

Post-Partum and Post-Conceptional Ovarian Activity of Dairy Cows: Evaluation Based on Progesterone Profiles

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Eldon, J: Post-partum and Post-conceptional ovarian activity of dairy cows: Evaluation based on progesterone profiles. Acta vet. scand. 1991, 32, 377-386. – The progesterone level of milk was measured daily in 20 Icelandic dairy cows for 50-90 days post-partum and for 30-50 days post-conception by radioimmunoassay. In 40% of the cows the 1st post-partum ovarian cycle was shorter and the progesterone level lower than in normal cycles. The average duration of the 1st post-partum luteal phase was significantly shorter and the progesterone values lower than in the 2nd and 3rd phases. The progesterone level rose to a plateau at the onset of luteal function within 5 ± 2 (mean \pm s.d.) days, whereas the decrease in progesterone at the end of the luteal phase was more rapid i.e. 3 ± 1 days. The progesterone value for a normal interluteal phase was 1.5 ± 1.3 nmol/l. However in 9 of 48 interluteal phases the progesterone values did not decline below 3 nmol/l. The average progesterone concentration was significantly increased during the first 30 days post-conception. In 4 of 20 cows a significant drop in progesterone concentration was found 15-19 days post-conception.

The results indicate that the luteal activity of the 1st post-partum ovarian cycle is inferior to that of the following cycles. There is a significant correlation in the intensity of the luteal activity between cycles. Ovarian cyclicity continues in early pregnancy in, at least, 20% of cows. The sampling frequency is of importance in studies concerning the luteal activity.

luteal phase; interluteal phase.

Introduction

The importance of luteal function for conception and maintenance of pregnancy in ruminants is well established. The progesterone environment on the days around oestrus influences the success of insemination in the ewe (Ashworth *et al.* 1987, 1989, Diskin & Niswender 1989) and in the cow (Folman *et al.* 1973). Sustained high oxytocin concentration can extend the luteal life-span in cattle and sustained high progesterone concentration will block oestrus and ovulation (Gilbert *et al.* 1989, Nanda *et al.* 1988). Prostaglandin (PGF_{2 α}) induces premature luteolysis, terminates pregnancy in inseminated animals and alters the interval between oestrus

periods (Kindahl *et al.* 1976, 1980, Lafrance & Goff 1988, Copelin *et al.* 1989).

As the concentration of progesterone in blood and milk is an indicator of ovarian activity, survey of progesterone levels are of importance in reproductive studies. Earlier studies of the post-partum ovarian function of Icelandic dairy cows (Eldon 1988) were based on samples collected every 5th day. This sampling frequency was too low for establishing accurately the length of the ovarian cycle, luteal phase, inter-luteal phase and changes in progesterone concentration during the oestrus cycle. The objective of this study was to establish the length of the oestrous phases and changes occurring in the

concentration of progesterone post-partum and early post-conception in the Icelandic dairy cow.

Materials and methods

Animals

Twenty Icelandic dairy cows were randomly selected from a herd of 70 cows. The age of the cows was 3-11 years (2nd-10th calving). During the study the animals were housed in tie stalls and fed hay, silage, concentrates and minerals. The cow shed was bright, warm and well ventilated. The calvings were normal and without any complicating diseases.

Milk sampling

Milk samples were taken daily for 90-100 days from calving. The milk was collected in 10 ml plastic vials. Each vial contained as a preservative 10 mg 2-bromo-2-nitro-1,3-propanediol (Sigma Chemical Co., St. Louis, MO). The samples were kept frozen at -20°C until assayed.

Progesterone assay

Progesterone was assayed by a radioimmunoassay technique as described by *Eldon & Olafsson* 1986. The hormone was determined in the fat free part of the milk. The tracer used was (1, 2, 6, 7, 21-³H(N))-progesterone (7 TBq/mmol; NEN Research Products, Kastrup, Denmark). An antibody raised in sheep against 11 α -hydroxyprogesterone-hemisuccinate conjugate (*Castellanos & Edqvist* 1978) was applied. Cross-reactivity was 100% with progesterone, 9.5% with 17 α -hydroxyprogesterone, 3.5% with 11 α -hydroxyprogesterone and less with other steroids. Diluted 1:20.000 the antibody bound 45% of the ³H-progesterone. Free hormone was separated from bound by dextran coated charoal 0.25% Activekohle (Merk) and 0.1% dextran T70 (Pharmacia,

Uppsala, Sweden) in PBS buffer without gelatin). The samples were analyzed on a Packard 1600 CA Tri-Carb Liquid scintillation Analyzer. Inter-assay coefficient of variation (CV%) was 12.2 for a sample containing 6.0 nmol/l of progesterone (n=50; mean \pm s.d., 5.9 \pm 1.2). Intra-assay CV% for duplicate samples was 6.7%. The sensitivity of the assay was 0.5 nmol/l and average recovery was 98%.

Progesterone profile analysis

The length of an ovarian cycle was evaluated from the day before progesterone rose above 3 nmol/l to the same day of the subsequent cycle (short cycle \leq 15 days; normal cycle \geq 16 days) and the length of the luteal phase from the day before progesterone rose above 3 nmol/l to the day it fell below the same value. The interluteal phase was estimated as the interval between the luteal phases. The area under the curve during luteal phase was evaluated by drawing the curves on a millimeter paper and then counting the mm².

Statistical analysis

The data were analysed using Single classification analysis of variance, analysis of variance for repeated measures, Student's t-test and test of significance for correlation coefficients as described in *Sokal & Rohlf* (1981).

Results

The average duration of 1st, 2nd and 3rd post-partum ovarian cycle is shown in Table 1. In 8 of 20 cows (40%) the 1st post-partum ovarian cycle was short (8-13 days), leading to a significantly lower average of the 1st (17 days) as compared to the 2nd (20 days) and 3rd (21 days) ovarian cycle.

At the beginning of the luteal phase the concentration of progesterone rose above 3 nmol/l, to a plateau. This rise took 5 \pm

Table 1. The length of 1st, 2nd and 3rd post-partum ovarian cycles.

	No. of obs.	Mean±S.D. (days)	Range (days)	Stat. sign.
1st pp ovarian cycle				
Normal	12	19.5±2.7	16-26	***
Short	8	10.7±1.7	8-13	
All	20	16.5±4.9	8-26	
2nd pp ovarian cycle				
	17	20.1±2.8	14-25	*
3rd pp ovarian cycle				
	9	20.9±2.5	17-22	ns

pp = post partum; No. of obs. = Number of observations; Mean±S.D. = Mean±1 Standard Deviation; Stat. sign. = Statistical significance between means according to Student's t-test, ns = not significant, * = $p < 0.05$, *** = $p < 0.001$.

Table 2. The length of 1st, 2nd and 3rd post-partum luteal and interluteal phases.

	No. of obs.	Mean±S.D. (days)	Range (days)	Stat. sign.
1st pp luteal phase				
Normal cycle	12	15±1.4	14-18	
Short cycle	8	10±2.4	6-13	***
All	20	13±3.2	6-18	
2nd pp luteal phase				
	17	17±1.3	14-18	
3rd pp luteal phase				
	11	17±1.6	14-19	ns
1st pp inter-lut. ph				
Following Short luteal ph.				
	8	4±1.1	2- 5	
Following Normal luteal ph.				
	12	5±1.4	3- 8	ns
All	20	5±1.8	1- 8	
2nd pp inter-lut. ph.				
	17	5±1.9	1- 8	ns
3rd pp inter-lut. ph.				
	11	4±1.0	3- 6	ns

Interlut. ph. = interluteal phase. pp = post partum; No. of obs. = Number of observations; Mean±S.D. = Mean ± 1 Standard Deviation; Stat. sign. = Statistical significance between means according to Student's t-test, ns = not significant, * = $p < 0.05$, *** = $p < 0.001$.

(mean±s.d.) days. The plateau values, 13 ± 3.3 nmol/l, lasted 8 ± 2.6 days. The decline below 3 nmol/l took 3 ± 1 days which was significantly faster ($p < 0.05$) than the rate of increase. No difference was found in the rate of rise or decline between 1st, 2nd and 3rd luteal phases. Interval from calving had no effect on this activity.

The average length of 1st, 2nd and 3rd post-partum luteal phases is shown in Table 2. The 1st post-partum luteal phase was significantly shorter (13 days) than 2nd and 3rd luteal phase (17 days).

There was no significant difference between the average length of the 1st, 2nd and 3rd interluteal phase (means = 4 and 5 days, Table 2). The length of the interluteal phase varied from 2-8 days and was not significantly shorter following a short luteal phase. Figure 1 shows progesterone profiles where the

interluteal phases are 8 and 2 days, respectively.

The progesterone values assayed during the luteal phases are shown in Table 3. The values of a short cycle were significantly lower than those assayed during a normal cycle. The average value of 1st post-partum luteal phase was significantly lower than those of the 2nd and 3rd luteal phase.

The area under the curve of the progesterone profile during the luteal phase increased from 572 ± 281 mm² during the 1st post-partum luteal phase to 1138 ± 162 mm² during the 3rd. The area of the 2nd post-partum luteal phase correlated significantly with the area of the 1st post-partum luteal phase ($y = 0.54x + 807$, $r = 0.62$). During normal interluteal phase average progesterone values were 1.5 ± 1.3 nmol/l. During 9 of 48

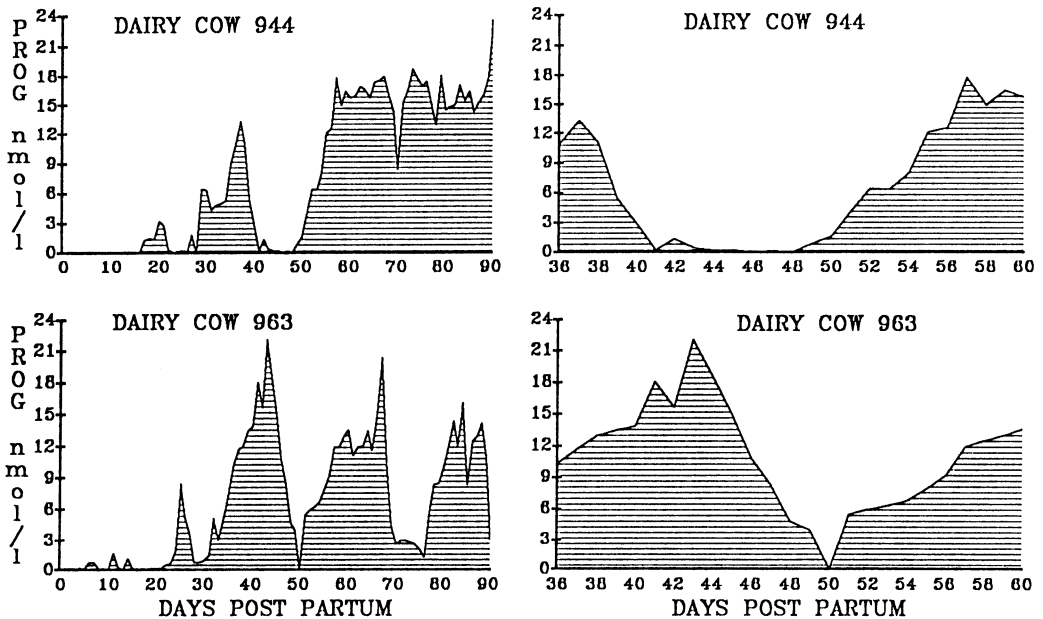


Figure 1. The duration of 2 interluteal phases. The profile from dairy cow 944 has interluteal phase of 8 days duration 41-49 days post-partum. The profile from dairy cow 963 has interluteal phase of 2 days duration 49-51 days post-partum.

Table 3. Progesterone values assayed during the luteal phase.

	No. of obs.	Mean±S.D. (nmol/l)	Range (nmol/l)	Stat. sign.
1st pp luteal phase				
Normal cycle	169	8.7±3.5	3.0-12.5	
Short cycle	119	6.3±2.8	3.0-19.0	***
All	288	8.0±3.6	3.0-19.0	
2nd pp luteal phase				
	289	11.0±4.6	3.0-19.5	***
3rd pp luteal phase				
	187	11.0±5.2	4.0-30.0	ns

pp = post partum; No. of obs. = Number of observations; Mean±S.D. = Mean ± 1 Standard Deviation; Stat. sign. = Statistical significance between means according to Student's t-test, ns = not significant, * = $p < 0.05$, *** = $p < 0.001$.

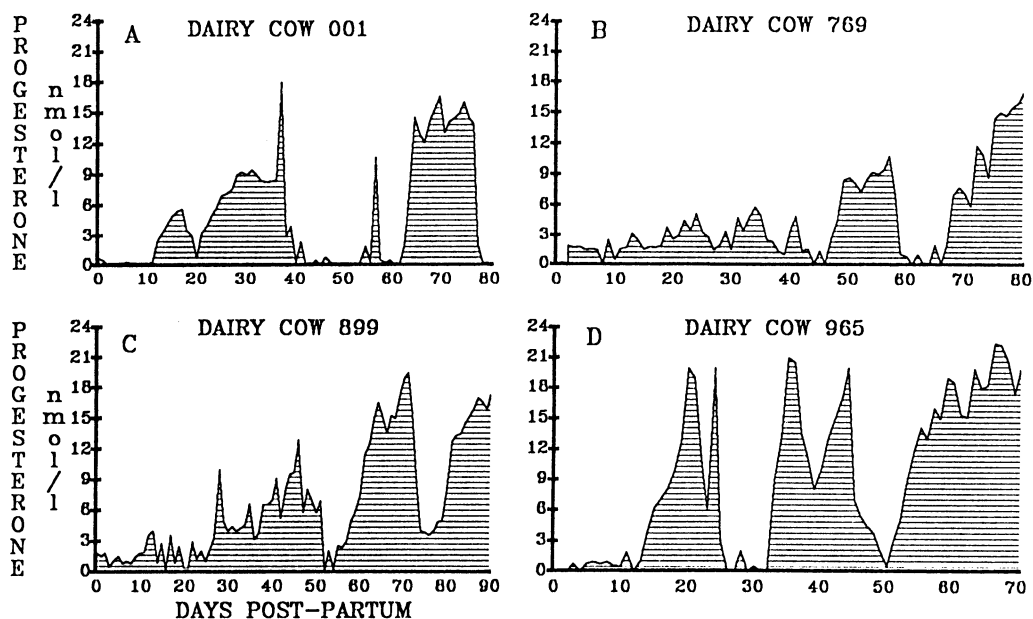


Figure 2. Irregularities in progesterone profiles. A. Sporadic progesterone production, cessation of luteal activity 40-56 days post-partum. - B. Suppressed luteal function during the first 45 days post-partum (luteal cyst?). - C. Prolonged luteal activity 25-52 days post-partum. High progesterone interluteal phase (> 3 nmol/l) 74-79 days post-partum. - D. A temporary drop (14 nmol/l) in progesterone concentration during 2 consecutive luteal phases.

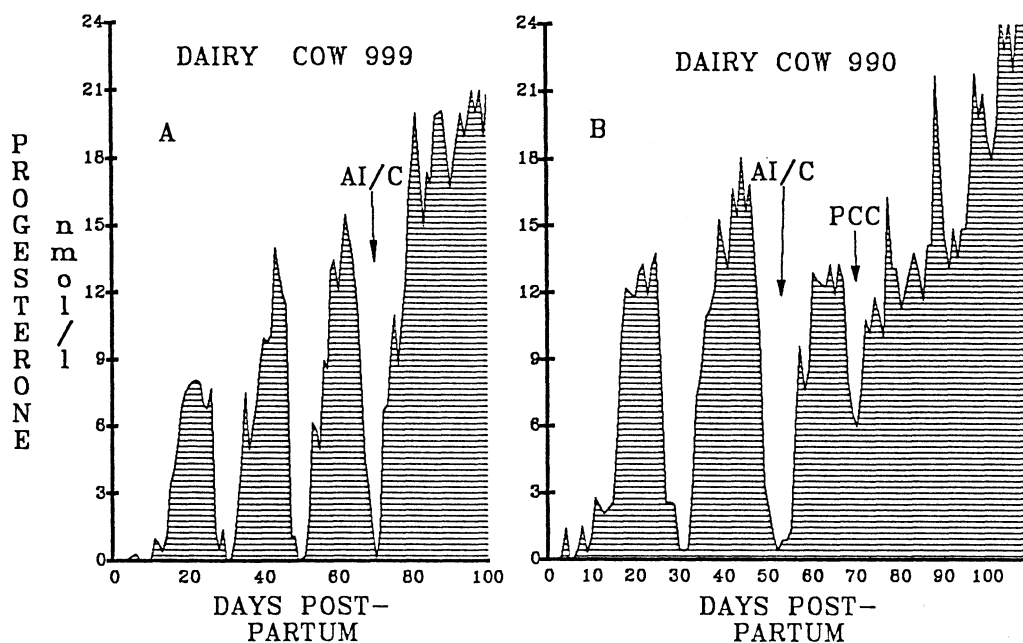


Figure 3. A. The progesterone profile from dairy cow 999. The profile shows increased luteal activity from 1st to 3rd luteal phase and increased luteal activity post-conception compared to 3rd luteal phase. - B. The progesterone profile from dairy cow 990. The profile shows continued cyclic activity 16 days post conception. The profile also shows increased progesterone concentration post-conception. AI/C = time of artificial insemination and conception; PCC = drop in progesterone concentration signifying cyclic activity post-conception.

interluteal phases the progesterone values did not decline below 3 nmol/l (Fig. 2 C).

Fourteen of 20 (70%) progesterone profiles showed regularity in pattern of progesterone concentrations during the luteal phases. Five profiles showed irregularity in luteal patterns. These irregularities were sporadic progesterone production, temporary cessation of cyclicity, suppressed and prolonged luteal function and a temporary drop in progesterone concentration of 14 nmol/l during 2 consecutive luteal phases (Fig. 2).

A significant increase ($p < 0.01$) in average progesterone concentration was detected during the first 30 days post-conception as compared to the last luteal phase preceding conception (Fig. 3 A). Comparable increase was not seen in non-pregnant cows during

the same time post-partum. The progesterone values dropped significantly ($p < 0.05$) in 4 profiles for 1-2 days, 15-19 days post-conception. The drop in progesterone concentration was 6-10 nmol/l. Values below 6 nmol/l were not observed (Fig. 3 B).

Discussion

In the present study 1st post-partum ovarian cycles were shorter than normal in 40% of cows, whereas the 2nd and 3rd were of normal length i.e. ≈ 21 days (Morrow *et al.* 1969, Eldon 1988, Copelin *et al.* 1989). The results are in accord with earlier studies both in Icelandic dairy cows (Eldon & Olafsson 1986, Eldon 1988) and other cow breeds (Mather *et al.* 1978, Larsson 1984). The short cycle was mainly due to a significantly

shorter luteal phase though variations in the rate of granulosa cell luteinization and onset of progesterone production after ovulation; variations in the time of onset and rate of luteolysis; the rate of folliculogenesis, time of onset and length of oestrus and time of ovulation decide the overall length of the ovarian cycle (Kindahl *et al.* 1980, Dieleman *et al.* 1986, Gustafsson *et al.* 1986, Spicer & Echternkamp 1986). Furthermore the progesterone level was significantly lower in the 1st as compared with the 2nd and 3rd luteal phase. The cause of the reduced functional capacity of the 1st luteal tissue formed post-partum have been reported to be incomplete restoration of luteinizing hormone (LH) release leading to altered follicular development and/or premature, prolonged or increased release of PGF_{2α} from the involuting uterus (Braden *et al.* 1989, Copelin *et al.* 1989, Schirar *et al.* 1989).

The mean values for luteal phase progesterone concentrations found in this study were higher than the findings in earlier studies of Icelandic dairy cows (Eldon & Olafsson 1986, Eldon 1988). This is probably due to differences in feed quality as dietary intake has been shown to alter progesterone concentrations in peripheral blood (Gombe & Hansel 1973, Carrol *et al.* 1988, Knutson & Allrich 1988).

The increasing duration and progesterone concentration from 1st to 3rd post-partum luteal phase and the increase in progesterone concentration post-conception found in this study is similar to the findings of Mather *et al.* (1978), Lee *et al.* (1985) and Robinson *et al.* (1989) and do reflect an increased activity of corpora lutea. The development of full luteal function from the beginning of progesterone production was slower than luteolysis and cessation of progesterone production. The progesterone level of the 1st post-partum luteal phase rose to a lower plateau than in the 2nd and 3rd luteal phases. But in

contrast to the findings of Schirar *et al.* (1989), who found that progesterone secretion increased at a slower rate early post-partum than later, at the resumption of ovarian activity in ewes, the rate of increase was similar in all luteal phases studied.

In 5 (25%) profiles irregularities in the progesterone concentrations were observed. This is an indication of hormonal imbalance which may lead to formation of ovarian cysts and ovulation failure (Eyestone & Ax 1984, Kassa *et al.* 1986, Stevenson & Call 1988). Another explanation for the luteal dysfunction may be bacterial infection of the uterus (Fredriksson *et al.* 1985, Paisley *et al.* 1986).

The variation in the duration of the 1st, 2nd and 3rd post-partum interluteal phases was not statistically significant. However this variation could affect the reproductive performance of the cows. During an interluteal phase lasting 2 days oestrus is inevitably short and may be hard to detect. A long interluteal phase increases the danger of too early service (Gustafsson *et al.* 1986). The cause for high progesterone (> 3 nmol/l) during the interluteal phase is not clear. The progesterone was not high enough to hinder folliculogenesis, ovulation and formation of a new corpus luteum though low circulating concentrations of exogenous progesterone (e.g. Prid's) are known to do so.

A continuation of the cyclic pattern seen in 4 profiles 15-19 days post-conception is in accord with the report of Claus *et al.* (1983), Eldon (1988) and Schallenberger *et al.* (1989). The cyclic patterns are probably due to interruption of luteolysis by luteotrophic and antiluteolytic effects of the conceptus (Garrett *et al.* 1988, Putney *et al.* 1988, Diskin & Niswender 1989, Wallace *et al.* 1989). Dobson (1989) reported oestrus behaviour in cows at all stages of pregnancy. However, he did not observe hormonal changes compara-

ble to those associated with oestrus in non-pregnant cows. Another possibility is that PGF_{2α} produced in the uterus may play a role (Zarco *et al.* 1988). The question is still open whether the cyclic pattern of progesterone concentrations seen in pregnant cows is preceded by a rise in uterine production of PGF_{2α} and whether this cyclic pattern can be seen in later stages of pregnancy.

It can be inferred from these results that the luteal activity of the 1st post-partum ovarian cycle is inferior to that of the following cycles; the intensity of the luteal activity is significantly correlated between cycles; ovarian cyclicity can be seen early post-conception in at least 20% of dairy cows. The sampling frequency is of importance in studies concerning the luteal activity, too infrequent sampling can lead to erroneous results.

Acknowledgement

I want to thank the farmers V. Ásgeirsson and S. Ásgeirsson for the milk sampling. I'm indebted to Dr. G. Georgsson, Institute for Experimental Pathology, University of Iceland, Keldur, for reviewing the manuscript. This study was supported by the Icelandic Science Fund.

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Sammanfattning

Äggstocksaktiviteten hos mjölkkor efter partus och tidigt i dräktigheten: Evaluering baserad på progesteronprofiler.

Progesteronnivån i mjölk från 20 isländska mjölkkor studerades. Hormonmätningen gjordes fortlöpande under tiden från förlossningen i mjölkprov som samlades dagligen i 50-90 dagar efter partus och i 30-50 dagar efter dräktighetspunkten. Hos 40% av korna var längden på den första brunstcykeln efter partus kortare och progesteronnivån lägre än i normal brunstcykel. Den första lutealfasen var signifikant kortare och progesteronnivån var lägre än den andra och den tredje lutealfasen. Vid början av lutealfasen höjdes progesteronkurvan till en jämn nivå

på genomsnitt mindre än 5 ± 2 dagar men sjönk vid lutealfasens slut på 3 ± 1 dagar. Progesteronnivån under en normal interlutealfas var i genomsnitt 1.5 ± 1.3 nmol/l, men i 9 av 48 interlutealfaser sjönk progesteronnivån inte under 3 nmol/l. Progesteronnivån var i genomsnitt signifikant ökad under de första 30 dagarna i dräktigheten. Den sjönk signifikant 15-19 dagar efter dräktighetspunkten hos 4 av 20 kor. Resultaten visar att den första brunstcykelns aktivitet är förminskad relativt till de följande. Signifikant korrelation hittades för luteal intensitet mellan cykler. Brunstcykelns påverkan märkes even tidigt i dräktigheten hos, åtminstone, 20% av korna. Prövtagningsfrekvensen är av stor viktighet när luteal aktiviteten studeras.

(Accepted October 8, 1990).

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