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Effect of *post partum* uterine involution on folliculogenesis, oestrus and conception in cows

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The ending of the puerperium has place when the first oestrus postpartum gestation is restored. To that end the anatomical and histological uterine involution must be completed and the hypothalamic-pituitary-gonadal axis must work properly to allow: oestrus, ovulation, conception, implantation, formation and persistence of *corpus luteum* of pregnancy. Therefore, early postpartum disorders such as NEB, abnormal gonadotropin secretion, uterine infection and other disorders could have subsequent deleterious effects on oocyte development and competence. However, low BCS coupled with severe NEB during this period suppresses pulsatile LH secretion, reduces ovarian responsiveness to LH stimulation and also reduces the functional competence of the follicle characterized by reduced oestradiol production and ultimately results in delayed ovulation. Normal oestrous cycles in cows coupled with overt signs of oestrus are essential so that insemination can occur at the appropriate time relative to ovulation. Also good nutrition management, environment conditions and welfare can support normal uterine postpartum involution and recurrence of cyclicity.

KEY WORDS: uterine involution / folliculogenesis / cows

The end of the *puerperium* is limited by the first oestrus postpartum when gestation is restored. To that end the anatomical and histological uterine involution must be completed and the hypothalamic–pituitary–gonadal axis must work properly to allow: oestrus, ovulation, conception, implantation, formation and persistence of corpus luteum of pregnancy.

The goal of reproduction management is to have cows become pregnant at a biologically optimal time and at an economically profitable interval after calving. The timing of examination of animals after parturition should allow for the normal process of involution, yet also provide sufficient time for treatment and response prior to the start of the breeding period. The aims of uterine disease treatments are to reverse inflammatory changes that impair fertility, whilst enhancing uterine defence and repair [29].

Involution of the genital tract after parturition also aids the resolution of uterine infection, and conversely may be delayed by uterine disease. In addition, evaluating uterine and cervical involution may help to differentiate between physiological and pathological

observations. In normal cattle, the cervix reopens after 1-week post partum [33]. Lochia is passed until 15-20 days post partum; over the course of involution, lochia changes from a red-brown fluid to a more viscous yellow-white material. Healthy cows achieve an uterine horn diameter of 3-4 cm by 25-30 days post partum, and cervical diameter <5 cm by 40 days post partum, but involution of the uterus and cervix is not complete until approximately 40-50 days post partum [27]. However, uterine involution can also be affected by age, breed, nutrition and other factors so that the delayed uterine involution is not a specific indicator of uterine disease [8].

Table

Involution of uterus after parturition

Days	Long (cm)	Diameter (cm)	Weight (kg)
1	100	40	10
3	90	30	8
9	45	8	4
15	35	5	1.5
25	25	3.5	0.8

Before parturition the uterine lumen is sterile and if bacterial invasion occurs, there is usually resorption of the fetus or abortion [30]. During parturition, the physical barriers of the cervix, vagina and vulva are compromised providing the opportunity for bacteria to ascend the genital tract from the environment as well as the animal's skin and feces.

There are classifications of postpartum diseases and their definitions.

Puerperal metritis is an acute systemic illness due to infection of the uterus with bacteria, usually within 10 days after parturition. Puerperal metritis is characterized by the following clinical signs: a fetid red-brown watery uterine discharge and, usually, pyrexia [7]; in severe cases, reduced milk yield, dullness, inappetance or anorexia, elevated heart rate, and apparent dehydration may also be present. The term *metritis* should be used for cows that have delayed involution and a fetid discharge, in the absence of detected fever.

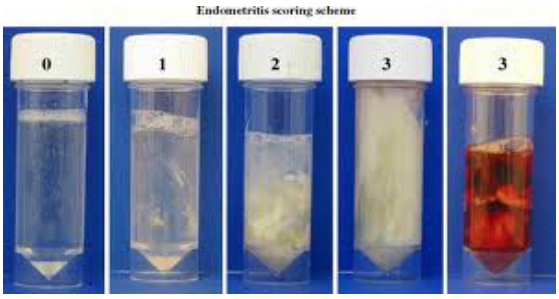

Fig. 1. Examples of vaginal mucus samples and their character score. Score **0** – clear or translucent mucus; **1** – clear mucus containing flecks of white pus; **2** – <50 ml exudate containing ≤50% white or cream pus; **3** – >50 ml exudate containing ≥50% white, cream, or bloody pus [27]

Clinical endometritis is characterized by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudate in the vagina, 21 days or more post partum, and is not accompanied by systemic signs. Diagnostic criteria for clinical endometritis in postpartum dairy cows have been validated by examining factors associated with an increased interval from parturition to conception [15].

Subclinical endometritis can be defined as endometrial inflammation of the uterus usually determined by cytology, in the absence of purulent material in the vagina [10].

Pyometra is characterized by the accumulation of purulent or mucopurulent material within the uterine lumen and distension of the uterus, in the presence of an active corpus luteum.

Therefore, early postpartum disorders such as NEB, abnormal gonadotropin secretion, uterine infection and other disorders discussed earlier could have subsequent deleterious effects on oocyte development and competence. Minerals, trace elements and vitamins play a vital role in the prevention of disorders at this time. Macro minerals are involved in the acid base status of the dairy cow and influence calcium metabolism. The use of anionic salts in combination with adequate calcium supplementation may help to improve dry matter intakes and reduce negative energy balance in the post-calving period as well as prevent hypocalcaemia.

One of the functions of calcium is to allow muscle to contract. Whilst milk fever may not actually present itself until plasma calcium reaches 4 mg/dl, it has been shown that plasma calcium concentrations of 5 mg/dl reduce abomasal motility by 70% and the strength of the contraction by 50%. Muscle tone in the uterus will also be adversely affected in cows experiencing prolonged calvings and retained placenta. Uterine involution may also be impaired [5] what would also give rise to fertility problems**.**

High-producing dairy cows have been selected to produce more milk, in large part through their ability to mobilize fat and muscle to support milk production in early lactation. It results in a loss of body condition score (BCS) and is associated with alterations in blood metabolites and hormone profiles, which in turn affect fertility [32]. Although resumption of normal ovarian cyclic activity is of critical importance, it may be associated with abnormal luteal activity. In previous studies, based on milk or serum progesterone (P_4) concentrations, negative energy balance (NEB) was associated with a greater incidence of irregular patterns of the postpartum luteal activity [31]. These irregularities consisted of a delayed first ovulation, prolonged luteal phase (PLP), short luteal phase and ovarian cysts [20]. Conversely, postpartum uterine infection might contribute to the early demise of the CL. Furthermore, the first CL to develop in cows with uterine disease secretes less P_4 than that in normal animals [34]. Kaneko and Kawakami [13] reported that an infusion of *A. pyogenes* into the uterus caused luteal regression; consequently, first-wave dominant follicles, which normally become atretic, ovulated in half of the infused cows. However, the CL did not regress in the remaining cows, and the mechanism determining the fate of CL remained unclear. The lifespan of the CL is regulated by the secretion of $PGF_{2\alpha}$ and prostaglandin E_2 (PGE_2) from the endometrium, with the latter having a luteotrophic role [22]. Kaneko and Kawakami [14] demonstrated that *A. pyogenes* in the uterus has the potential to stimulate the release of PGs and induce ovulation. With regard to that event, $PGF_{2\alpha}$ plays a more important role than PGE_2, and the increased ratio of $PGF_{2\alpha}$, rather than the absolute

amount, may decide on the fate of the CL. In the present study, serum concentrations of P_4 in cows with PLP were higher than in NLA, indicating that the occurrence of PLP in the clinically healthy high-producing dairy cows was not related to uterine infection.

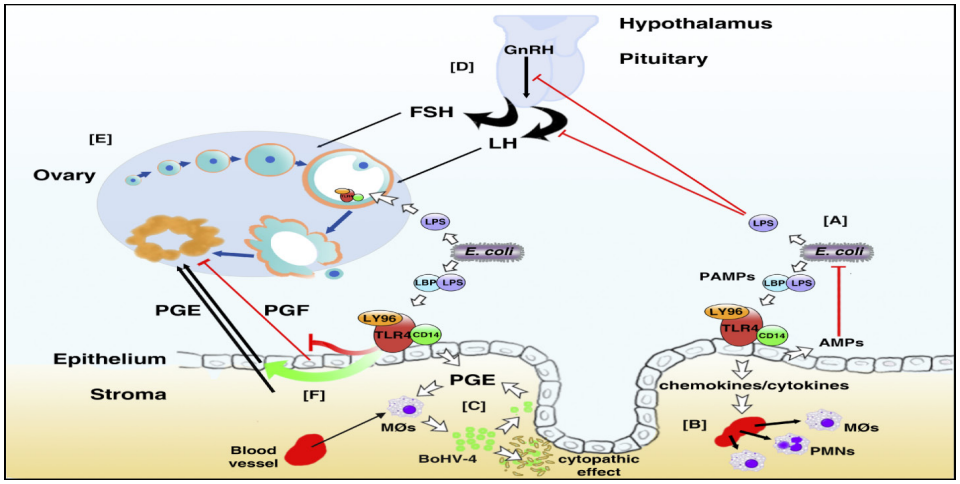

Fig. 2. Effect of uterine infection on folliculogenesis in cow [28]

Although feeding strategies had no effect on PLP incidence [4], in the present study, the decrease of BCS in clinically healthy cows with PLP was higher than NLA. Consistent with our finding, Hommeida et al. [12] reported a greater loss of BCS in cows with PLP. Moreover, the likelihood of clinically healthy cows with PLP increased by 1.8-fold for each 1 mmol/l increase in the 1st wk serum concentrations of βHB. Follicles in cows affected with severe NEB were reported to be smaller and produce less estrogen [1]. However, some studies have indicated that PLP was not associated with any obvious clinical reproductive disorder. Clinically healthy high-producing dairy cows with PLP had a greater calving to first service interval compared to those with NLA. However, the latter had a slightly delay to first ovulation and furthermore, they were high-producing cows. Moreover, calving to conception interval was extended in cows with PLP. Martin et al. [17] reported that PLP was positively related to an increased number of days open. Moreover, a higher artificial insemination submission rate, conception rate, and pregnancy rate between 44 and 100 d postpartum were reported in dairy cows with normal ovarian cycles in the first 44 d postpartum than those with PLP [20]. Therefore, delay in the first ovulation of clinically healthy high-producing dairy cows with NLA reduced intervals to first insemination and conception.

High milk producing dairy cows experience a substantial increase in energy requirements to facilitate the dramatic increases in daily milk yield, which peaks between 4 and 8 weeks postpartum.

This requirement is only partially met by increased feed consumption (due to limitations in intake and appetite) with remainder being met by mobilization of body reserves

resulting in animals entering negative energy balance (NEB) [11]. The consequences of severe NEB are an increased risk of metabolic diseases, which largely occur within the first month of lactation, reduced immune function and a reduction in subsequent fertility. Body condition score is the internationally accepted, subjective visual and tactile measure of body condition and temporal changes in BCS are used to monitor nutritional and health status of high producing cows during their productive cycle. Cows in low BCS at calving, or that suffer from excess BCS loss early postpartum, are less likely to ovulate, have a reduced submission rate to artificial insemination, conception rate to first service, 6-week in-calf rate and also have an increased likelihood for pregnancy loss and increased calving to conception interval. Fertility in cows that are over conditioned at calving (BCS≥3.5; 5-point scale) is also compromised as they have reduced dry mater intake (DMI) just prior to calving, take longer to increase DMI postpartum, tend to have greater fat mobilization and therefore a more severe NEB early postpartum than cows with an optimum BCS at calving [25]. Heat stress can further exacerbate the effects of NEB. Minimizing BCS loss in the first few weeks post partum is an imperative. It is recommended that cows have a BCS of 2.75-3.0 (scale 0-5) at calving and that they are managed to suffer a BCS loss not more than 0.5 between calving and first service [2].

Normal oestrous cycles in cows coupled with overt signs of oestrous are essential so that insemination can occur at the appropriate time relative to ovulation. However, that percentage of oestrous animals that stand to be mounted has declined from 80% to 50% and duration of detected oestrus has reduced from 15 h to 5 h over the past 50 years [6]. Coupled with poor expression, an inability to easily detect oestrus further hinders insemination at the correct time. Different methods of oestrus detection yield different detection efficiencies. Based on visual observation for standing heat and use of tail paint as an aid to detection, an average oestrus detection rate of 70% has been reported with individual herd rates ranging from 25 to 96% [18]. Studies that investigated oestrus detection rates using pedometers reported efficiencies between 80 and 100% [23]. A number of physiological events also affect the expression of oestrus. Firstly, high producing dairy cows (≥39.5 kg/day) have shorter oestrus (6.2 h vs. 10.9 h), less total standing time (21.7 s vs. 28.2 s) and lower serum oestradiol concentrations (6.8 pg/ml vs. 8.6 pg/ml) compared to lower producing dairy cows (≤39.5 kg/day) [16]. Nulliparous heifers have higher circulating concentrations of oestradiol around the time of oestrus and this may account for the longer duration of oestrus (11,3 ±6,9 h) and longer standing oestruses (16.8 ±12.8) observed in nulliparous heifers when compared to multiparous cows [19].

Fertilization rates in the 1980s in Holstein-Friesian dairy cows were greater than 95%. In contrast, fertilization rates in heifers have remained consistently high at and greater than 90% [26]. Similarly, cows exposed to heat stress prior to AI were 31-33% less likely to conceive than those not exposed to heat stress [3]. Similar fertilization rates between lactating and non-lactating cows have been observed (87.8 and 89.5%) during the cool season; however, lactating dairy cows had lower fertilization rates than heifers during high ambient temperatures (55.6 and 100%, respectively) [26].

A „normal“ post partum dairy cow can be defined as one which has resolved uterine involution, resumed ovarian follicular development, ovulated a healthy dominant follicle early postpartum and continues to have normal oestrous cycle at regulars intervals of

approximately 21 days, coupled with homeostatic concentrations of insulin, IGF-I and glucose [24]. Up to 50% of modern dairy cows have abnormal oestrous cycles postpartum resulting in increased calving to first insemination intervals and decreased conception rates [9]. In mastitic and lame cows, a delay in the resumption of cyclicity could add an extra 7 and 17 days, respectively to the calving to conception interval. Compared to their healthy herdmates, cows with clinical endometritis were 4.5 times more likely to have delayed resumption of ovarian cyclicity and 4.4 times more likely to have prolonged postpartum luteal phases [20]. Endometrial epithelial cells respond to uterine infection by altering the secretion and thereby function of prostaglandins from luteolytic (prostaglandin F2alfa) to luteotropic (prostaglandin E2) action. This mechanism has been proposed to explain delayed resumption of cyclicity in infected cows. Pulsatile secretion luteinizing hormone (LH) early postpartum is necessary for pre-ovulatory follicle growth, oestradiol secretion and ovulation of the dominant follicle.

However, low BCS coupled with severe NEB during this period suppresses pulsatile LH secretion, reduces ovarian responsiveness to LH stimulation and also reduces the functional competence of the follicle characterized by reduced oestradiol production and ultimately results in delayed ovulation [21].

In cattle, postpartum contamination of the uterine lumen is ubiquitous, and persistence of pathogenic bacteria commonly causes clinical disease. The consequence is subfertility associated with delayed ovulation after parturition, persistence of the corpus luteum once it forms, and lower conception rates. Thus, effective diagnosis and treatment of uterine disease is essential in reproductive control programs. Also good nutrition management, environment conditions and welfare can support normal uterine postpartum involution and recurrence of cyclicity. The challenge for the future is to use our knowledge of the risk factors for uterine disease to design prevention and control programs to reduce the incidence of disease.

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