days 11 and 14 after first PGF) for these cows was subjected to frequency analysis and then the embryo recovery rates were determined for cows in each progesterone quartile. Recovery rate was influenced significantly ($P < .01$) by the average concentration of blood progesterone on days 11 and 14 after the first PGF (Figure 4.2). For example, embryo recovery rate ranged from 28.6% for cows in the lowest progesterone quartile to 85.7% for cows in the highest (Figure 1).

Among the 56 cyclic cows, progesterone was influenced by sampling period ($P < .01$) and by embryo recovery status ($P < .05$), and there was no period by embryo recovery status interaction (Figure 4.3). Progesterone increased significantly during the estrous cycle preceding embryo recovery, reaching a peak during the period from 6 to 4 days before estrus. Prior to estrus, progesterone was greater in cows from which an embryo was recovered than in those from which embryo recovery was unsuccessful (Figures 4.3 and 4.4); however, after estrus, there was no difference between embryo status groups (Figure 4.3). When progesterone was examined during the period between the two injections of PGF, independent of day of estrous cycle for each cow, it increased at a greater rate and reached a greater peak for cows which subsequently yielded an embryo (Figure 4.5).

Seven cows that exhibited estrus after the 2nd PGF injection were classified as acyclic during PGF treatment based on low levels of progesterone before the 2nd PGF injection. These cows showed estrus during the target window following the 2nd injection of PGF, but it was not possible to attribute this response to the luteolytic action of PGF. The average milk progesterone concentration for samples collected thrice weekly for 16 days prior to estrus in these cows was 0.17 ng/ml, and their average blood concentration of progesterone for days 11 and 14 after the first PGF was <1.0 ng/ml. Nevertheless, embryos were recovered from 6 of 7 (85.7%) of these cows.

Figure 4.2. Percentage of embryos recovered from cyclic cows divided into quartiles based on average blood progesterone on days 11 and 14 after the 1st PGF.

Figure 4.3. Progesterone concentrations in milk samples collected before and after estrus for cows from which an embryo was recovered and for cows from which no embryo was recovered.

Figure 4.4. Average progesterone concentrations in milk samples collected before estrus from cyclic cows from which an embryo was recovered and from those from which no embryo was recovered.

Figure 4.5. Progesterone concentrations in milk samples collected before and after the second injection of PGF in cows from which an embryo was recovered for those from those from which no embryo was recovered.

Embryo stage and quality. None of the factors examined influenced stage of embryo development or embryo quality. Among 41 embryos recovered, 24 (58.5%) were at an appropriate stage of development and 17 (41.5%) were retarded or degenerate. Among the 24 embryos at an appropriate stage, 17 (74%) were of excellent quality or had minor defects. Number of cells differed between stages (Figure 4.6) and degenerate embryos had few cells.

Discussion

Prior studies by our groups (Folman et al., 1973; Fonseca et al., 1983) indicated that fertility at first insemination was positively related to concentration of progesterone in blood during the estrous cycle preceding insemination. Results from Experiment 4 showed that there was a positive relationship between concentration of progesterone and likelihood of recovering an embryo on day 7 after estrus among cyclic cows. Cows with higher concentrations of progesterone had higher recovery rates, and recovery rate increased for each progesterone quartile. When progesterone was compared between cows from which embryo recovery was successful or unsuccessful, progesterone was higher during the cycle preceding estrus in cows that yielded an embryo, but progesterone did not differ between groups during the 7 days after estrus.

Mechanism(s) through which greater concentrations of progesterone may influence recovery of an embryo are unknown. Progesterone might affect uterine or oviductal function related to spermatozoa transport and survival, fertilization of the oocyte, embryo transport, or embryonic development. Or it might influence follicular function and oocyte quality by regulating the persistence of follicle waves through its effect on pulse frequency of LH (Glencross, 1987).

Recent studies that have focused on fertility of cattle given various forms of exogenous progestogens (including progesterone) have revealed that doses adequate to control synchrony of estrus do not adequately regulate growth of the dominant follicle (Kojima et al., 1995; Sanchez et al., 1993; Sanchez et al., 1995; Wehrman et al., 1993). Cows given inadequate doses of progestogen have prolonged growth of the dominant follicle and larger dominant follicles, and this results in lower fertility. Whether such a phenomenon happens under natural circumstances because of natural differences in progesterone is unclear, but the our results would be consistent

Figure 4.6. Number of cells for embryos classified by stage of development at recovery on day 7.

with such an effect. Higher concentrations of progesterone could have caused more frequent emergence of new follicle waves and consequently resulted in better quality oocytes. A recent study with ewes given exogenous progesterone indicated that oocyte quality was strongly related to concentration of progesterone during preovulatory follicle growth (McEvoy et al., 1995).

Six of seven cows that were classified as acyclic between the two injections of PGF but that showed estrus after the 2nd PGF yielded embryos. These results were unexpected, but may indicate that emerging follicles capable of eliciting estrus are also capable of producing high quality oocytes. One would not have expected such follicles to persist in the absence of low levels of progesterone to inhibit LH.

Embryos were recovered from 65.1% of the cows detected in estrus. A recovery rate (embryos / corpus luteum) of 75.5% was reported for healthy superovulated donors that had ≤ 10 corpora lutea (Hasler et al., 1983). Based on this estimate, our recovery rate was of the same magnitude as that obtained in commercial embryo transfer. Thus, the strong relationship we observed between embryo recovery rate and progesterone concentration prior to the 2nd PGF appears to be a true physiological phenomenon.

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Evaluation of the Research Achievement

This project comprised three primary experiments focused on how progesterone prior to estrus and AI influenced fertility in high producing cow. In previous studies, we had observed that: 1) concentration of progesterone prior to first AI was positively correlated with conception rate at first AI, 2) supplemental progesterone provided by insertion of a PRID in cows with low endogenous progesterone improved fertility, and 3) fertility during the breeding period was inversely correlated with body condition loss during the first 5 weeks postpartum. In the current project, we conducted a replicated experiment to confirm and extend our previous observations and conducted complementary experiments focused on mechanisms of action.

Experiment 1 was conducted to confirm our prior observations regarding influence of progesterone on fertility. The experiment was replicated on a large scale (3 herds, 815 cows) in both countries and it also assessed how reproductive performance of high-producing cows subjected to similar management procedures differed between the US and Israel. The three herds utilized for Experiment 1 were managed similarly to account for similar environmental conditions in the US and Israel. For example, cows in both countries were not inseminated from mid June to early October to avoid the confounding effects of heat stress on fertility.

The reproductive performance of cows in both countries in Experiment 1 was amazingly similar. This provided persuasive evidence that the reproductive traits of US and Israeli Holsteins are essentially identical under well-controlled conditions. This is an important finding because it will allow liberal extrapolation of research results between countries in the future.

The results from Experiment 1 confirmed that the concentration of progesterone prior to first AI influenced fertility in a significant way. Thus, conception rate at first AI for cows that had “high” progesterone was 55.8% compared with 44.4% for cows with “low” progesterone. In contrast to our earlier observations, insertion of a PRID for 6 days did not improve fertility in “low” progesterone cows. We have no clear explanation for this finding except that PRID insertion was not able to raise progesterone to quite the same level as that seen in “high” progesterone cows that did not receive a PRID, and the duration of elevated progesterone in the

PRID-treated “low” progesterone cows was shorter than that seen in “high” progesterone cows. These results indicated that it would not be cost-effective to use a PRID routinely to improve fertility.

Changes in body condition during the first 5 weeks after calving did not consistently influence fertility during the breeding period in Experiment 1. Based on our previous work, we had anticipated that cows that lost more body condition would have lower fertility, but this was not the case. Cows in the current studies may have been fed differently from cows in our earlier studies, because cows in the current experiment were fed total-mixed rations and in earlier studies the feedstuffs had been provided separately. Also, the relative change in body condition from calving to week 5 in the current study was less than that seen earlier, indicating that management of dry cows and early postpartum cows may have been better than when earlier studies were conducted. In general we conclude that moderate losses in body condition during the first 5 weeks postpartum are not detrimental to fertility during the breeding period.

It was clear from Experiment 1 that fertility during October-November was lower than during December-January, February-March and April-June. Cows treated in October-November had lower conception rates 5 to 14% lower than cows bred in the other months. Days open for cows treated in October-November exceeded values for other months by 6 to 13 days. The period of October-November is beyond the normal period of heat-stress in both countries, but the lower fertility may be a carry-over effect of heat stress through a long-term effect on follicular development. The percentage of noncycling cows was actually lower for cows inseminated in October-November, so the lower fertility was not associated with acyclicity. These results imply that cows calving in late summer under heat-stress conditions experience a carry-over effect into the cooler season.

Experiment 3 provided evidence that supplemental progesterone provided through a PRID may exert part of its potential effect locally rather than systemically. Cows inserted with a PRID had higher concentrations of progesterone in uterine tissue and fluids, but these higher concentrations were not always reflected in higher systemic concentrations. Thus, route of

administration of exogenous progesterone may have an important influence on its effectiveness.

Experiment 3 also showed that low concentrations of progesterone may cause lower fertility through effects on follicular development. Cows in Experiment 3 that had low progesterone during the early and mid-luteal phase of the estrous cycle had persistent follicles that had exaggerated production of estradiol. Such follicles would be expected to produce poor-quality oocytes as has been shown in other studies, and this should be associated with poorer fertility. Overall, the findings of Experiment 3 show that progesterone may influence fertility through several mechanisms, but the most important is probably its effect on the developing preovulatory follicle.

Experiment 4 showed that embryo recovery on day 7 after synchronized estrus, induced by two injections of PGF given 14 days apart, was strongly related to systemic concentrations of progesterone during the luteal phase in cyclic cows, but not in acyclic cows that showed a spontaneous estrus after the second injection of PGF. In cows with the highest concentrations of progesterone (highest quartile) and in cows that exhibited spontaneous estrus, the embryo recovery rate was 83 to 85%. In contrast, embryo recovery rate was 29% for cyclic cows in the lowest progesterone quartile. These data provide strong evidence that luteal phase concentrations of progesterone preceding estrus influence factors associated with fertilization and embryo development. We speculate that low levels of endogenous progesterone in cyclic cows may be associated with ovulation of a persistent follicle, as predicted by results of Experiment 3. In contrast, cows with high progesterone and those that experienced a spontaneous estrus after being acyclic should have ovulated a new follicle.

Conclusions

Overall, results of our collaborative efforts have solidified the concept that progesterone concentration during the luteal phase has a profound effect on fertility at the next estrus in high-producing dairy cows. It can no longer be argued that progesterone prior to estrus is of negligible importance. Our findings show that cows with highest levels of progesterone: 1) have higher fertility, 2) have normal rather than exaggerated systemic concentrations of estrogen during the follicular phase, 3) have follicles that are of normal rather than enlarged size, 4) have follicles that produce normal rather than exaggerated quantities of steroids in vitro, and 5) are much more likely to have a recoverable embryo on day 7 after estrus. Although we found that high levels of systemic progesterone were necessary for normal follicular development, highest conception rates and highest rates of embryo recovery, we did not find a consistent effect of supplemental progesterone on fertility. It may be that the period of treatment was insufficient, or that we were unable to reach a threshold that would give more positive results.

We also found that synchronized cows had much better reproductive performance than non-synchronized cows, primarily because of earlier insemination. Improved methods to increase percentage of cows that respond to estrous-synchronization protocols would be of great value to dairy farmers.

Finally, we found that reproductive performance of cows in the US and Israel was amazingly similar. Conception rates and intervals to first AI and conception were nearly identical. Moreover, seasonal differences in reproductive performance were parallel in the two countries, indicating a potential carry-over effect of late-summer heat stress on fertility during the autumn.

List of Publications

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