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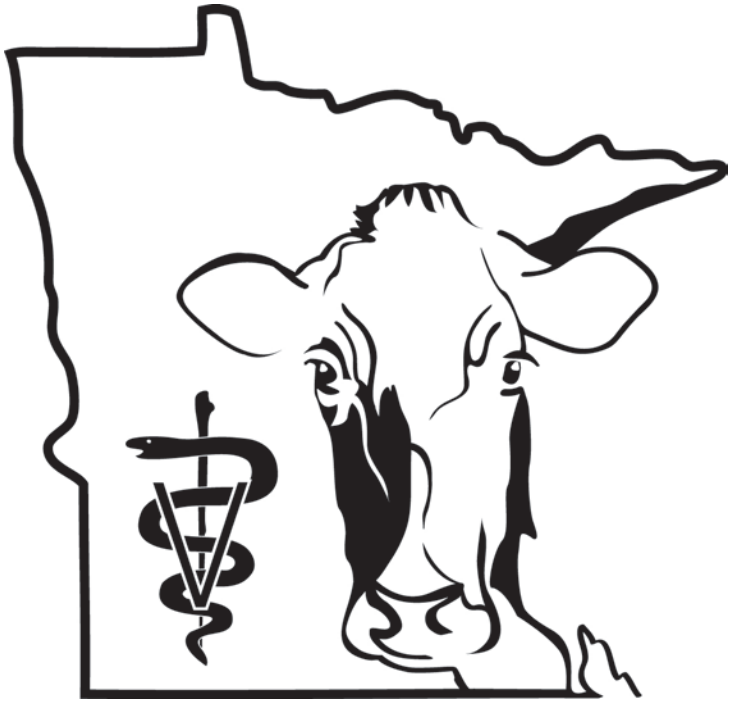

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HORMONAL THERAPY IN THE POSTPARTUM COW – DAYS 1 to 10. FACT OR FICTION ?

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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, dihydroepandrostenedione) 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 ocurs.[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 placentome.[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 inappetant 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 diestrous 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 numbers 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, co-ordinated 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

meclofanamic 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 suppressive 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 is 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 (activity 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 supraphysiological.[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-cesarean 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 if 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 then 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 observed 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 effect 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 day11.[168] However, luteolytic doses of PGf2a (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 <u>in vitro</u> 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 involution (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, 16, 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 then 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 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 estrous, 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 estrous 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 reported 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 post-partum 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, & 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 to 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 estraiol-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 I.M. 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 counter-productive 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]

References

1. Addis M, Chiesa F, Colombo G & Oberosler R. Motilita spontanea dell'utero di bovina durante il ciclo estrale. Nuova Vet 40:343-353 (1964)
2. Aiken J: Aspirin and indomethacin prolong parturition in rats. Evidence that prostaglandins contribute to expulsion of the fetus. Nature 240:21-25 (1972)
3. Akers R & Lefcourt A: Milking- and suckling- induced secretion of oxytocin and prolactin in parturient dairy cows. Horm Behav 16:87-93 (1982)
4. Bp36 Al-Eknah M & Noakes D: Uterine activity in cows during the oestrous cycle, after ovariectomy, and following exogenous oestradiol and progesterone. Br Vet J 145:328 (1989)
5. Anderson K, Hemeida N, Frank A & Whitmore H: Collection and phagocytic evaluation of uterine neutrophilic leukocytes. Therio 24:305-317 (1985)
6. Archbald L, Tran T, Thomas P & Lyle S. Apparent failure of prostaglandin F2a to improve the reproductive efficiency of postpartum dairy cows that had experienced dystocia and/or retained fetal membranes. Theriogenology 34:1025-1034
7. Arthur GH. Retention of the afterbirth in cattle: a review and commentary. Vet Ann 19:26-36 (1979)
8. Bar-Peled U, Maltz E, Bruckental I, Folman Y, Kali Y, Gacitua H, Lehrer A, Knight C, Robinzon B, Voet H & Tagar H: Relationship between frequent milking or suckling in early lactation and milk production of high producing dairy cows. J Dairy Sci. 78:2726-2736 (1995)
9. Bazar FW & First NL. Pregnancy and parturition. J.An.Sci. 57:Suppl 2, 425-460. (1983)
10. Beam SW & Butler WR: Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod. 56:133-142 (1997).
11. Beam SW & Butler WR: Energy balance effects on follicular development and first ovulation in postpartum cows. In: Reproduction in domestic ruminants IV. J Reprod Fertil (Suppl) 54 (1998)
12. Benesch F. The graphic representation of the uterus – normal, and strengthened or weakened by drugs – in living bovines during the period of involution. Proc 11th Intl Vet Congress 367-368 (1930)
13. Berridge MJ. Inositol phosphate and diacylglycerol as second messengers. Biochem J. 220:345-360 (1984)
14. Bengtsson B. Factors of importance for regulation of uterine contractile activity. Acta Obstet Gynecol Scand Suppl. 108:13-16 (1983)
15. Bretzlaff KN, Whitmore HL, Spuhr SL et al. Incidence and treatment of postpartum reproductive problems in a dairy herd. Therio 17:5:527-535 (1982)
16. Bretzlaff KN, Ott RS: Postpartum reproductive problems in a large dairy herd. Bovine Clin. 1:4 (1981)
17. Broome AW, Winter AJ, McNursh and Casida LE. Variation in uterine response to experimental infection due to the hormonal state of the ovaries. II. The mobilization of leukocytes and their importance in uterine bactericidal activity. Am J Vet Res 16:675-682 (1960).
18. Bruckmaier R & Blum J: Oxytocin release and milk removal in ruminants. J Dairy Sci. 81:939-949 (1998)
19. Bulbring E & Tomita T. Catecholamine action on smooth muscle. Pharmacol Rev 39:50-96 (1987)
20. Burton MJ, Uterine Motility in Periparturient Dairy Cattle. PhD Thesis. Universitity of Minnesota, 1986.
21. Burton M, Herschler R, Dzuik H, Fanning M & Zemjanis R. Effect of fenprostalene on postpartum myometrial activity in dairy cows with normal or delayed placental expulsion. Br Vet J. 143:549-554 (1987)
22. Burton MJ, Dzuik HE, Fahning ML and Zemjanis R. Effects of oestradiol cypionate on spontaneous and oxytocin-stimulated postpartum myometrial activity in the cow. Br. Vet. J. 146:309-315 (1990).

23. Cai T, Weston P, Lund L, Brodie B, McKenna D & Wagner W. Association between neutrophil function and periparturient disorders in cows. Am J Vet Res 55:934 (1994)
24. Callahan C & Horstman L: Treatment of early postpartum metritis in a dairy herd: Response and subsequent fertility. Bovine Practitioner 22:124-128 (1987)
25. Callahan C – personal communication (1998).
26. Carson R, Wolfe D, Klesius P, Kemppainen & Scanlon C: The effects of ovarian hormones and ACTH on uterine defense to C.pyogenes in cows. Therio 30:91-97 (1988).
27. Chan W. Relationship between the uterotonic action of oxytocin and prostaglandins: oxytocin addition and release of PG-activity in isolated nonpregnant and pregnant rat uteri. Biol Reprod. 17:541-548 (1977)
28. Chan W Uterine and placental prostaglandins and their modulation of oxytocin sensitivity and contractility in the parturient uterus. Biol Reprod 29:680-688 (1983)
29. Chen T, McDonald M & Hawes R. Mechanical and electrical activities of the female bovine genital tract in vivo. Can J Anim Sci. 46:25-32 (1965)
30. Cole WC & Garfield RE. Evidence for physiological regulation of myometrial gap junction permeability. Am J. Physiol. 251:C411-420 (1986)
31. Croker KP & Shelton JN. Influence of stage of cycle, progestagen treatment and dose of oestrogen on uterine motility in the ewe. J.Reprod. Fertil. 32:521-524 (1973)
32. Csapo A. The four direct regulatory factors of myometrial function. In:Progesterone:Its regulatory effect on the myometrium. Ciba Foundation Study Group 34, J&A Churchill, London, pp13-42
33. Currie W. Physiology of uterine activity. Clin Obstet Gynecol 23:33-49 (1980)
34. Davis AJ, Fleet IR, Harrison FA and Maule Walker FM. Pulmonary metabolism of prostaglandin F2a in the conscious nonpregnant ewe and sow. J. Physiol 301:86P (abstract) (1980).
35. Dersken FJ: Anecdotes and clinical trials: the story of clenbuterol. Eq Vet J 26:4:256-257 (1994).
36. Dialogue newsletter, Pharmacia & Upjohn Animal Health. Vol 7 No.1 (1998).
37. Dialogue newsletter, Pharmacia & Upjohn Animal Health. Vol 8 No.2 (1999)
38. Dielman S & Bevers M: Development of preovulatory follicles in the cow from luteolysis until ovulation. In: Roche J& O'Callaghan D(eds): Follicular growth and ovulation rate in farm animals. Dordrecht, Netherlands, Martinus Nijhoff, p31 (1987).
39. Docke F. Untersuchungen zur Uteruskontraktilitat beim Rind. Arch Exp Vet Med. 16:1205-1209 (1962)
40. Downing SJ, and Porter DG. Evidence that inhibition of myometrial activity by oestradiol in the rat is mediated via an RNA synthetic pathway. J.Endocr 78:119-124 (1978).
41. ECP sterile solution package insert. Revised March 1998. Pharmacia & Upjohn Company, Kalamazoo, MI. 49001, USA.
42. Edqvist LE, Kindahl H and Stabenfeldt GH. Release of prostaglandin F2a during the bovine periparturient period. Prostaglandins 16:1:111-119 (1978).
43. Ehrenreich H, Ruesse M, Schams D, Hammerl J and Herz A: An opiod antagonist stimulates myometrial activity in early postpartum cows. Therio 23:309-324 (1985)
44. Eiler H, Oden J, Schaub R, Sims M. Refractoriness of both uterus and mammary gland of the cow to prostaglandin F2a administration: Clinical implication. AJVR 42:314-317 (1981)
45. Eiler H, Byrd W & Hopkins F: Uterokinetic activity of fenprostalene (a prostaglandin F2a analog) in vivo and in vitro in the bovine. Theriogenology 32:5:755-764 (1989).
46. Eiler H, Hopkins FM, Armstrong-Backus CS et al. Uterotonic effect of prostaglandin F2a and oxytocin on the postpartum cow. AJVR 45:1011-1014 (1984)
47. Eiler H & Hopkins F: Bovine retained placenta: Effects of collagenase and hyaluronidase on detachment of placenta. Biol Reprod 46:580 (1992)
48. Eley DS, Thatcher WW, Head HH, Collier RJ, Wilcox CJ, and Call EP. Periparturient and postpartum endocrine changes of conceptus and maternal units in Jersey cows bred for milk yield. J. Dairy Sci. 64:312-320 (1981).
49. Eulenberger Von-K, Wilhelm J, Schulz J, et al. Uterotonica im puerperium des rindes. Mh Vet-Med 41:371-377 (1986)
50. Evans, E. & Miller F. Uterine motility in the cow. Am. J. Physiol. 116:44-45 (1936)
51. Fairclough RJ, Moore LG, McGowan LT et al. Temporal relationship between plasma concentrations of 13,14-dihydro-15-keto-prostaglandin and neurophysin I/II around luteolysis in sheep. Prostaglandins 20:199-204 (1980)
52. Flint AP and Sheldrick EL. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. J.Reprod Fertil 67: 215-225 (1983)

53. Fosgate OT, Cameron NW, and McLeon RJ. Influence of 17-alpha-hydroxyprogesterone-n-caproate upon postpartum reproductive activity in the bovine. J. Anim. Sci. 21:791-793 (1962).
54. Fourichon C, Seegers H & Malher X: Effect of disease on reproduction in the dairy cow: A Meta-Analysis. Therio 53:1729-1759 (2000)
55. Frank T, Anderson KL, Smith AR et al. Phagocytosis in the uterus: a review. Theriogenology, 20:103-110 (1983)
56. Fredriksson G: Some reproductive and clinical aspects of endotoxins in cows with special emphasis on the role of prostaglandins. Acta Vet Scand. 25:365 (1984)
57. Fuchs A, Periyasamy S, Soloff M. Systemic and local regulation of oxytocin receptors in the rat uterus, and their functional significance. Can J Biochem Cell Biol 61:615-624 (1983)
58. Fuchs A, Rust W & Fields M: Accumulation of cyclooxygenase-2 gene transcripts in uterine tissues of pregnant and parturient cows: stimulation by oxytocin. Biol Reprod. 60:341-348 (1999).
59. Gajewski Z & Faundez R: Characteristics and analysis of uterine electromyographic activity in pregnant cattle. Theriogenology 37:1133-1145 (1992).
60. Gajewski Z, Thun R, Faundez R & Boryczko Z: Uterine motility in the cow during puerperium. Reprod Dom Anim 34:185-191 (1999)
61. Garcia-Villar R, Marnet P, Laurentie M & Toutain P: Fenprostalene in cattle: Evaluation of oxytocic effects in ovariectomized cows and abortion potential in a 100-day pregnant cow. Theriogenology 28:467-480 (1987).
62. Garcia A, Barth A, & Mapletoft R. Induction of parturition in the cow: effects of prostaglandin treatment on the incidence of retained placenta. Theriogenology 31:195 (1989)
63. Garcia A, Barth A & Mapletoft R: The effects of treatment with cloprostenol or dinoprost within one hour of induced parturition on the incidence of retained placenta in cattle. Canadian Vet J. 33:175-183 (1992)
64. Garfield RE, Rabideau S, Challis JR & Daniell EE. Ultrastructural basis for maintenance and termination of pregnancy. Am.J.Obstet.Gynecol. 133:308-315 (1979)
65. Gilbert R & Schwark W. Pharmacologic considerations in the management of peripartum conditions in the cow. In: Applied Pharmacology and Therapeutics II. Vet Clin N. Am: Food An Pract. 8:1:29-56 (1992)
66. Gillespie A, Brummer HC, Chard T. Oxytocin release by infused prostaglandin. Brit Med J 1:543-544 (1972)
67. Gillette DD Holm L. Prepartum to postpartum uterine and abdominal contractions in cows. Am J Physiol 204:1115-1121 (1963)
68. Glencross R and Pope S. Concentrations of oestradiol-17B and progesterone in the plasma of dairy heifers before and after cloprostenol induced and natural luteolysis and during early pregnancy. Animal Reproduction Science 4:93-106 (1981)
69. Gross T, Williams W & Morehead T. Prevention of the retained fetal membrane syndrome (retained placenta) during induced calving in dairy cattle. Theriogenology 26:365 (1986)
70. Grunert E. Placental separation/retention in the bovine. Proc 10th Intl Congr Anim Reprod AI , Illinois. X117 – X124 (1984)]
71. Grunert G. Etiology and Pathogenesis of retained bovine placenta. In: Current Therapy in Theriogenology. Ed. Morrow DA, 1st ed. WB Saunders Co, pp237-242 (1986)
72. Guilbault LA, Thatcher WW, Drost M and Hopkins SM. Source of F series prostaglandins during the early postpartum period in cattle. Biol Reprod 31:879-887 (1984).
73. Guilbault LA, Thatcher WW, Drost M and Haibel GK. Influence of a physiological infusion of PGF_{2a} into postpartum cows with partially suppressed endogenous production of prostaglandins. 1. Uterine and ovarian morphological responses. Theriogenology 27:6:931-946 (1987).
74. Guilbault L, Villeneuve P & Dufour J. Failure of exogenous prostaglandin F2a to enhance uterine involution in beef cows. Can J Anim Sci. 68:669-676 (1988)
75. Gustafsson B and Ott R: Current trends in the treatment of genital infections in large animals. Compend Contin Educ Pract Vet. 3:S147 (1981)
76. Gustafsson BK. Therapeutic strategies involving antimicrobial treatment of the uterus in large animals. J Am Vet Assoc. 185:1194-1198 (1984)
77. Hanzen C. Electrical activity of the bovine uterus prior to and post parturition. Vet Res Commun 5:2:143-150 (1981)
78. Hanzen C. Uterine motility in cattle prior to and post parturition (preliminary reports). Curr Topics Vet Med Anim Sci 20:61-66 (1982)
79. Hawk HW, Turner GD and Sykes JF. The effect of ovarian hormones on uterine defence mechanism during the early stages of induced infection. Am J Vet Res. 21:644-648 (1960)

80. Hawk HW, Brinsfield T, Turner G, Whitmore G & Norcross M: Effect of ovarian status on induced acute inflammatory responses in cattle uteri. Am J Vet Res 25:362 (1964)
81. Hawk HW. Uterine motility and sperm transport in the estrous ewe after prostaglandin induced regression of corpora lutea. J.Anim. Sci. 37:1380-1385 (1973).
82. Hawk HW & Echternkamp SE. Uterine contractions in the ewe during progestagen-regulated oestrus. J.Reprod.Fertil. 34:347-349 (1973).
83. Hawk HW. Hormonal control of changes in the direction of uterine contractions in the estrous ewe. Biol Reprod 12:423-430. (1975).
84. Hemeida NA, Gustafsson BK, and Whitmore HL. Therapy of uterine infections: Alternatives to antibiotics. In: Current Therapy in Theriogenology. 2nd ed. Morrow, DA. WB Saunders Co. pp 45-47 (1986)
85. Herschler RC, Lawrence JR A prostaglandin analogue for therapy of retained placentae. Vm/SAC 79:822-826 (1984)
86. Hickey GJ, White ME, Wickenden RP and Armstrong DA. Effects of oxytocin or placental retention following dystocia. Vet Rec 114:189-190 (1984)
86a. Hollinger K and Ekperigin H: Mycotoxins in food producing animals, In: Chemical Food Borne Hazards and Their Control, L.Tollefson, ed. Vet Clin N. Am. 15:1:133-165.
87. Jordan WJ. The puerperium of the cow. A study of uterine motility. J. Comp. Pathol 62:54-68 (1952)
88. Kaker ML, Murray RD, and Dodson H. Plasma hormone changes in cows during induced or spontaneous calvings and the early postpartum period. Vet Rec. 115:378-382 (1984)
89. Kehrli M, Nonneke B & Roth J: Alterations in bovine neutrophil function during the periparturient period. Am J Vet Res 50:207 (1989)
90. Kelly EK & Verhage HG. Hormonal effects on the contractile apparatus of the myometrium. Am.J.Anat. 161:375-382 (1981)
91. Kindahl H, Fedriksson G, Madej A & Edqvist L. Role of prostaglandins in uterine involution. Proc. 10th ICAR, Urbana, Il. VolIV, XI 9-19 (1984)
92. Kindahl H, Odensvik K, Aiumlamai S & Fredriksson G: Utero-ovarian relationships during the bovine postpartum period. Anim Reprod Sci. 28:363-369 (1992)
93. Kindahl H, Bekana M, Kask K, Konigsson K, Gustafsson H and Odensvik K: Endocrine aspects of uterine involution in the cow. Reprod Dom Anim. 34:261-268 (1999).
94. Knickerbocker J, Drost M & Thatcher W: Endocrine patterns during the initiation of puberty, the estrous cycle, pregnancy and parturition in cattle. In: D.Morrow, Current Therapy in Theriogenology 2. WB Saunders Co. Philadelphia, PA. Pp117-125 (1986).
95. Ko J, McKenna D, Whitmore H, Chen C, Gustafsson B & Smith R. Effects of estradiol cypionate and natural and synthetic prostaglandins on myometrial activity in early postpartum cows. Theriogenology 32:537-543 (1989).
96. Kojima N, Stumpf T, Cupp A, Werth L, Roberson M, Wolfe M, Kittok R and Kinder J. Exogenous progesterone and progestins as used in estrous synchrony regimens do not mimic the corpus luteum in regulation of luteinizing hormone and 17B-estradiol in circulation of cows. Biol Reprod 47:1009-1017 (1992).
97. Kundig H, Thun R, Zerobin K & Bachmann B. Uterine motility in the cow during late pregnancy, parturition and puerperium. I. Spontaneous motility. Schweiz Arch Tierheilk. 132:77-84 (1990).
98. Kundig H, Thun R & Zerobin K. Uterine motility in the cow during late pregnancy, parturition and puerperium. II. Drug Influence. Schweiz Arch Tierheilk. 132:515-524 (1990).
99. Lamming G and Mann G: Control of endometrial oxytocin receptors and prostaglandin F2a production by progesterone and oestradiol. J Reprod Fertil 103:69-73 (1995)
100. Lander MF, Hansen P & Drost M: Effects of stage of the estrous cycle and steroid treatment on uterine immunoglobulin content and polymorphonuclear leukocytes in cattle. Theriogenology 34:1169-1184 (1990)
101. Lander Chacin M, Hansen P & Drost M: Effects of stage of the estrus cycle and steroid treatment on uterine immunoglobulin content and polymorphonuclear leukocytes in cattle. Therio 34:1169-1184 (1990)
102. Larsson K, Wagner C & Sachs M: Oestrogen synthesis by bovine foetal placenta at normal parturition. Acta Endocrinologica 98:118 (1981)
103. Leung S and Wathes D: Oestradiol regulation of oxytocin receptor expression in cyclic bovine endometrium. J Reprod Fertil 119:287-292 (2000).
104. Lewis, G. Uterine Health and Disorders, In: Symposium: Health problems of the postpartum cow. J Dairy Sci. 80:984-994 (1997).
105. Lindell J, Kindahl H., Jansson L & Edqvist L. Post-partum release of prostaglandin F2a and uterine involution in the cow. Theriogenology 17:237-245 (1982)

106. Lindell J, Kindahl H. Exogenous prostaglandin F2a promotes uterine involution in the cow. Acta Vet Scand 24:269-274 (1983)
107. Lye SJ, Sprague CL, Challis JR. Modulation of ovine myometrial activity by estradiol-17B. The possible involvement of prostaglandins. Can J Physiol Pharmacol. 61:729-735. (1983)
108. Lye SJ, Wathes DC, Porter DG. Oestradiol-17B both inhibits and stimulates myometrial activity in ewes in vivo. J Reprod Fertil 67:335-341 (1983)
109. Lye SJ. Initiation of parturition. Anim Reprod Sci 42:495-503 (1996)
110. Madej A, Kindahl H, Woyno W, Edqvist L & Stupnicki R. Blood levels of 15-keto-13, 14-dihydro-prostaglandin F2a during the postpartum period in primiparous cows. Theriogenology 21:279-287 (1984)
111. Mann G and Lamming G: Effect of the level of oestradiol on oxytocin-induced prostaglandin F2a release in the cow. J Endocrinol 145:175-180 (1995)
112. Marion GB, Norwood JS and Gier HT. Uterus of the cow after parturition: Factors affecting regression. Am J. Vet Res. 29:71-75. (1968)
113. Markusfeld O. Factors responsible for postpartum metritis in dairy cattle. Vet Rec. 114:539-542 (1984)
114. Martin LR, Williams WF, Rusek E and Gross TS. Postpartum uterine motility measurements in dairy cows retaining their fetal membranes. Therio 15:5:513-524 (1981)
115. McCracken JA Hormone receptor control of PGf2a secretion by the ovine uterus. Adv Prosta Thrombox Res. 8:1329-1333 (1980)
116. McCracken JA. Update on luteolysis – receptor regulation of pulsatile secretion of PGf2a from the uterus. Res Reprod 16:1-2 (1984)
117. Merial ltd. 2100 Ronson Rd, Iselin, NJ.
118. Mihm M: Delayed resumption of cyclicity in postpartum dairy and beef cows. Reprod Dom Anim. 34:278-284 (1999).
119. Miller BJ, Lodge JR Effect of oxytocin on retained placentas. Abstr 551 J. An Sci 53 (suppl) 350 (1981)
120. Miller B & Lodge J. Postpartum oxytocin treatment for prevention of retained placentas. Theriogenology 17:237-243 (1982)
121. Mitchell MD, Flint AP, Turnbull AC: Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep. Prostaglandins 9:47-56 (1975)
122. Mitchell MD, Flint AP Use of meclofenamic acid to investigate the role of prostaglandin biosynthesis during induced parturition in sheep. J. Endocr. 76:101-109 (1978)
123. Molnar M & Hertelendy E. Regulation of intracellular free calcium in human myometrial cells by prostaglandin F2a; comparison with oxytocin. J. Clin Endocrinol Metab 71:1243-1250 (1990)
124. Olson J, Bretzlaff K, Mortimer R & Ball L. The metritis-pyometra complex. In: Current Therapy in Theriogenology 2: Diagnosis, Treatment and Prevention of Reproductive Disease in Small and Large Animals. WB Saunders Co., Philadelphia, PA. Pp227-236 (1986)
125. Olson JD, Ball L and Mortimer RG. Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. Am J Vet Res. 45:2251-2255 (1984).
126. Oxenrider SL. Evaluation of various treatments for chronic uterine infections in dairy cattle. Proc Ann Mtg Soc Therio, 1982; 64-71
127. Paisley LG, Mickelsen WD, Anderson PB. Mechanisms and therapy for retained fetal membranes and uterine infections of cows: A review. Therio 25:353-381 (1986)
128. Patil RK, Sinha SN, Einarsson S, Settergren I. The effects of PGf2a and oxytocin on bovine myometrium in vitro. Nord Vet Med 32:474-479 (1980
129. Pimentel S, Pimentel C, Weston P et al: Progesterone secretion by the bovine fetoplacental unit and responsiveness of corpora lutea to steroidogenic stimuli at two stages of gestation. Am J Vet Res. 47:1967 (1986)
130 Plumb DC: Veterinary Drug Handbook 3rd ed. Iowa State University Press, Ames IA. Pp 253-255. (1999).
131. Porter DG. The myometrium and the relaxin enigma. Anim Reprod Sci. 2:77-96 (1979)
132. Porter DG, Downing SJ & Bradshaw J: Relaxin inhibits spontaneous and prostaglandin-driven myometrial activity in anaesthetized rats. J Endocrin 83:183-192 (1979)
133. Porter DG, Lye SJ, Bradshaw JM et al. Relaxin inhibits myometrial activity in the ovariectomized non-pregnant ewe. J. Reprod Fertil 61:409-414 (1981)
134. Puri CP and Garfield RE Changes in hormone levels and gap junctions in the rat uterus during pregnancy and parturition. Biol Reprod 27:967-975 (1982)
135. Rao M & Sanborn B. Relaxin increases calcium efflux from rat myometrial cells in culture. Endocrinology 119:435-437 (1986)

136. Risco C, Drost M, Thatcher W et al. Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postpartum dairy cows. Therio 42:183 (1994).
137. Roberts, SJ. Injuries and diseases of the puerperal period. In: Veterinary Obstetrics and Genital Diseases – Theriogenology. 3rd ed. Ed. SJ Roberts. David and Charles Inc. VT. Pp353-396. (1986).
138. Rodriguez-Martinez H, Ko J, McKenna D, Weston P, Whitmore H, Gustaffson B & Wagner W: uterine motility in the cow during the estrous cycle. II. Comparative effects of prostaglandins F2, E2, and cloprostenol. Theriogenology 27:349-358 (1987)
139. Rodriguez-Martinez H, Ko J, McKenna D, Weston P, Whitmore H, Gustaffson B & Wagner W: uterine motility in the cow during the estrous cycle. III.Effects of oxytocin, xylazine, and adrenoceptor blockers. Theriogenology 27:359-368 (1987)
140. Rodriguez-Martinez H, Ko J, McKenna D, Weston P, Whitmore H, Gustaffson B & Wagner W: uterine motility in the cow during the estrous cycle. I.Spontaneous activity. Theriogenology 27:337-348 (1987)
141. Rodriguez-Pinon M, Tasende C, Meikle A & Garofalo E: Estrogen and Progesterone receptors in the ovine cervix during the postpartum period Therio 53:743-750 (2000)
142. Roth J, Kaeberle M, Appel L et al. Association of increased estradiol and progesterone blood values with altered bovine polymorphonuclear leukocyte function. Am. J. Vet. Res. 44:247 (1983).
143. Ruckebusch Y and Bayard, F. Motility of the oviduct and uterus of the cow during the oestrus cycle. J. Reprod.Fert. 43:23-32 (1975).
144. Ruesse M. Der Geburtsablauf beim Rind. Arch Exp Vet Med. 19:763-870 (1965)
145. Ruesse M. Myometrial activity postpartum. In: K Karg and E Schallenberger (eds):Factors influencing fertility in the postpartum cow. Curr. Topics Vet Med An Sci. 20:55-60 (1982)
146. Ruzycky A & Crankshaw D. Role of inositol phospholipid hydrolysis in the initiation of agonist-induced contractions of rat uterus: effect of domination by 17B-estradiol and progesterone. Can J. Physiol Pharmacol 66:10-17. (1988)
147. Samuelsson B, Granstrom E, Green K, Hamberg M and Hammarstrom S. Prostaglandins. Ann Rev Biochem. 44:669-695 (1975).
148. Schams D & Prokopp S. Oxytocin determination by RIA in cows around parturition. Anim Reprod Sci. 2:267-270 (1979)
149. Schallenberger E, Schams D, Bullerman B and Walters P. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. J. Reprod and Fertil. 71:493-501 (1984)
150. Schindler D, Lewis G, Rosenberg M, Tadmor A, Ezov N, Ron M, Aizinbud E & Leher A: Vulvar electrical impedence in periparturient cows and its relation to plasma progesterone, oestradiol-17b and PGFM. Anim Reprod Sci. 23:283 (1990).
151. Schrey M, Comford P, Read A & Steer P. A role for phosphoinositide hydrolysis in human uterine smooth muscle during parturition. Am J Obstet Gynecol 159:964-970 (1988)
152. Senger PL: Placentation, the Endocrinology of Gestation and Parturition. In:Pathways to Pregnancy and Parturition. 1st Ed. P.Senger. Current Conceptions, Inc. Washington State University, Pullman WA.pp242-243 (1999)
153. Shaw RN Pituitary extract in cattle practice with particular reference to its use in cases of retained placenta. Vet Bulletin VIII:9 (1938)
154. Singh LP, Sadiku A, Verma OP. Prostaglandin F2a – induced response of the bovine ovary, oviduct (uterine tube), and uterus. AJVR 40:1789-1791 (1979)
155. Slama H, Vaillancourt D and Goff A. Leukotriene B4 in cows with normal calving, and in cows with retained fetal membranes and/or uterine subinvolution. Can J Vet Res. 57:293-299 (1993).
156. Slama H, Vaillancourt D and Goff A. Metabolism of arachidonic acid by caruncular and allantoic tissues in cows with retained fetal membranes (RFM). Prostaglandins 45:57-75 (1993).
157. Smith B, Donavon G, Risco C, Little R, Young C, Stanker L & Elliott J. Comparison of various antibiotic treatments for cows diagnosed with toxic puerperal metritis. J Dairy Sci 81:1555-1562 (1998)
158. Soloff MS. Uterine receptor for oxytocin: Effects of estrogen. Biochem Biophys Res Comm 65:205-212 (1975)
159. Soloff MS, Fernstrom MA, Periyasamy S, Soloff S, Baldwin S and Weider M. Regulation of oxytocin receptor concentration in rat uterine explants by estrogen and progesterone. Can J Biochem Cell Biol 61:625-630 (1983)
160. Steffan J, Adriamanga S & Thibier M. Treatment of metritis with antibiotics or prostaglandin F2alpha and influence of ovarian cyclicity in dairy cows. Am J Vet Res 45:1090 (1984)
161. Stevens R & Dinsmore R: Treatment of dairy cows at parturition with prostaglandin F2alpha or oxytocin for prevention of retained fetal membranes. J Am Vet Med Assoc. 211:1280-1284. (1997).

162. Steward R & Stevenson J. Hormonal, estral, ovulatory, and milk traits in postpartum dairy cows following multiple daily injections of oxytocin. J Anim Sci. 65:1584-1585 (1987).
163. Stocker H & Waelchli R: A clinical trial on the effect of prostaglandin F2a on placental expulsion in dairy cattle after caesarean operation. Vet Record 132:507-508 (1993).
164. Subandrio A, Sheldon I & Noakes D: Peripheral and intrauterine neutrophil function in the cow: The influe