

Hormonal Therapy in the Postpartum Cow – Days 1 to 10 - Fact or Fiction?

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Introduction

In 1986 the late Prof. Raimunds Zemjanis from the University of Minnesota was the advisor for a PhD thesis entitled, “Uterine Motility Patterns in the Postpartum Dairy Cow” (Burton, MJ). In my opinion this work contains some of the most definitive data to date about the effects of exogenous hormones on the postpartum bovine uterus. Thus, much of the information presented in this paper is based on that thesis. Unfortunately some good work by our European colleagues is not readily accessible. It is often published in foreign language journals in which there is no more than a brief English abstract. One renowned group (Kundig, Thun & Zerobin) has validated Burton’s conclusions. The author has had this series of uterine motility papers translated so that pertinent information could be included in this review.^{97,98,168}

Normal Physiology

The initiation of parturition involves activation of the fetal hypothalamic-pituitary-adrenal axis.^{109,167} Elevated fetal corticoids initiate changes in the enzyme systems (hydroxylase, lyase and aromatase) within the cotyledons, and increase the capacity of the bovine placenta to convert C-21 steroids (progesterone, pregnenolone) into C-19 estrogen precursors (androstenedione, dihydroepiandrosterone) and estrogens as the parturient cascade progresses.^{94,102,129,152} This results in a dramatic prepartum elevation of plasma estrogen, estrogen sulfate and estrogen precursors. Estrogens are known to stimulate the production of PGF_{2a}.⁹ The caruncular tissue is a very active site of synthesis of PGF_{2a}.⁷² Approximately 1 week prior to parturition prostaglandin metabolite (PGFM) concentrations gradually increase in maternal plasma. As the PGFM level peaks there is an abrupt decline in progesterone levels associated with regression of the corpus luteum.^{42,48,150} This eliminates the “progesterone block” and parturition ensues.

The postpartum period in the cow involves contraction of the uterine musculature, sloughing of excess caruncular tissue, and regeneration of the endometrial epithelium – uterine involution.¹²⁴ This process occurs during three distinct phases with respect to the hormonal milieu. The puerperal period is defined as that period extending from calving until the pituitary gland becomes responsive to GnRH at 7 to 14 days postpartum. Uterine infections carried over from this period may result in chronic infertility problems. The length of the intermediate period (onset of pituitary responsiveness to GnRH through to the 1st postpartum ovulation) varies tremendously, depending on many factors including nutrition and energy balance. The post-ovulatory period is self-explanatory, and extends until about 45 days postpartum when uterine involution is complete.¹²⁴ It is only in this latter period when luteal tissue is present on the ovary that prostaglandins have a proven effect on the postpartum cow.

PGFM levels peak by day 3 postpartum, then gradually decline to baseline by days 10 – 15 postpartum.^{72,88,110,150} The systemic estrogen levels fall precipitously at parturition, and are at baseline levels (<5 pg/ml) within 2-3 days.^{48,88,150} Recovery of the hypothalamo-pituitary-ovarian axis is influenced by several factors, including nutrition. FSH secretion resumes within the first week and cohorts of follicles begin to emerge. The first dominant follicle may be selected as soon as 10-12 days postpartum.^{10,118} This dominant follicle acquires LH receptors on its granulosa cell layer, and thereby attains enhanced steroidogenic capacity compared with the other members of the cohort. However, continued growth and increased estradiol production from the dominant follicle depends on LH pulse frequency – otherwise it will become atretic. There is a clear relationship between the timing of the negative energy balance nadir and the LH pulse frequency.^{10,118} Once the negative energy nadir is passed and energy levels rise there is an increasing LH pulse frequency which is able to support dominant follicle growth and steroidogenesis. Thus, if a dominant follicle is selected during the recovery period

from negative energy balance, LH pulses can occur and drive the follicle to maturity. The increased estradiol synthesis induces a gonadotrophin surge, and 75% of the time ovulation occurs.¹⁰ The first ovulation postpartum occurs 7-14 days after the energy balance nadir. Thus, if the lowest point of the negative energy balance occurs between the 1st and 2nd weeks postpartum then most cows can be expected to ovulate by 21-30 days.¹¹ This understanding of postpartum follicular dynamics explains why the estradiol level in normal postpartum cows is basal for at least 10 to 14 days.

The normal hormonal milieu that has just been described must be remembered when postpartum uterine motility is being discussed. If one accepts that “mother nature” knows best, then it seems ludicrous to insist that uterine involution can be enhanced by estrogen injections. It is obvious that uterine involution typically proceeds quite uneventfully when the postpartum cow has minimal estrogen in her system. While there is little – if any – data to support a beneficial role for estrogen therapy in the immediate postpartum period, several studies have demonstrated the negative impact of progesterone. Uterine involution can be delayed by the administration of progesterone.^{53,112} This is not surprising if it is remembered that removal of the “progesterone block” is necessary to permit enhanced myometrial tone prior to birth.

Retention of fetal membranes is associated with a failure of dissolution of the collagen within the placenta.⁴⁷ However, strong myometrial contractions are still important since they aid in the mechanical separation of the cotyledonary villi from the caruncle by intermittently spreading the maternal tissue such that the crypts are distended. Nutrition related conditions such as hypocalcaemia impede this involution process, as does myometrial fatigue following a protracted dystocia.¹³⁶ Pain (endorphins) and fear (adrenaline) associated with dystocia manipulations are known to impede uterine motility.^{43,145} In fact, a slow intravenous infusion of epinephrine (10 ml of 1:1,000) can be used to facilitate manual prolapsing of the postpartum uterus. Relaxation of the uterus is detectable within 1 to 2 minutes of initiating the infusion.⁷² The same inhibitory effect on myometrial activity has been demonstrated when adrenaline is administered intravenously to a cow in estrus.¹⁴³ Adrenaline exerts a beta-mimetic effect on the estrogen primed uterus (beta2-receptors), thereby suppressing motility.^{19,181,182}

The Sick Postpartum Cow

There is no doubt that dystocia and hypocalcaemia predispose a cow to postpartum uterine problems. Persistent uterine infection and inflammation tends to be associated with the presence of a flaccid, atonic uterus

(delayed involution). Metritis, by definition, indicates that all layers of the uterine wall are inflamed – not just the endometrium (endometritis).^{15,104,113,157} Acute puerperal metritis is usually diagnosed within the first week postpartum, and is characterized by fever, depression, and a fetid, watery uterine discharge. The flaccid, fluid-filled uterus can't be retracted. Toxemia and/or bacteraemia cause the affected cow to become inappetent and milk production declines.^{56,157,166,179} Decreased rumen fill in the postpartum period predisposes the cow to abomasal displacement. In severe cases, cows with toxic metritis may become recumbent and susceptible to all of the negative features associated with the Downer Cow Syndrome. Few cows actually die from uterine infections, but certainly cows with uterine infections are more likely to be culled for poor reproductive performance in the future.¹⁰⁴ Thus, there is a tendency for researchers to focus on reproductive end points such as conception rate at 1st service; number of services per conception; and days open.⁵⁴ However, in the short term the animal's immediate health – not future fertility – is the major reason why clients seek veterinary intervention for cows with toxic metritis. This postpartum metritis-delayed uterine involution syndrome is extremely frustrating for a veterinary clinician to manage since there is no scientifically proven protocol that will enhance uterine contraction and promote evacuation of the fetid uterine contents. Supportive measures (anti-inflammatory medication and systemic antibiotics) may help to maintain the cow's appetite and rumen motility, but this author remains unconvinced that any current hormonal therapy actually works. As a profession we desperately need scientific data that demonstrates conclusively which therapeutic agents – if any - are uterotonic in the postpartum cow. Dose and route of administration are factors that must be considered in any meaningful study.

Uterine Motility Studies

As early as the 1930's strong uterine contractions were documented at the time of estrus.⁵⁰ Recordings were made using a balloon technique, and these investigators were able to demonstrate that contractions became very weak during the progesterone dominated diestrus period. Since then, several studies of uterine motility have been performed on the involuted uterus of non-pregnant, cycling cows.¹⁴³ Although this is useful information, it is important not to assume that these same mechanisms apply in the immediate postpartum period when the uterus is a vastly different organ in both size and activity. There is some variability in the data available from periparturient studies. The reasons for this are three-fold - differences in the recording equipment employed; variability in the duration of recording sessions; and the limitations inherent when small num-

bers of animals are studied.^{12,20,22,60,67,77,78,87,97,98,114,165,168,171,180,181} Several types of data recording equipment have been employed to study the mechanisms of postpartum uterine involution. The conclusions need to be interpreted in light of many physiologic factors that can bias the results. Studies have been performed with intraluminal and intramural balloons or catheters, stretching measuring strips, strain gauges, and electromyographic data recorders.^{1,12,20,22,29,39,59,60,67,77,78,87,114,143,144,165,171,180,181} Some of these methods are prone to artifacts attributable to respiration, rumination, postural or excretory activity, as well as local myometrial irritation around the surgical site. Balloons and catheters are useful to record pressure information. Multiple intraluminal balloons, strain gauges or electrodes can record the frequency and direction of contraction waves.

One major disadvantage that all these studies have in common is that the animals being studied were healthy postpartum cows. It is impossible to say what - if any - of the conclusions actually apply to the atonic uterus that characterizes the toxic metritis cow.

Uterine Motility

(i) Effects of Hormones

Since the cellular mechanisms involved in periparturient myometrial contractility have not been well characterized in the cow, the temptation has been to extrapolate findings from other species.²⁰ Myometrial excitation and uterine contractility are suppressed by the "progesterone block".³² Progesterone prevents development of oxytocin receptors.^{57,159} Fetal cortisol initiates changes in placental enzymatic activity such that serum levels of progesterone decline and estrogen levels increase.^{9,129} Estradiol binding to myometrial receptors causes changes in muscle cell polarization and increases the number of gap junctions.¹³⁴ These gap junctions are intercellular connections with low electrical impedance. Enhanced movement of electrolytes and small molecules between adjacent myoepithelial cells leads to increased contractility.^{14,30,64,90,134} Estrogens are also known to stimulate the production of PGF_{2a} from caruncular tissue.^{9,72} Prostaglandin induced luteolysis removes the remaining effects of the progesterone block and estrogen becomes the dominant steroid a few hours prior to parturition.^{42,48} This results in a marked increase in the number of oxytocin receptors. There is a significant increase in myometrial excitability within 24 hours of parturition, and uterine tone increases.^{32,57,115,158,159} As parturition approaches, a rapid membrane depolarization results in the onset of strong, coordinated uterine contractions that characterize the first-stage of labor.

The physiologic role of elevated postpartum prostaglandin levels is unclear. Prostaglandins and thromboxanes are metabolites of the cyclooxygenase pathway

of arachidonic acid metabolism. The metabolism of PGF_{2a} itself is very rapid. The postpartum period in the normal cow is characterized by high concentrations of PGFM in the peripheral circulation.⁹² Whether prostaglandins have a direct effect on periparturient uterine activity has been a contentious issue amongst researchers.^{20,44,45,46,60,72,73,74,93,97,98,105,106,127,128,154,168} There is a complex interaction between oxytocin and PGF_{2a}. PGF_{2a} is integrally involved in a feedback loop with oxytocin, even though the molecules have different receptors on the myometrium, and different second messengers within the cells.^{27,45,123} Once oxytocin binds to its receptor on the myometrial cell there is an increased synthesis of prostaglandin.¹⁵¹ Recent data reveal that bovine parturition is associated with a marked induction of cyclooxygenase-2 in the uterus.⁵⁸ In periparturient ewes, circulating oxytocin binds to myometrial receptors, leading to rapid uterine contraction and a rise in PGF_{2a} levels.^{116,115,121} Research in ewes and rats has indicated that PGF_{2a} stimulates the release of more oxytocin (ovary; pituitary?) and also enhances the sensitivity of the myometrium to oxytocin.^{28,51,52,66}

This complex interaction is demonstrated by the fact that the prostaglandin synthetase inhibitor (meclofenamic acid) blocks corticosteroid induced parturition in sheep, but uterine motility can be reactivated by administration of oxytocin. It appears that by inhibiting PGF_{2a} production the meclofenamic acid indirectly blocks uterine activity. The low PGF_{2a} levels don't promote further release of oxytocin and thus there is an indirect inhibitory effect on the myometrium.^{2,122} In the presence of prostaglandin synthetase inhibitors the myometrium will still respond if exogenous oxytocin is administered.^{28,45} In an *in vitro* study prostaglandin-desensitized uteri responded significantly to oxytocin challenge, and vice versa, suggesting that there are separate uteri receptors for oxytocin and prostaglandin.⁴⁵ In postpartum cows that have been treated with a cyclooxygenase inhibitor (flunixin meglumine) the response to a low dose of oxytocin (5 IU intravenously) is attenuated - but the rate of uterine involution is not affected.¹⁶⁸ This study suggests that although the action of oxytocin is closely associated with prostaglandin levels, high levels of PGF_{2a} are not a pre-requisite for uterine involution. If a hysterectomy is performed within 8 hours of parturition the PGFM levels fall dramatically and become undetectable within 5 hours. This conclusively demonstrates that the uterus is the source of the postpartum prostaglandin production.⁷²

(ii) Myometrial Activity

There is minimal uterine activity during the final week of gestation.^{20,78,97,165} The concentration of relaxin, a proteohormone produced by the corpus luteum, increases in the days leading up to parturition. It has a mostly sup-

pressive impact on uterine motility possibly by increasing the efflux of calcium ions out of the myometrial cells.¹³⁵ The high relaxin levels serve to increase collagenase activity in the uterus and other tissues (cervix, pelvic symphysis and ligaments). Collagenase activity is an essential feature of the placental maturation process, leading to rapid detachment of the fetal membranes following fetal expulsion.⁴⁷ It appears that fetal movement results in localized myometrial contractions, possibly associated with positioning the fetus in preparation for delivery.^{20,97,181} In the final 6 hours of stage I uterine activity (contractions) are present about 70% of the time.²⁰ The falling levels of progesterone and high levels of estrogen result in regular, strong waves of contraction – each lasting 5 to 15 minutes. The rate of tubocervical wave propagation becomes more rapid and the propagation index (percentage of contractions of the uterine body that form the end of a tubocervical wave sequence) approaches 75%.^{20,97} Between 8 and 4 hours prior to fetal expulsion the contraction size increases from 10% and 30% of the size at fetal expulsion. At the onset of stage II the contractions increase to almost 80% of the size at expulsion. In short, the amount of uterine work (force and frequency of contractions) increases markedly during the 12 hours prior to delivery of the calf.^{20,22,67,78,97,165,180}

Oxytocin, which is mainly secreted into the blood stream in the expulsion phase, increases the contractile activity of the myometrium. This occurs subsequent to an increased influx of calcium ions into the smooth muscle cells, and also increased calcium availability within the cells.^{13,33,146,148} Maternal straining (contraction of the abdominal muscles) is almost always associated with large sustained uterine contractions that are most commonly associated with the uterine body. Rupture of the amnion, and loss of the remaining fetal fluids leads to a transient reduction in uterine activity – until the calf itself enters the cervico-vaginal canal. The frequency and amplitude of the contraction waves then increase markedly. The activity index increases to over 80%; the mean duration of contractions increases to about 75 seconds; and the propagation index increases to about 75%. The speed of wave propagation (propagation time) down the horn is about twice as rapid as that prior to the onset of the second stage of labor. This is probably the result of oxytocin binding following its reflex release. Although propagated contractions always start at the tip of the uterine horn, the rate of propagation is so rapid that all parts of the uterus tend to contract simultaneously – approaching 1 every 2 minutes.^{20,97}

Immediately after delivery of the calf there is a marked change in the activity of the uterus. The propagation index approaches 100%, meaning that almost all tubocervical waves end with a contraction of the uterine body.^{20,22,78,165,180} The frequency of contractions becomes extremely regular, slowing to approximately 1

every 2.5 minutes.^{20,22,97} Although the propagation time (wave passing from horn tip to uterine body) becomes more rapid over the final 18 hours prior to parturition, it suddenly slows to almost a minute once the calf is expelled. The mean duration of contractions at the tip and at the uterine body is increased (1.5 mins) compared to those during delivery.^{20,22,97} The contraction size increases quadratically as parturition progresses, and continues to increase after the calf is expelled. By 2 hours post-partum the contraction size is about 1.5 times greater than at fetal expulsion. This increasing contraction size is reflected in a similar increase in the level of uterine work being performed. The frequency of contractions declines steadily as their size increases. The mean figures for day 1 postpartum are a contraction frequency of 1 every 6 minutes - each lasting approximately 2.3 minutes. Frequency, amplitude, and duration of contractions are highest at one hour postpartum and decrease progressively thereafter.¹¹⁴ The strong propagated postpartum contractions serve to rapidly involute the uterus and promote placental expulsion.^{20,22,97,181}

Early in stage III these organized postpartum contractions are still propagated mainly (70% to 90%) in a tubo-cervical direction.^{20,22,77,97,181,165} The greater frequency of contractions at the tip of the uterine horn compared with the uterine body may serve to invert the apices of the fetal membranes and lead to a gradual peeling of the cotyledonary villi from the caruncular crypts in progressive tubo-cervical direction such that the membranes are expelled “inside-out”.^{20,71,181} Passage of the fetal membranes by 3-8 hours causes a rapid decrease in uterine activity.^{60,97} The mean figures for day 1 postpartum are just over half of the uterine body contractions occurring at the end of a tubo-cervical wave, and each wave taking about 1.6 minutes to travel down the horn.^{20,22} Spontaneous postpartum uterine activity consists predominantly of single-peak, propagated contractions throughout the first week after calving. Discrete myometrial contractions can be detected up until at least 7 days postpartum, but the frequency of contractions and the rate of contraction propagation (propagation index) decreases with time.^{12,20,22,60,67,77,78,87,97,114,165,171,180,181} By day 12 to 13 uterine activity begins to increase again. This is believed to be in response to the onset of ovarian activity and the subsequent rise in follicular estrogen levels.^{60,97}

Although dexamethasone induction of parturition leads to retention of fetal membranes, the drug itself has not been shown to alter uterine activity in the immediate peripartum period.²⁰ The presence of fetal membrane retention does not appear to affect the duration of individual uterine contractions.²⁰ However, retention of fetal membranes doubles the rate (short propagation time – 60 seconds from uterine horn to cervix) and increases the frequency of uterine contractions, resulting in a higher relative percentage of uterine activity (ac-

tivity index), and a larger amount of uterine work^{20,114,171} By 24 hours postpartum the amount of uterine work normally decreases by over 50%, but if the membranes are retained then uterine work remains at approximately 80% of the activity at 6 hours postpartum.²⁰ On day 1 postpartum a third of the uterine body contractions form at the end of a tubocervical wave, whereas two-thirds occur in cows with retained membranes. By day 3 the cows with retained membranes still have a third of the uterine body contractions forming at the end of a tubocervical wave whereas only about 6% occur in normal cows. On day 5 this tubocervical wave propagation has ceased in normal cows but 13% of the waves in retained cows still propagate through to the uterine body.²⁰ On days 2-5 postpartum the amount of relative uterine work is twice as great, and the frequency of contractions at the body of the uterus is 3.5 times as great in cows with fetal membrane retention. Irrespective of the presence of fetal membranes, contraction frequency and activity index (percentage of recording time occupied by uterine activity) are significantly greater at the tip of the gravid horn than at the body of the uterus.²⁰ However, in cows with retained membranes 10-15% of the contractions at the uterine body actually initiate a reverse wave that progresses in a cervico-tubal direction. These reverse waves are detectable for the first two days postpartum.²⁰ The significance of this abnormal contraction pattern is not known.

Oxytocin

Oxytocin is perhaps the most overdosed (NOT over utilized) hormone available in veterinary practice. Products are routinely formulated at concentrations of 20 United States Pharmaceutical (USP) units/ml and package inserts recommend dosages of 100 USP (5 ml).¹³⁰ An injection of a mere 1.0 I.U. oxytocin causes blood concentrations comparable with those occurring physiologically during milking. Suckling has been shown to be a more potent oxytocin stimulus than milking.^{3,8} Even doses as low as 10 – 20 USP are actually suprphysiological.¹⁸ In the period from 2 days before to 2 days after estrus as little as 2.5 IU of oxytocin intravenously will cause the proximal ends of the uterine horns to respond within 30 to 50 seconds. The increased frequency of myometrial activity persists for up to 80 minutes.¹⁴³ If the same dose is administered during estrus itself the latency period is reduced to 10 seconds, and the frequency of the prolonged rhythmic activity is doubled for about 2 hours.¹⁴³

The pain (endorphins) and fear (catecholamines) associated with dystocia manipulations are known to impede uterine motility via an oxytocin block.^{43,145} This early postpartum uterine atony appears to be preventable by the administration of 20 IU oxytocin. Post-ce-

sarean section fetal membrane retention was reduced from 35% (controls) to 7% (treatment group) when cows received 20 IU oxytocin IM immediately following surgery and again in 2 to 4 hours later.¹⁴⁵ Whether this hormone is effective in cows that have already developed toxic metritis remains to be determined.^{84,137} Although some authors suggest that an injection of oxytocin immediately after calving may reduce the incidence of fetal membrane retention, there is limited data to support this approach, and the reports are contradictory.^{7,51,70,71,86,119,120,153,162} As previously mentioned in this review, the mere presence of retained fetal membranes doubles the rate and increases the frequency of uterine contractions.²⁰ As little as 5 IU of oxytocin intravenously will initiate a more intense rhythm of contraction in these cows.⁹⁸ In two fetal membrane retention studies that reported no beneficial effect of postpartum oxytocin therapy the authors used what has been demonstrated to be a "spasm" inducing dose of 60 – 100 IU.^{20,86,119} Few studies have attempted to determine what is the most physiologic uterotonic dose of oxytocin.^{20,46,49,98}

During the first 6 days postpartum intravenous doses of oxytocin ranging from 2 USP up to 40 USP will increase the frequency of myometrial contractions, with the onset of response occurring approximately 30 seconds after injection. The magnitude of this increase is dependent on both dose and day of treatment.^{20,98} Each successively larger dose produces a significantly greater increase in contraction frequency, ranging from 1 every 6.5 minutes (2 USP) up to 1 every 3 minutes (40 USP). The last detectable responses to doses of 2, 5, 10, 20, and 40 USP of oxytocin were observed on postpartum days 6, 7, 8, 9 and 10, respectively. The percentage of uterine body contractions that formed at the end of a propagated tubo-cervical wave (propagation index) was also increased by all doses of oxytocin. An intravenous injection of 25 USP oxytocin at 12 hours postpartum increases the propagation index to 80% - up from the baseline 50% of contractions reaching the uterine body. The same dose of oxytocin (25 USP) consistently caused an increased contraction frequency ($P < 0.01$) and higher tubo-cervical wave propagation ($P < 0.01$) on treatment days 1 to 5. The initial response to oxytocin (during the 1st hour after injection) was similar on days 1 to 5.²⁰

On postpartum days 1 to 6 the mean overall duration of response following injection of 20 or 40 USP of oxytocin (approx 2 hours and 25 minutes) was significantly greater than that following the lower doses (approx 1.5 hours). When 25 USP oxytocin was injected intravenously the uterine response lasted at least 2.0 hours on days 1 to 4, but had decreased to 1.5 hours on day 5. Although the overall duration of response was similar following injection of either 20 or 40 USP, the higher dose caused an initial tetanic-like spasm that lasted 6 to 10 minutes. This tetanic effect was only ob-

served at the 40 USP dose and was most marked on the first 3 days postpartum. Burton, Kundig and Gajewski have independently reported that oxytocin's effect is to increase the frequency of uterine contractions, and the percentage of these contractions that travel completely down the horn to the uterine body.^{20,98,60} Kundig reported that an intravenous dose of 5 IU oxytocin resulted in a rapid and strong increase in contractility during the first 2 to 3 days postpartum, but that by days 4 and 5 the amplitude and duration of response began to decrease.⁹⁸ Since the 40 USP dose causes an initial tetanic spasm it would appear that most cows are currently being overdosed. The overall duration of response at 2 days postpartum is approximately 3 hours, decreasing and plateauing to 1.5 hours by days 5 to 6.²⁰ Thus, the most efficacious oxytocin therapy may need to be adjusted with days postpartum. A possible Day 2-3 protocol may be repeated 20 USP (1.0 ml) oxytocin injections administered at least 3 hours apart – or three doses evenly spaced between milkings. By day 4 the dose could be increased to 30 USP and the frequency increased to every 2 hours. Although this frequent low dose therapy may be impractical, it certainly would be more physiologic than the widely used infrequent, tetany-inducing doses.^{75,76} Since flunixin meglumine attenuates the uterine response to an intravenous injection of 5 IU oxytocin, doses lower than 20 IU are probably not appropriate when sick cows are being concurrently treated with anti-inflammatory medication.¹⁶⁸ It must be emphasized, however, that in cows which have been treated with flunixin meglumine, uterine involution progresses normally.¹⁶⁸

It is noteworthy that the increased frequency of uterine contractions following oxytocin administration in the postpartum cow is exactly the same observation as that seen when stage II of labor commences. The presence of the fetus in the vaginal canal is known to stimulate endogenous oxytocin release (Ferguson Reflex).^{145,148} The research from workers such as Burton, Gajewski and Kundig has refuted the often quoted view that oxytocin is only uterotonic during the first 1 to 2 days postpartum.^{7,20,22,60,97,98,137} It is unfortunate that the work of Burton, Kundig and Gajewski was not conducted using the more typical intramuscular route of oxytocin administration. However, Burton did confirm that the myometrial response following injection of 20 to 30 USP of oxytocin is similar following administration via the IV, IM, or SQ routes.²⁰ Since serum estrogen levels decline rapidly in the postpartum period it would appear that the dogma about estrogen-primed receptors warrants serious questioning.⁸⁸ Perhaps the estrogen-induced oxytocin receptors persist on the postpartum uterus for several days?^{158,159} It is interesting to speculate that the characteristic tone of the estrus uterus is rapidly lost not so much because of a fall in post-ovulatory estrogen concentrations, but rather because of the inhibitory ef-

fect of a rising progesterone level. Irrespective of the mechanism, the previously mentioned studies clearly demonstrate that there can be no valid argument with respect to the oxytocin receptors that would support the use of exogenous long-acting estrogen formulations in the postpartum dairy cow.^{20,60,97,98}

Prostaglandins

In 1986 Gross *et al* reported that routine administration of prostaglandin F_{2a} immediately after dexamethasone induced calving was a successful means of reducing the incidence of retained fetal membranes.⁶⁹ Numerous studies since have failed to confirm these results, and many suggest that prostaglandin has no effect. Although several other authors also report that prostaglandin therapy may enhance uterine involution and promote the passage of fetal membranes, the results are far from conclusive.^{6,21,44,46,62,63,74,85,91,93,95,127,106,160,161,163,173,178} Many reports must be considered anecdotal because of the small number of animals used, lack of controls, and the concurrent use of other medications. Certainly the results of a study that alluded to a beneficial effect of PGF_{2a} after cesarean section were clouded by the concurrent use of a smooth muscle relaxant (isoxsuprine) during surgery.¹⁶³ In many field studies it is not acceptable to the owner that a group of cows receive no treatment at all. Concurrent use of intrauterine medication, or traction on the membranes, is a common study flaw.

Intramuscular injections of 25 mg prostaglandin F_{2a} (Dinoprost) have no effect on uterine motility.^{20,98} When the prostaglandin doses were doubled (50mg PGF_{2a}) there was still no uterotonic effect detected.²⁰ This is not really surprising when one considers that there are already high endogenous levels of prostaglandins in the postpartum cow.^{42,110} The concentration of prostaglandin metabolite (PGFM) drops slowly after the first two days postpartum, reaching baseline levels by day 11.¹⁶⁸ However, luteolytic doses of PGF_{2a} (25mg Dinoprost) administered by rapid intravenous (bolus) injection are uterotonic in postpartum cows, increasing both the frequency of contractions and the amount of tubo-cervical wave propagation.^{20,60} When 15mg of Dinoprost was injected intravenously it resulted in a strong, but delayed (10-20 minutes), stimulation. However, the stimulatory effect was markedly diminished by day 4 postpartum.^{98,168} This treatment would be impractical in a toxic cow since there are dramatic side effects (uneasiness; dyspnea; frequent urination; milk ejection; and salivation).⁹⁸ In contrast to the natural prostaglandin (Dinoprost), an intravenous injection of the synthetic PGF_{2a} derivative (Cloprostenol 0.25 mg) resulted in a minimal increase in uterine activity on day 1.⁹⁸ The lack of a uterotonic effect from Cloprostenol has been reported by other investigators.⁴⁶

The myometrial response to intravenous prostaglandin F_{2a} may explain why *in vitro* studies have demonstrated a uterotonic effect of PGF_{2a} .^{128,154} The half-life of PGF_{2a} is very short – reportedly less than 1 minute.¹⁴⁷ Intramuscular injections of PGF_{2a} may not be uterotonic because the PGF_{2a} is metabolized almost entirely into PGFM upon a single passage through the lungs.³⁴ Thus, gradual absorption of PGF_{2a} from an injection site, followed by immediate metabolism by the lungs, may mean that levels equivalent to the bolus IV effect are never achieved. The half-life of PGFM itself is approximately 18 minutes.⁷²

Fenprostalene, a synthetic analog of PGF_{2a} with a prolonged plasma half-life, has been recommended as a treatment for fetal membrane retention.⁸⁵ Peak plasma levels of fenprostalene concentrations are reached approximately 10 hours after injection, and the elimination half-life is reported to be 18-23 hours.^{20,21,65} Interestingly, SQ injections of this long-acting synthetic prostaglandin have not been shown to produce any significant changes in the percentage of recording time that is occupied by uterine activity (activity index); the percentage of contractions of the uterine body which form at the end of a tubo-cervical contraction wave (propagation index); or the amount of time taken for propagated tubo-cervical contraction waves to pass along the length of the uterus (propagation time).²⁰ Repeated SQ injections of fenprostalene (1 mg) at 12, 36, 60 and 84 hours postpartum did not produce any cumulative uterotonic effects, and did not promote passage of the fetal membranes.²⁰ Even when the fenprostalene dose was doubled (2 mg) there was still no uterotonic effect detected.²⁰ It was not uterotonic after intravenous injection either.⁴⁵

Thus, IM and SQ injections of either natural or synthetic prostaglandins do not appear to be uterotonic in the postpartum cow.^{20,45,46,97,98,168} What is especially intriguing is that prostaglandin does appear to have a uterotonic effect in the non-pregnant cow if estrogen is dominant (follicular phase; estrogenized ovariectomized cows).^{20,44,45,46,61,138}

Another factor that speaks against a direct uterotonic effect for PGF_{2a} in the postpartum cow is that although the administration of the cyclooxygenase inhibitor, flunixin meglumine (days 1-10 postpartum) will significantly decrease the levels of prostaglandin metabolite (PGFM), the rate of uterine involution is not affected.^{73,168} In one study the overall reduction in prostaglandin production exceeded 80%.¹⁶⁸ These studies indicate that partial suppression of prostaglandin synthesis early in the postpartum period does not affect the rate of decrease in the cervical and uterine horn diameter, nor the location of the uterus within the pelvic canal.^{73,168} It would appear that high levels of PGF_{2a} are not an essential factor for normal uterine involution. The physiologic processes involved in uterine in-

volution (vasoconstriction, myometrial contractions, collagen tissue re-organization) seem to progress normally even if anti-inflammatory medication has lowered the normal prostaglandin level. This is despite the fact that spontaneous uterine motility and the response of the myometrium to oxytocin and intravenous PGF_{2a} is attenuated.¹⁶⁸ When 8 cows were treated twice daily with flunixin meglumine for 10 days, uterine involution was actually completed in significantly less time than the control animals.¹⁶⁸ Reports that imply an association between PGF_{2a} and uterine involution have not demonstrated a cause and effect relationship. A study suggesting that a large dose of flunixin meglumine (1.5g) after cesarean sections increases the incidence of fetal membrane retention may have been compromised by the fact that a smooth muscle relaxant (isoxsuprine) was administered prior to surgery.¹⁷³

Advocates may argue that prostaglandins could have beneficial effects on the postpartum uterus that don't relate to the uterine motility controversy. Phagocytosis by neutrophils and subsequent killing of ingested bacteria is important in the elimination of infection. Since prostaglandins are an integral part of the inflammatory process it may be that exogenous prostaglandin therapy enhances the effects of other inflammatory mediators. Scientific investigations are needed to determine if these anecdotal reports are valid.

Estrogens

Since estrogen levels fall dramatically once the calf is expelled, it would appear that uterine involution can actually progress without estrogenic influence in the normal cow. Thus, it is strange that the administration of exogenous estrogens as a treatment for metritis has been in vogue – off and on – for a many years. Advocates claim that estrogen therapy will improve uterine tone.^{7,84,116,126,137} However, a field study involving 374 cows was not able to demonstrate a beneficial effect of 6mg ECP, prostaglandin and ECP, or oxytocin and ECP.²⁴ Some recent anecdotal reports from bovine veterinarians suggest that estrogen therapy in the immediate postpartum period may be useful in the treatment of delayed uterine involution and metritis.¹⁶⁹ Unfortunately solid scientific evidence is lacking to support use of this estrogen product – even at the lower 4mg dose that is currently being advocated.^{36,37,41} Field trials that use subjective assessment of clinical response should be blinded (observers are unaware of the treatment) so that the possibility of placebo effects is eliminated. There must be an untreated control group.³⁵

The rationale for ECP therapy is based on an unsubstantiated belief that estrogens will enhance the response of the postpartum uterus to uterotonic agents such as oxytocin. The evidence for the effects of estrogen on

myometrial activity in the postpartum cow is primarily subjective – based on clinical impression.^{20,22} The expectation for beneficial postpartum effects may result from an over-extrapolation of the data from non-pregnant, cycling cows.¹³⁹ Estradiol has a positive effect on the ability of the uterus to secrete PGF_{2a} in response to oxytocin, but the nonpregnant uterus must have been exposed to progesterone first (luteal phase).^{99,111} The whole concept of estrogen priming requires intensive study since the role of estradiol in the regulation of oxytocin receptor synthesis remains controversial. Although estradiol will induce an increase in oxytocin receptors, uterine oxytocin receptor concentrations have been shown to be high in ovariectomized ewes.¹⁷⁰ Concentrations will decline soon after progesterone replacement therapy is initiated. This work supports the notion that removal of the “progesterone block” may be more important than stimulation by estrogen. A recent study on the cyclic bovine endometrium demonstrated that estradiol speeds up the spontaneous upregulation of oxytocin receptor expression via the estradiol receptor - but it is not essential for this process. Local factors from the endometrium may be necessary to regulate oxytocin receptor expression via interaction with the estradiol receptor.¹⁰³ Studies specifically looking at the postpartum myometrium are required before we can extrapolate these exciting findings from the cycling animal. The estradiol receptors may well be down-regulated in the postpartum cow.¹⁴¹ Certainly spontaneous upregulation of endometrial oxytocin receptors occurs in the absence of estradiol. In fact, some now believe that estradiol may not be the primary regulator of oxytocin-receptor gene expression.¹⁰³

Observations on the uterine motility of sheep and rabbits have confirmed some interesting features about the non-gravid, estrogen-dominated uterus. In estrous rabbits the majority of uterine contractions move from the cervix towards the oviducts.¹⁷⁶ This cervico-tubal contraction pattern has also been reported in estrous ewes.^{31,81,82} In the estrous ewe the number of contractions average 5 – 6 per minute. In early estrus at least two-thirds of the contractions originate in the uterine body and progress anteriorly. In late estrus, only a third of the contractions originate in the uterine body. In contrast, two days after estrus some three quarters of the contractions originate at the tip of the horns and move in a tubo-cervical direction.⁸³ These tubo-cervical contractions are possibly an extension of the oviduct contractions that carry the embryo down into the uterus.¹⁴³ Administration of estradiol-17B during late estrus prevents the change in direction of the contractions. Ovariectomies performed during the luteal phase of the cycle, in conjunction with estradiol injections, will initiate the typical cervico-tubal estrus contractions within 48 hours.⁸³ Estrogen induced reverse peristalsis is probably the reason for the high incidence of salpingitis re-

ported when the 10mg labeled dose of ECP was widely used to treat metritis.^{25,41,75}

Hormonal control of the direction of uterine contractions has been confirmed in the cycling cow as well. Open-tipped catheters have been employed to demonstrate a relationship between the motility pattern of the uterine horn and the phases of the estrous cycle. It was shown that maximal rhythmic activity occurs during estrus, with contractions running from the cervix towards the oviduct (cervico-tubal). The direction was reversed at the end of estrus.³⁹ Another study showed that in the 48 hours prior to the onset of estrus there is a gradual transition from local, non-propagating electrical activity to propagating electrical activity with an increase in the duration of contractions, and then of their amplitude.¹⁴³ This transition coincides with a rapid decrease in progesterone level from 5 to 10 ng/ml to less than 0.1 to 0.4 ng/ml. Bursts of activity (5 minutes) start near the cervix, then progress towards the oviduct. The prevailing direction of uterine contractions through until late estrus is cervico-tubal.¹⁴⁰ These findings are not unexpected since cervico-tubal contractions during estrus will assist with sperm transport. In fact, vaginal stimulation during estrus leads to a myometrial response that spreads over the whole of the uterus and into the lower part of the oviduct. These contractions last from 5 to 30 minutes beyond the time of stimulation.¹⁴³ In metestrus the majority of contractions appear to originate in the oviduct near the uterotubal junction and to propagate towards the cervix. Perhaps this facilitates expulsion of extraneous foreign protein (sperm) prior to the arrival of the embryo? The strength, but not the frequency of activity, diminishes progressively for 2-3 days after estrus, and then relative inactivity ensues.¹⁴³

The myometrial effects of an intramuscular injection of 5mg estradiol cypionate (ECP) at 18 hours postpartum has been compared with baseline motility, and with oxytocin responses prior to, and on the first day after injection of ECP.^{20,22} The estrogen treatment had a statistically significant and negative impact on uterine motility. Contraction frequency was reduced from 9.6/hour to 2.9/hour ($P < 0.01$) and duration of each contraction was increased from 141 seconds to 422 seconds ($P < 0.05$). The ECP treatment changed the normal motility pattern from predominantly single-peak contractions into a sustained contraction pattern - with multiple superimposed small peaks. The uterus could be best described as in spasm since all parts of the uterus tended to contract simultaneously. Despite this, the contractile force was probably reduced since the mean amplitude of contraction curves was lowered significantly ($P < 0.05$). These uterine effects of ECP became apparent by approximately 4 hours after treatment, and they persisted until day 5. Only then did some discrete, single-peak

contractions return.^{20,22} When 25 USP oxytocin was administered (IV) on day 2 postpartum (6 hours after the ECP treatment), the myometrial activity returned to the normal, single-peak, propagated contraction waves. The effect of 25 USP oxytocin on the contraction frequency (17.5/hour) in this ECP primed uterus was no different than the 6-day mean (17.3/hour) for 20 USP oxytocin on the normal uterus.^{20,22} This tends to dispel the notation that ECP enhances the myometrial effect of oxytocin. Estrogen priming actually caused a slight suppression in the post-oxytocin mean contraction duration (119 seconds) and propagation index (72%). The mean duration of myometrial response to oxytocin in the ECP primed uterus was not significantly different from that of the normal postpartum uterus. Burton concluded that there were no detectable differences between myometrial response to oxytocin administered before, and 6 hours after the ECP (5mg) injection. Oxytocin was then administered daily following the ECP (5mg) priming to determine whether there was any delayed positive effect on postpartum myometrial activity. No changes were detected.²² Pretreatment with ECP (5mg) did not result in either PGF_{2a} (25mg IM) or fenprostalene (1mg SQ) becoming uterotonic. There were no significant changes in postpartum myometrial activity. The prostaglandin injections were repeated daily for 5 days to determine if there was an effect of the ECP treatment, but no effect was detected.²⁰

Another proposed benefit of estrogen therapy is stimulation of natural uterine defense mechanisms.^{7,23,26,55,80} Advocates hypothesize that exogenous estrogens may improve uterine blood flow and thus bring more neutrophils to the site of infection. It has also been suggested that exogenous estrogens will improve the phagocytic capacity of these neutrophils. Yet again, the evidence is inconclusive and the variability in the methods used to assess neutrophil function makes it difficult to reach a definitive answer. The antibacterial action of neutrophils recovered from the uterine lumen has been measured by their chemotactic, phagocytic and killing ability.^{5,101,164} A recent study reported that there was no consistent influence of the reproductive state on the resistance of the uterus to infection, as measured by differences in either peripheral or intrauterine neutrophil function.¹⁶⁴ Comparisons were made between responses obtained at estrus and diestrus, and following the administration of exogenous estradiol and progesterone to ovariectomized cows. Leukotriene B4 (LTB4) may play an important role in both placental separation and uterine involution in cattle.¹⁵⁵ It is a metabolite of the 5-lipoxygenase pathway of arachidonic acid metabolism, and is a potent chemoattractant of polymorphonuclear cells (PMN). This association with prostaglandin metabolism may explain why oxytocin has been shown to stimulate LTB4 synthesis during the early post-partum

period in cattle.¹⁵⁶ Perhaps that is why repeated small doses of oxytocin may have some therapeutic merit? Caruncular tissue taken from the previously gravid horn produces less LTB4 if it is treated with progesterone, but estrogen treatment has no effect, neither increasing or decreasing LTB4 synthesis.¹⁵⁵ Once again it may be the absence of progesterone's inhibitory action - rather than the presence of estrogen - that enhances the uterine defense mechanisms when a cow is in estrus.^{17,79} The inhibitory effect of progesterone may also explain the increased incidence of clinical endometritis in cows if the first ovulation occurs early in the postpartum period.^{89,100,125,142} Luteolytic doses of prostaglandin in the post-ovulatory period are beneficial to return the cow to estrus. In this instance the estral uterine tone and characteristic mucus flow appear to be therapeutic.⁴

My conclusion is that there is little - if any - scientific data to support the use of estrogen therapy in the postpartum cow. Veterinarians should consider the tissue half-life of long-acting estrogens when using these products. Since estradiol-17B has a very short half-life (<5 minutes) it is marketed in commercial preparations (cottonseed or sesame oil) in one of several esterified forms (E2-17B benzoate, E2-17B valerate, and E2-17B cypionate).^{38,172} Although ECP is an old drug (1950's), there is limited information available on its pharmacokinetics for any of the veterinary species.¹³⁰ Esterified estrogens such as ECP have delayed absorption after IM administration. Estrogens are distributed throughout the body and accumulate in adipose tissue.¹³⁰ ECP is highly fat soluble.⁴¹ Only after slow hydrolysis in the liver is the active estradiol-17B released.¹⁷² The eventual elimination of the steroidal estrogens occurs principally by hepatic metabolism. Estrogens and their metabolites are primarily excreted in the urine, but are also excreted into the bile, where most is then reabsorbed from the GI tract.¹³⁰ In short, the various esterified forms are long-acting formulations of estrogen.

The well-known Syncro-Mate-B progestogen ear implant was approved to permit synchronized breeding in cycling beef cattle and non-lactating dairy heifers. The package insert specifically warned that the product was NOT to be used in cows producing milk for human consumption.¹¹⁷ The protocol included an IM injection that is administered at the time of insertion of the 6mg norgestomet ear implant. The 2ml injection contained 5 mg estradiol valerate and 3mg norgestomet. In a study that investigated the impact of progestins on luteinizing hormone release it was determined that the estradiol valerate resulted in elevated estradiol-17B levels that persisted for several days.⁹⁶ Levels peaked at over 80 pg/ml estradiol-17B on day 2, and then slowly declined to what are maximal follicular estrogen derived levels by day 5.⁹⁶ There is one report that specifically looked at the plasma estradiol-17B concentrations in the cow during

induced estrus and after injection of estradiol-17B benzoate and estradiol-17B cypionate (ECP).¹⁷² The objective of that study was to use plasma estradiol-17B levels attained during the normal estrous cycle as a baseline in making withholding recommendations for esterified estrogens. The peak estradiol levels in cycling cows are reported to be in the range of 25-28 pg/ml, with mean values of approximately 16 pg/ml.^{68,149,172,175} An intramuscular injection of 10mg ECP (5ml) resulted in maximal estradiol-17B levels of 56 to 128 pg/ml over a range from 13 hours and 5 days. The concentrations then decreased steadily to estral levels by 5.6 to 9.6 days (135 to 231 hours). In some cows there were two peaks in the E2-17B plasma concentration following an ECP injection. This biphasic curve warrants further investigation since it may be a reflection of an initial redistribution of the mobilized ester, followed by an elimination phase.¹⁷² Estradiol benzoate (10mg) caused a higher initial E2-17_B peak level (82 to 320 pg/ml) but also a more rapid decline, with a return to estral levels within 3.6 to 6.0 days (87 to 143 hours).¹⁷² The marked variability in the peak numbers, and in the return to estral levels warrants further investigation. Only 5 cows were evaluated in this study.¹⁷² It may be that there is substantial biological variation in how cows metabolise these estrogen esters, possibly related to the level of body fat and liver function.¹³⁰ The work needs to be repeated in postpartum dairy cows – especially since the long-acting steroids may well be concentrated in the butter fat component of the milk. A recommendation for a 10 day withdrawal period was proposed for the 10mg (5ml) ECP injection - based on adding twice the standard error of the mean to the average time taken for concentrations of E2-17_B to return estral levels.¹⁷² The author has not been able to find any references that address the biological clearance of ECP at the 4mg (2ml) level that is currently in vogue.^{36,37,169} There are no labeled ECP withholding recommendations for meat or milk.⁴¹

Conclusion

Unfortunately the period of interest – days 1 to 10 postpartum – has not been well studied, and current data is inconclusive. One is faced with anecdotal reports, testimonial type papers or book chapters that espouse the benefits of a particular drug or protocol, yet lack any controls to verify their efficacy. The scientific literature contains peer-reviewed papers that report conflicting results and diametrically opposed conclusions. The end point, uterine involution, is typically determined by palpation per rectum and that, by its very nature, is subjective. The variability in classification of an infection as metritis makes interpretation and comparison of data extremely difficult. Thus, at this time it is impossible to be dogmatic about the efficacy of any treatment modality

for management of the large, atonic bovine uterus that is an integral part of the toxic metritis syndrome.

Several research groups have demonstrated that although there appears to be no increase in the force of uterine contractions if the fetal membranes are retained, both the frequency of these contractions and their rate of propagation along the length of the uterus are significantly greater than in cows that have expelled their membranes normally. Studies have demonstrated conclusively that fetal membrane retention causes an overall increase in myometrial contractile effort. Thus, it seems illogical to advocate hormone use in cows with fetal membrane retention on the pretext that this therapy will enhance uterine activity. Uterine effort is already greater than normal in these animals! However, the question still remains – What to do with that flaccid atonic uterus in a cow with toxic metritis?

In the author's experience (7 yrs mixed practice; 13 yrs university hospitals) the delayed uterine involution - toxic metritis syndrome is probably one of the most frustrating conditions to treat. This is because so little is known about the pathophysiology of the diseased postpartum uterus. Clinicians are faced with a dilemma since clients expect treatment - yet there is no scientifically proven therapy! Certainly no one would argue with the concept that prevention is better than cure, and it is well accepted that attention to dry cow nutrition can markedly reduce the incidence of these postpartum problems. Early intervention before the cow succumbs to the systemic effects of toxic metritis would appear to be a rational goal. Daily monitoring of rectal temperatures in postpartum cows can identify the high-risk animals. Anti-inflammatory medication and broadspectrum antibiotic coverage have merit.

I fully expect that my concluding statement will be controversial. This review of the literature has not convinced me that there is any scientific evidence to support the widespread use of estrogen or prostaglandin in the first 7 to 10 days postpartum. It is unlikely that prostaglandin injections prior to the formation of a functional corpus luteum will have any beneficial effect on the postpartum cow. There is no scientific evidence that estrogens stimulate the type of rhythmic tubo-cervical contractions required to empty the postpartum uterus. Research to date does not support the theory that ECP enhances the myometrial response to oxytocin or prostaglandin. In fact, an intramuscular injection of ECP (5mg) has been shown to actually inhibit the normal spontaneous, co-ordinated myometrial activity of the postpartum cow!! This is obviously counterproductive when uterine emptying is the desired response. A recent evaluation of the 4mg ECP dose as a prophylactic treatment at 24 hours postpartum was not encouraging. There were no measurable benefits, and a negative effect on days to pregnancy by 200 days was

detected.¹⁷⁴ The author is aware of at least two recently completed, but not yet published studies that have found no beneficial effect of ECP treatment in cows with puerperal metritis. Since a low dose of oxytocin (20 USP) is uterotonic up to 9 days postpartum, it has the most scientific validity as a therapeutic agent. Estrogen priming is not indicated!! Repeated low dose oxytocin therapy (20 USP every 3 hours) would ensure that the uterus remains under the influence of rhythmic contractions. This protocol may be impractical under field conditions. Well-controlled scientific studies are desperately needed.

Recent research has determined that many chemicals in the environment (and in our food) possess estrogenic activity. It is the cumulative effect of exposure that may impact on human health. Competitive binding to estrogen receptors in target tissues (eg. uterus, breast) may result in insidious, long-term effects.^{86a} Veterinarians should be cognizant of the increasing public concern about hormone contamination of food products. Thus, estrogen treatment should be used judiciously since it may unnecessarily contribute to the overall burden of human exposure to chemical entities with estrogenic activity. It may be prudent to avoid the use of estrogen treatment until there is convincing evidence of its efficacy.⁶⁵

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