

# The Role of the Bovine Practitioner in Synchronization and Twinning in Cattle

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## Introduction

There are four main areas which should be discussed: (a) The practitioner needs to understand the physiological aspects of the problem. (b) The practitioner needs to know the economics and production aspects at the farm level. (c) The practitioner has to be familiar with the limits on the use of drugs imposed by regulatory bodies such as FDA. (d) The practitioner requires expert advice on numerous aspects of endocrine control and reproductive activity.

In this paper only (a) will be discussed in detail. Part (b) depends on local considerations of farm management, and (c) and (d) depend on the interest and capacity of the practitioner to keep up-to-date and learn new techniques.

In general, examples given in this paper will be from my own work. For further reading the following references should be consulted (Gordon, et al. 1962; Hafez, et al. 1964; Mauleon, 1967; Foote & Onuma, 1970; Turman, et al. 1971; Vincent & Mills, 1972; Bellows and Short, 1972; Henricks, et al. 1973).

## The Physiological Problem

### 1. The normal estrous cycle

It is necessary to outline present knowledge concerning the estrous cycle of the cow. Since the physiological problem is largely of an endocrine nature, we will concentrate on the endocrine aspects. The critical point in time in the estrous cycle of the cow is the day when the corpus luteum begins to regress. At present it is not known precisely what causes regression on the 16th to 18th day of an estrous cycle, but certain facts are pertinent. The rate of change in plasma progesterone levels varies between animals during the period of luteal regression. In some cows, blood levels decline from about 10 ng/ml to less

than 1 ng/ml in a half day, whereas in others regression may extend over at least two days. There are breed differences in the rate of decline, the Angus having a more abrupt cessation of progesterone secretion than the Hereford (Lamond, et al. 1971). The most important feature of the decline of progesterone is the minimum threshold level above which progesterone will inhibit follicle maturation and hence estrogen production and estrus. This is between 1 to 2 ng depending on the individual cow. In my view, the point in time that progesterone concentrations decline below the threshold is the focal point in the estrous cycle. It can be assumed that from this moment onwards one or more follicles will develop to maturity.

Estrogen production begins during the period of regression of the corpus and increases to a maximum a few hours before heat is first detected (Henricks, Dickey & Hill, 1971; Echternkamp & Hansel, 1971; Shemesh, et al., 1972; Mason, et al., 1972; Wetteman, et al., 1972; Glencross, et al., 1973). The rate of increase in estrogen production varies between cows, in some cases taking as little as one day and in others three to four days to reach the blood concentration which triggers estrus. At the present time workers are finding differences between estrinol and estradiol during this period of active estrogen secretion. Production of estrinol appears to be greater than estradiol but it is not yet known which of these two causes the hypothalamic-pituitary system to release large quantities of ovulating hormone early in estrus. It is certain that the mechanism controlling release of ovulating hormone is set in motion before the onset of estrus.

The peak of LH release is observed in some cows before the beginning of estrus, though in the

majority it is released during the first 12 hours of estrus (Hansel & Snook, 1970; Henricks, et al., 1970; Swanson & Hafs, 1971). Ovulation tends to occur about 24 hours after the peak in LH is reached. This explains why ovulation occurs at the end of or soon after estrus.

Plasma levels of progesterone, estrogen and LH reach base-line levels by the time ovulation occurs. Within the next few days there may be a secondary output of estrogen. This transitory rise in estrogen is related to the fact that progesterone blood levels have not yet reached an inhibitory level and at any stage of the cycle there are follicles capable of producing estrogen if given the right stimulus. By about the fourth day after ovulation, blood levels of progesterone have reached 2 to 5 ng at which time further maturation of follicles, at least so far as the secretion of estrogen is concerned, tends to be inhibited. There is still some argument as to what is the exact relationship between follicle size and estrogen secretion but the most likely explanation is that largest follicles are not necessarily producing estrogen.

During the progestational phase of the cycle, progesterone levels reach peaks between 5 and 20 ng depending on breed and individual characteristics (Stabenfeldt, et al., 1969; Pope, et al., 1969; Henricks, et al., 1971). Beef heifers tend to have much higher blood levels of progesterone than dairy cows. Furthermore, the curves which relate blood levels of progesterone to the stage of the cycle are not smooth as is generally presented in the literature. Considerable day-to-day variation in concentration occurs. There is no good explanation for this variability but it does indicate that more factors than the secretory ability of the corpus

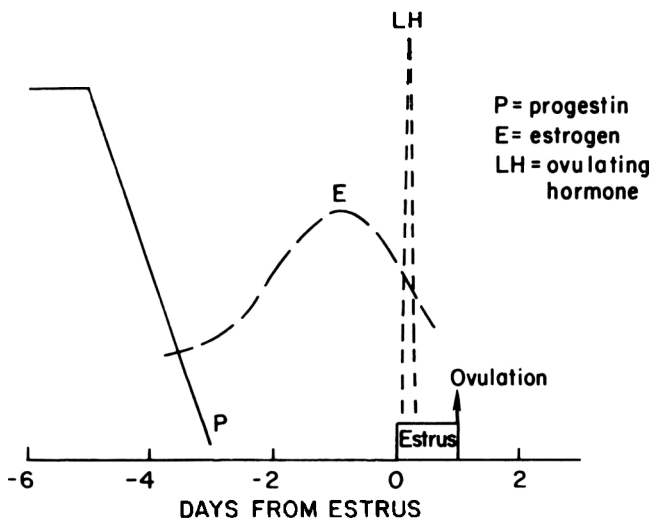


Figure 1. Endocrine relation during the period of follicular maturation.

luteum determine the amount of progesterone getting into the peripheral circulation.

Figure 1 is a diagrammatic representation of the critical events during the few days prior to and during estrus. I have not put any scales on the ordinate because there is so much variability between cows that the numbers could be misinterpreted. The important thing is to understand the general relationships shown in the figure. I want to emphasize the following:

a. The period from P, which is the point when progesterone levels reach their minimum threshold value, to the onset of estrus is controlled by the following factors:

i. *The rate of decline from the maximum to P.*

Progesterone has a half-life in blood of 3 to 5 minutes, though some hours elapse before it is depleted from reservoirs such as fat depots. Synthetic progestins vary widely, not only in half-life but also in rate of removal from reactive sites.

ii. *The breed of cow.*

The Hereford has a shorter period from P to the onset of estrus than the Angus (by about one day). When cows have estrous cycles of 22 to 24 days in length some of the variation may be due to a prolonged period between P and the onset of estrus. However, it is also possible that in some cows with long cycles the length of life of the corpus luteum may be prolonged. A good deal needs to be learned yet about individual differences in the period between P and the onset of estrus. For example, it can be assumed that success in synchronization and twinning will depend on knowing whether this is a two-day or four-day period.

iii. *Season.*

It has been well established that during the late winter and fall, the period from P to the onset of estrus is longer than during the spring and summer. This is due to the seasonal effect of the suppressive action of progesterone on the hypothalamic-pituitary system.

iv. *The action of progesterone on the central nervous system.*

In the natural system, one would not expect any significant difference between cows in the action of progesterone on the hypothalamus. However, when progesterone is given by injection at intermittent intervals or when analogues of progesterone such as melengestrol acetate (MGA) or chlormadinone acetate (CAP) are administered, the responsiveness of the hypothalamic-pituitary system to the progestins will differ, and this will

have the effect of influencing the period from P to estrus.

In general, the interval from minimum threshold progestin values to the onset of estrus can be predicted on the basis of known differences amongst breeds and seasons, and knowledge of the physiological action of the progestins. It is obvious that factors such as lactation and undernutrition could lengthen the period from P to estrus but little quantitative information is available in the cow. On the other hand it has been well established in sheep that undernutrition will delay the period from P to the onset of estrus.

**b. Interval from P to ovulation.**

It is generally assumed that ovulation occurs about 24 to 30 hours after the beginning of estrus. However, it has to be realized that to a large extent this is a coincidental relationship. The factors which primarily control the interval from P to ovulation are:

*i. The rate of change in the secretion of estrogen.*

This is related to the rate of growth of the follicle which is destined to ovulate. Little is known about variation in rates of development of follicles, however, there is a lot of variability in the size of the follicles when progesterone reaches its low threshold level, P. We have simulated follicular development in the estrous cycle of the cow on the computer and, while many assumptions were made, it was possible to test the hypothesis that considerable differences could exist at P in both the size of follicles and the capacity to respond to gonadotropin by producing estrogen.

*ii. The interval from the peak of estrogen secretion to LH release.*

It has been shown in other animals that a fairly precise interval exists between the threshold "trigger" level of estrogen, which is reached a day or so before estrus, and the release of ovulating hormone. Preliminary work in the cow indicates that the principle holds and about 24 hours probably elapses between the trigger level and release of LH.

*iii. The state of development of the follicle when LH is released.*

It would seem obvious that in the normal cow there would be a good synchronization between maturation processes in the follicle and the release of LH. However, estrogen is released during a hypertrophic phase in the *theca interna* of the follicle, and this is followed by a period of relative metabolic inactivity, when enzyme systems are switching off and on, in line with the shift in

endocrine function from the *theca interna* to the *theca granulosa*. If for some reason the release of LH is too soon or too late relative to specific timed changes in the follicle, then ovulation may not occur. Our studies on the vascular anatomy of the ovary indicate a structural basis for the inability of some animals to ovulate at the appropriate time. This would occur in cows in which branches of the ovarian artery are unable to meet the blood flow requirements associated with follicle maturation, and estrogen and LH production.

It can be seen from the above discussion that the kind of progestin that is used to suppress ovarian function will determine when estrus occurs and if the progestin or the progestational regime influences in any way the rate of development of follicles or the sensitivity of the hypothalamic-pituitary system to estrogen then there may not be perfect synchronization between estrus and ovulation.

It is this potential lack of precise timing which is at the basis of the problem of synchronization and of hormonal induction of multiple ovulation. In my opinion, it will not be possible for anyone to utilize the gonadotropins and the steroids effectively for synchronization and multiple ovulation until they appreciate the subtleties of this complex set of relations.

**The Effect of Progestins on the Bovine Ovary**

In order to emphasize that progestins may influence follicular function and hence upset the time-related changes which take place after cessation of progestin treatment, some data on ovarian changes will be discussed. In the early work with synthetic progestins and also with injections of progesterone it was observed that the intervals to estrus were variable and that ovulation did not always occur at the expected time. Further research showed that the progestins altered the follicle population, at least temporarily.

a. The effect of MGA on the ovarian follicle population. In a study carried out by Hill, et al. (1971), 0.5 mg MGA per head per day MGA was fed to beef heifers for 14 days commencing on the 4th or the 15th day of the estrous cycle. In Table 1

Table 1  
Ovarian Follicles in Beef Heifers Treated with MGA (From Table 1, Hill, et al., 1971)

Treatment	No. of Follicles	
	< 10 mm	> 10 mm
Controls	13.5	0.0
MGA 0.5 mg from Day 4	9.6	0.6
MGA 0.5 mg from Day 15	3.6	1.0

are shown the numbers of follicles observed in untreated and MGA-treated animals at the end of the MGA treatment. It can be seen that untreated heifers did not have follicles greater than 10 mm in diameter. MGA, especially when treatment commenced on Day 15 of the cycle (hence for about two weeks the ovary was under the influence of MGA alone) reduced the number of follicles but in every case there was a large one (approximately 15 mm diameter). Some variation was also observed in the development of atresia in the follicles and it is still not clear whether many follicles which had hyperplasia of the follicle thecal layer were atretic or not.

b. The effect of MGA on cervical mucus. In the same experiment outlined above (Hill, et al., 1971), cervical mucus was taken daily from the end of the progestin treatment to the onset of estrus. It can be seen from Table 2 that control

the ovary. It can be assumed that for every herd of cattle there will be a dose of each progestin which will be optimum. However, it is equally obvious that one selected dose of the progestin cannot possibly be optimum for all herds under all conditions. When it is realized that only slight deviations from normal endocrine activity in the genital tract will cause infertility, any approach which does not seek the optimum dose of progestin for each set of conditions can be regarded as neither scientific nor sensible.

#### Synchronization in Anestrous Cows

In non-cycling cows a special situation exists because a progestin has to be administered in order to prime the genital tract and possibly also the hypothalamic-pituitary system to respond to estrogen during the induced follicular stage. It would be expected that the optimum dose of progestin during anestrus would not be the same as

Table 2  
Effect of MGA on Cervical Mucus Index (From Table 2, Hill, et al., 1971)

Treatment	No. Heifers	Days after final MGA to CMI* peak				Type of Peak				Peak and Estrus
		2,3	4,5	6,7	8+	Single	Prolonged	Double	None	
Control**	17	5	8	4	0	13	1	3	0	17
0.5mg from Day 4	14	6	7	0	0	8	4	1	1	10
0.5 mg from Day 15	15	3	4	2	0	9	0	0	6	3

\*CMI - cervical mucus index (chloride/protein ratio)

\*\*Days 16 to 22 of cycle

animals had a peak cervical mucus index on Days 19, 20 and 21 of the cycle, and that this peak occurred only once in the majority and it coincided with estrus. In the MGA-treated animals the occurrence of the peak (or its non-occurrence) and the relationship between the CMI peaks and estrus were markedly different from the controls. From this we can assume that the progestin caused changes in cervical mucus which could lead to problems in gamete transport. In some of these heifers mucus flow continued for period of up to four days. Some heifers attracted the bull but would not let him mount and in some cases ovulation did not occur after estrus.

#### The Problem of Synchronization

In order to obtain suppression of ovarian function and subsequent synchronization of estrus associated with normal fertility, it is necessary to select a progestin regime (the optimum suppressive dose - OSD) which will inhibit maturation of follicles and hence block estrogen secretion and ovulation, but which at the same time does not interfere with normal follicle dynamics, i.e. the normal rates of growth and atresia of follicles in

the OSD but relevant data are not available. In many instances, particularly in cows experiencing lactation anestrus, and/or when the nutritional status of the cow is below optimum, a gonadotropin will be required in addition to the progestin. The principles governing the use of progestins and gonadotropins are similar to those for inducing multiple ovulations. Thus if one uses a gonadotropin the timing of its injection in relation to the cessation of progestin treatment is critical. It is necessary to select an optimum dose and give it at such a time that the induced follicles can develop at the correct rate through the final stages of maturation after the progestin blood levels reach their minimum thresholds. If the follicles reach maturity too early or too late relative to the changes in the tract and in the hypothalamic-pituitary system, then the egg and sperm may not make contact at all, or at best will do so under conditions not conducive to normal development of the zygote.

#### Induction of Twin Ovulations

An exogenous source of a gonadotropin is required to induce more than one ovulation. The

most practicable methods for inducing two to four ovulations depend on the use of a progestin such as MGA or CAP or progesterone, and the administration of a gonadotropin at a precise time relative to the end of the progestin treatment.

**1. The action of PMS.**

When given on Day 16 of the estrous cycle PMS causes considerable variation in follicle development and ovulation. In Table 3, the dose of PMS is

Table 3  
The Effect of PMS on Estrus, Ovulation, Follicle Development and Fertilization (n = 8 Heifers)  
(From Henricks, et al., 1973)

Item	1600 i.u. on Day 16 of a normal cycle	3200 i.u. on Day 16 of a normal cycle
Interval (days) from Day 16 to estrus	4.9 ± 1.4*	4.0 ± 1.6
Mean ovulations	1.0 ± 0.0	11.0 ± 11.5
Mean yield of fertilized ova	1.0 ± 0.0	4.8 ± 5.7
Mean follicles >10 mm diameter	0.5 ± 0.5	22.0 ± 13.0

\*± S.E.

shown to influence number of ovulations and numbers of large follicles which did not ovulate. The number of fertile ova was much the same regardless of dose. Variability in ovulation rate and in numbers of fertilized ova was very great as can be seen from the standard errors in the table. The two important pieces of information which are clearly demonstrated in Table 3 are, first, that increasing doses of PMS cause marked increase in follicular development but not all follicles ovulate; and secondly, there seems little point in giving large doses of PMS to cause marked follicular development when the yield of fertilized ova is not improved. One of the most important problems facing the entire area of ovum transfer (artificial in ovulation) in valuable cattle is this problem of variation in the yield of fertilized ova.

**2. Interaction between progestin and PMS.**

In Figure 2 the results of a study described by Lamond (1972) are outlined. Hereford and Angus heifers were given 5 or 10 mg of CAP/head/day for 16 days and near the end of the progestin treatment were injected with one of three doses of PMS or three dose levels of FSH. The FSH was a porcine pituitary preparation obtained from Armour-Baldwin Labs. The FSH doses were given in two divided injections 24 hours apart. The following general conclusions are representative of the field of progestin-gonadotropin interaction.

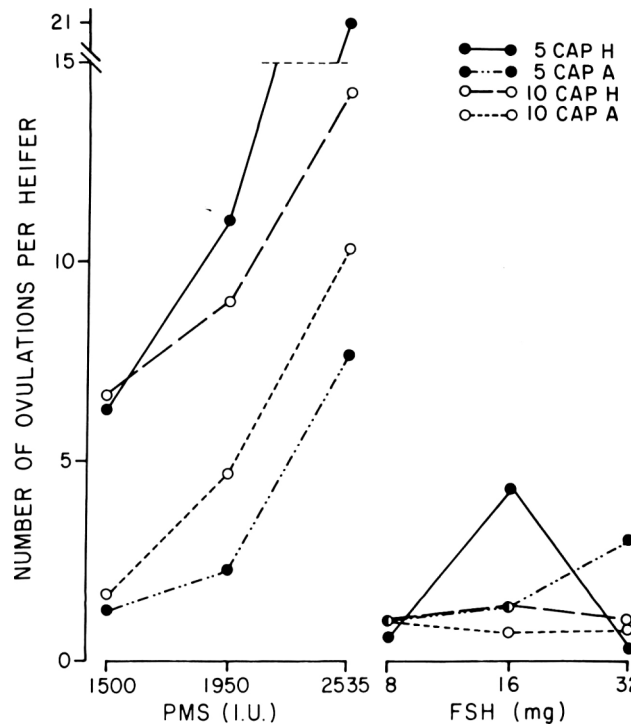


Figure 2. Mean ovulations in groups of three heifers (Angus, A, or Hereford, H) given 5 mg or 10 mg CAP per head per day, and PMS or FSH three days before the end of the CAP treatment.

PMS gave greater numbers of ovulations than FSH, nevertheless, there appeared to have been a response to FSH in a very narrow range (16 mg FSH with 5 mg of CAP in Hereford heifers, and 32 mg of FSH with 5 mg of CAP in Angus heifers). There were no multiple ovulations with FSH at the higher dose of CAP.

With PMS, the most obvious result was that the dose-response line was very steep. Herefords produced more ovulations than Angus. Dose of CAP interacted with the breeds, because at the low dose of CAP there were large numbers of ovulations in the Herefords and low numbers in the Angus.

In terms of obtaining control of multiple ovulation (two to four ovulations) it can be seen that the dose of PMS would have to be lower than 1500 i.u. in the Herefords, whereas in the Angus a dose of about 2000 i.u. combined with 5 mg of CAP would have given adequate control, at least in the particular set of conditions of this experiment.

One point of considerable interest not shown in this diagram is that the variability between animals was not as great as one normally gets with PMS given on Day 16 of the estrous cycle. Further work has substantiated the fact that Herefords are extremely sensitive to CAP-PMS combinations.

**3. Progesterone blood levels in multiple ovulated cattle.**

Table 4  
 Plasma Progesterone Levels in Beef Heifers on  
 Day 13 After Induced Multiple Ovulations (From  
 Lamond & Gaddy, 1972)

No. of ovulations	No. of heifers	Progesterone ng/ml (range)
1	5	2-14
2	4	4-40
4	2	26,42
5	1	138
6	1	20
7	4	15-160
8	1	26
9	1	54
11	1	60
16	2	45,155
18	3	100-260
20	1	105
26	1	460
28	1	110

One of the hidden problems in multiple ovulation in cows is that the greater the number of corpora lutea produced the higher are the blood levels of progesterone. The data shown in Table 4 indicate that even with the highly desirable objective of two to four ovulations, plasma concentrations of progesterone could be expected to be above normal levels in the majority of cattle. The question whether these rather high blood levels could have any influence on maintenance of pregnancy has not yet been answered.

#### The Role of Prostaglandins and Releasing Factors

During the last few years a great deal of research has been conducted into the biological properties of prostaglandins and releasing factors. The prostaglandins comprise a family of fatty acids which occur naturally in many body tissues, and particularly in semen. The compound which is of greatest interest to reproductive physiologists is prostaglandin  $F_{2a}$  ( $PGF_{2a}$ ). This compound has the property of causing regression of the corpus luteum in a matter of hours. A good deal of research is now being conducted on the effects of  $PGF_{2a}$  as a luteolysin in most species of importance. In the human,  $PGF_{2a}$  given into the amnion in early pregnancy causes abortion. If injected intravenously, intramuscularly, or subcutaneously into the pregnant cow during the first six or seven months, doses of 20 to 30 mg  $PGF_{2a}$  will cause luteolysis and abortion. Amounts of the order of 2 to 5 mg administered directly into the uterus will also cause luteolysis. In the mare, less than 0.5 mg administered into the uterus will cause luteolysis. It is clear that the compound is extremely potent and since it and many of its

analogues can be produced at a reasonable cost it can only be a matter of time before they will be made available to the veterinary profession.

As is well known, a new compound has to pass rigorous tests of efficacy as well as safety, but since the prostaglandins are naturally occurring substances and are rapidly metabolized in the body to non-effective metabolites, I believe that there should be no serious problem in obtaining approval for their use in farm animals. However, anyone who has had much contact with the Food and Drug Administration must be aware that there are powerful consumer interests which can make simple tasks not only complex but even impossible.

The primary action of  $PGF_{2a}$  in the cow is not known. It is likely that when injected intravenously the compound has a direct action on the luteal cells because the effective blood levels of the compound persist for less than ten minutes. On the other hand, when given into the uterus the compound may have some other form of action because normally the blood levels rise by rather small amounts. Research in the sheep strongly implicates a counter-current mechanism for the transfer of  $PGF_{2a}$  from the uterine venous drainage system to the ovarian artery which is very closely attached to the ovarian (uteo-ovarian) vein. However, there are a number of unanswered questions concerning the exact method by which  $PGF_{2a}$  causes luteolysis.

In general, it has been found that an intravenous injection of the material is effective in most breeds of cattle. There is some evidence that the Holstein and Brown Swiss do not respond as effectively as some of the other breeds. Usually blood progesterone levels begin to decline within two hours of administration of  $PGF_{2a}$  and estrus occurs two to three days later. Occasionally estrus is delayed to the fifth day but synchronization is satisfactory in cows which are treated in the appropriate stage of the cycle.

It is important to realize then only active corpora lutea which are greater than six days old will regress as a result of  $PGF_{2a}$  treatment. This is an intriguing situation because the corpus luteum by about Day 3 or 4 is quite large and is producing considerable amounts of progesterone yet no four-day CL and not many five-day CL will respond to  $PGF_{2a}$  treatment.

Although only limited data are yet available on the fertility of cattle after luteolysis with  $PGF_{2a}$  there is no indication that fertility is depressed (Table 5). This is in contrast to the generally-accepted view that the use of progestins for

Table 5  
Fertility in Jersey Heifers Given 20 mg PGF<sub>2a</sub>  
Intravenously in the Luteal Phase of the Estrous  
Cycle (D. R. Lamond, unpublished)

No. of heifers treated	9
CL regressed*	9
Estrus detected 2 to 7 days after treatment and inseminated	7
Pregnant	6

\*Based on blood progesterone data

synchronization results in lower fertility at the synchronized heat. Presumably, even though regression of the corpus after PGF<sub>2a</sub> is extremely rapid there is no interference to ovarian function and neither the ovarian nor the genital tract is exposed to unusual endocrine influences.

The releasing factors are produced by the medium eminence of the hypothalamus and are transported via the portal system to the anterior pituitary gland. These compounds are decapeptides and appear to have the property of causing release of specific pituitary hormones. There are probably three gonadotropic releasing factors (GRF's): follicle stimulating hormone RF, luteinizing hormone RF and prolactin RF. However, there is a good deal of argument as to whether one releasing factor releases FSH and LH. A synthetic material believed to be identical to the natural compound causes release of both FSH and LH. There is no doubt, however, that gonadotropins can be released from the pituitary gland with these materials. The problem that has arisen is how best to utilize them effectively. Very precise timing of the releasing factor relative to the growth phase of the follicle is required in order not to interfere with normal follicular maturation and ovulation. It is for this reason that careful monitoring of follicle growth is needed. There is a good deal of variability in the results so far obtained.

One interesting aspect of GRF's is that, in theory, it should be possible to grow an extra follicle or two and then at the appropriate time ovulate these extra follicles. Thus, the GRF's ought to offer an ideal method for hormonal induction of two to four ovulations. Unfortunately, I know of no information that supports this theory and it may be some time before a method is worked out and it may then require detailed monitoring. However, there is little point in developing techniques which require expensive and detailed monitoring before they can be applied in the field. This has always been a problem with endocrinology and explains the variety of shotgun endocrine treatments used. Consequently, practitioners should not hold out too much hope for the

GRF's at least in the area of inducing multiple births.

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