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REVIEW

Possibility of Diagnosing Uterine Function in Cows

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Abstract

Declining in reproductive efficiency is a worldwide problem, although remarkable progress has been made in reproductive techniques including artificial insemination programs, estrous cycle synchronization, and effective embryo production. One means of solving this problem is to select breeding cows based on highly precise diagnosis for high reproductive ability, including ovarian and uterine function. Many recent studies have documented the regulation of uterine function, with the results suggesting that said functions might be an effective diagnostic index for reproductive performance. Therefore, the production of oxytocin-induced prostaglandin $F_{2\alpha}$ and epidermal growth factor in bovine endometrium, such as the physiological reaction *in vivo*, may be useful in detecting repeat breeder cows. Although the diagnosis is possible using relatively simple techniques such as biopsy, culture, and hormone assay, it is difficult to apply such diagnosis to the field conditions. However, further improvements in the exhaustive gene expression analysis of uterine function in repeat breeder or low fertility cows, so as to determine the genes associated with physiological reactions, could lead to new diagnostic indexes resulting in improved reproductive efficiency.

Discipline: Animal Industry
Additional key words: endometrial biopsy, epidermal growth factor, oxytocin, prostaglandin $F_{2\alpha}$

Introduction

The declining conception rate among cows is a problem not only in Japan and Western countries but also worldwide. Various policies intended to improve the conception rate have recently been implemented, as evidenced by an increasing trend, but the rate still remains lower than that of 30 years ago. Many researchers have demonstrated that lower conception rates may arise from changes in nutritional requirements due to the demand for increased milk production (Butler 1998, Butler 2000, Leroy et al. 2008) and such external factors as heat stress (Liang et al. 2013, Megahed et al. 2008, Sakatani et al. 2012) in dairy cows. In addition, a similar occurrence was reported in beef cattle (Biggers et al. 1987, Burke et al. 2001).

The stable production of calves is important for farming and the supply of food; therefore, cows must be carefully selected for breeding and their fertility accurately diagnosed. A morphological or functional evaluation of the ovary and uterus is generally conducted based on a clinical diagnosis (using rectal palpation and ultrasonography) or changes in steroid hormones, prior to the selection of each cow. The ovary undergoes remarkable change during the estrous cycle. The diagnostic precision of ovaries is higher than that of the uterus due to morphological changes in the ovaries, such as follicular wave, ovulation, or significant growth of the corpus luteum, whereas few morphological changes occur in the uterus during the estrous cycle. On the other hand, changes in the steroid hormones are an objective functional index (Kesler et al. 1980, Schams & Berisha 2002), but uterine function is not used as a diagnostic index because these indexes primarily reflect ovarian function. And although many reports have been made about bovine uterine function, there is little feedback on these results in clinical applications. If the evaluation of uterine function becomes possible in the future, we may be able to more fully evaluate reproductive function than at present, and could expect a more effective use of breeding cows, early disclosure, and the treatment of low fertility.

In this review, the authors discuss the possibility of

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bovine uterine function diagnosis with various indexes and survey the study of uterine function diagnosis using bovine endometrial biopsy, including the influence of ovarian function and later fertility by biopsy.

Repeat breeder cows

The repeat breeder cows were defined according to the following criteria: the cow experienced estrus within normal intervals after mating with a fertile bull, artificial insemination was performed in at least three apparently normal estrous cycles, and no pathological findings were detected by rectal palpation and ultrasonography of the genital tract. These criteria of repeat breeder cows are in line with a generally accepted nomenclature (Hafez 1987). Recently, many researchers have discussed various factors related to repeat breeding, such as an ovum and embryonic abnormalities (Hosseini et al. 2013, Diskin & Morris 2008), and internal secretion abnormality (Båge et al. 2002). In addition to these factors, breeding management (Rekwot et al. 2000) and environmental factors (De Rensis & Scaramuzzi 2003, Ali et al. 2009) have been found related to repeat breeding. Particularly, in terms of heat stress, the influence of ovum quality or the internal secretion environment in repeat breeder cows is reportedly more significant than in normal cows (Ferreira et al. 2011).

Physiological role of oxytocin on ovarian and uterine function

Oxytocin (OT) is a peptide of nine amino acids produced by the paraventricular nucleus and the supraoptic nucleus of the hypothalamus, and is stored and secreted by the posterior pituitary gland. OT mainly stimulates smooth muscle contraction and facilitates uterine contraction at delivery and lactation. In veterinary medicine, it is used for the treatment of hypotonic contractions, retained afterbirth, uterine prolapse, or milk release.

Armstrong et al. (1959) reported for the first time that OT plays an important role in the regulation of corpus luteum function; later it was found that OT stimulates prostaglandin F (PGF) secretion in the ewe uterus (Roberts et al. 1976). It is now well known that OT is synthesized and secreted by the corpus luteum in ruminants (Flint & Sheldrick 1982, Walters et al. 1984), and that luteal function during regression is controlled through positive feedback between luteal OT and endometrial PGF (McCracken et al. 1999). After OT injection, since PGF secretion from the uterus differs on the day of the estrous cycle, the difference relates to an endogenous ovarian steroid hormone condition (Silvia & Taylor 1989). On the other hand, OT receptors (particularly in the endometrium) increase at 17-18 days and peak at 21 days, and then decrease over 1-6 days of estrus (Meyer et al. 1988). Ovarian progesterone and estrogen regulate the expression of OT receptors in the estrous cycle. The expression of OT receptors in the estrous cycle are inhibited under the high progesterone and low estrogen environment of the luteal phase, but becomes higher under the low progesterone and high estrogen environment with luteal degeneration (Goff 2004). Thus, the important roles that OT plays in ovarian and uterine function are well established.

Uterine function diagnosis for repeat breeder cows

We conducted experiments to determine whether abnormalities in physiological reactions to OT can be diagnostic indexes of uterine function for repeat breeder cows. We used relatively simple techniques such as tissue culture and hormone measurement with enzyme immunoassay. Endometrial samples were obtained from live cows by biopsy under local anesthesia.

1. Biopsy

In general, biopsy using a fine needle or forceps is widely used in the diagnosis of cancer and inflammatory conditions in various tissues. In veterinary medicine, it has been used for the pathological diagnosis of endometritis in cows for more than 40 years (McQueen 1967). The existing collection of bovine endometrium using biopsy forceps (Rantala et al. 2014), endoscope instruments, or a cytobrush (Kasimanickam et al. 2014) was recently used for physiological analysis by tissue culture and gene expression analysis, as well as pathological diagnosis. As endometrial biopsy is conducted on live cows and collected repeatedly during the estrous cycle, it is the most suitable method of bovine reproduction (Chapwanya et al. 2010).

2. Effects of serial biopsies of endometrium on ovarian function and subsequent fertility

We investigated the effects of endometrial biopsy on subsequent reproductive performance, including ovarian function and fertility, in Japanese short horn (beef cattle). The animals were sedated with xylazine and lidocaine was used to induce epidural anesthesia. Biopsy forceps was introduced in a clean manner via the cervix to the uterine horn (corpus luteum side), aided by manipulation through the rectum. Endometrial biopsy samples were obtained on day 10 (one biopsy) or on days 8-16 (three biopsies at 2-4 day intervals) after estrus. The results suggest that a biopsy of the endometrium in a cycle not only affected the cycle's length but also the subsequent pregnancy rate (Table 1). Moreover, when endometrial biopsy was conducted one or three times during the luteal phase of the estrous cycle, the concentrations of progesterone were unaffected, and the follicular wave was found twice (Fig. 1). The results suggest that endometrial biopsy does not influence the length of the estrous cycle, ovarian function, or subsequent fertility.

3. Possibility of diagnosing uterine function with a physiological reaction to oxytocin

The endometrial tissues of normal and repeat breeder cows were obtained by biopsy. The collected tissues were cut in small pieces and plated individually in 4-well dishes, and then cultured in DMEM/Ham's F-12 + 0.1% bovine serum albumin supplemented with/without 10^{-7} mol/L OT under 5% CO_2 in air at 38.5°C. After 6 h of culture, the concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was directly determined in the medium with an enzyme immunoassay as described by Uenoyama et al. (1997). The production of $PGF_{2\alpha}$ from the endometrial tissue of normal cows was significantly increased by the addition of OT. However, OT did not stimulate the production of $PGF_{2\alpha}$ in the endometrial tissue of repeat breeders (Fig. 2). The results suggest that the endometrial tissue of repeat breeders does not have

Table.1 Comparisons of the length of estrous cycle before and after the endometrial biopsy. and the subsequent pregnancy

Designs of biopsy				Av. length of estrous cycle (day)				
Total no. of biopsies*	Interval of biopsy	Day of Ex. (after estrous)	No. of cows	before biopsy	after biopsy	No. of matings**	No. of pregnancy***	No. of normal calves
1	None	10	2	23.5	22	2	1	1
3	2-4days	8-16	5	22.2	21	1	1	1

* The endometrial biopsy was conducted for one estrous cycle.

** After endomaterial biopsy, cows were mated with a bull.

*** Pregnancy diagnosis was carried out on dya 35 after mating using a ultrasonography.

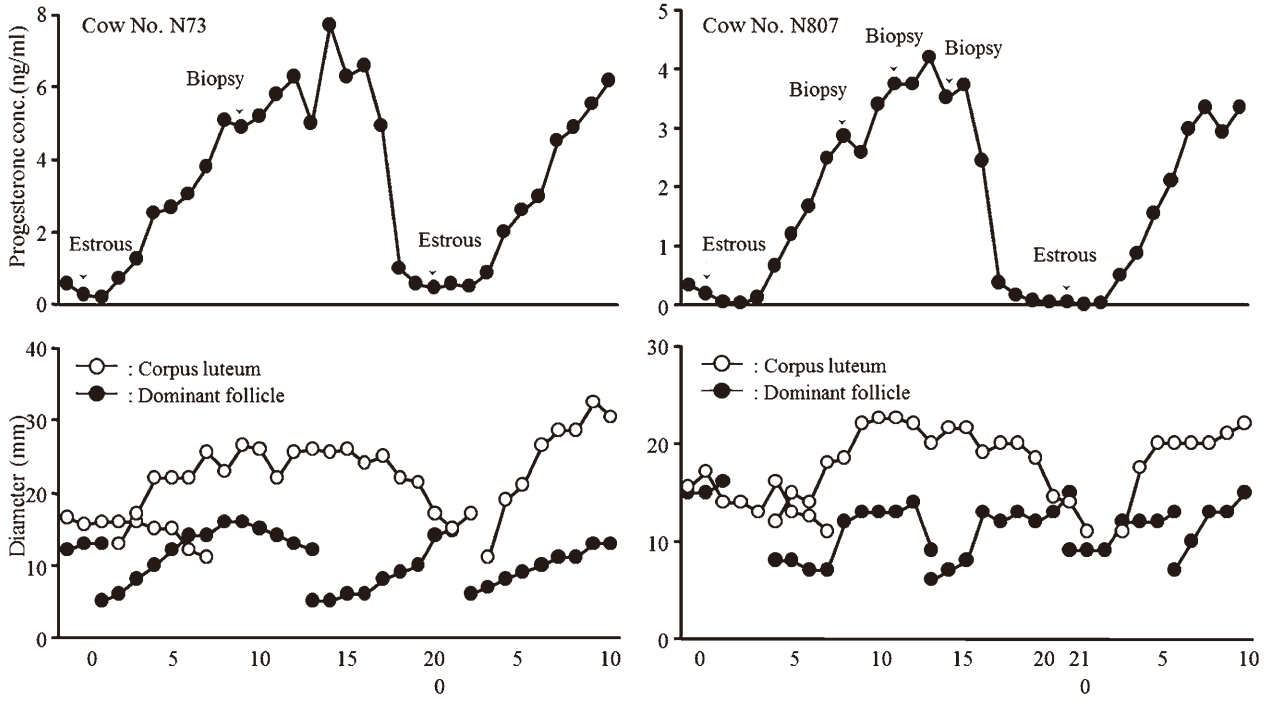

Fig. 1. Effects of bovine endometrial biopsy on the concentration of progesterone, follicular wave, and diameters of the corpus luteum and dominant follicle. Endometrial biopsy was conducted once on days 10 (N73) or three times on days 8-14 (N807) after estrous, respectively. The concentration of progesterone is determined by enzyme immunoassay, and the diameters of the corpus luteum and dominant follicle are measured by ultrasonography.

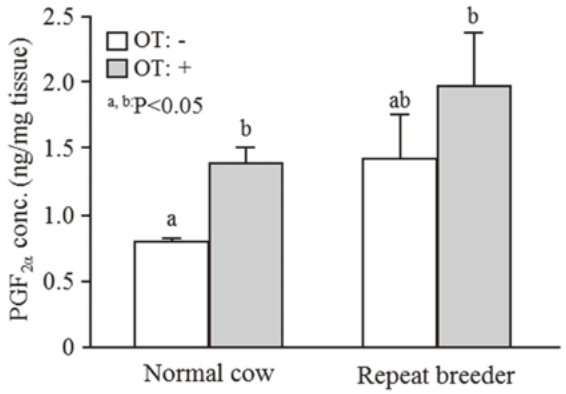

Fig. 2. Effect of oxytocin (OT) on prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) production of endometrial tissues obtained from normal (n = 6) and repeat breeder cows (n = 6) by biopsy forceps via cervical canal for days 16-18 after estrus. The tissues were cultured in DMEM/Ham's F-12 + 0.1% bovine serum albumin with/without 10^{-7} mol/L OT, and the production of $PGF_{2\alpha}$ by enzyme immunoassay was determined.

a normal uterine physiological reaction to OT; therefore, these reactions to OT may be a potential diagnostic marker for repeat breeder or low fertility cows.

Possibility of diagnosing of uterine functions using various new approaches

Many researchers have recently studied uterine functions using various methods that include the analysis of tissue gene expression (Killeen et al. 2014) and cells (Kasimanickam et al. 2014), immunohistochemistry (Kim et al. 2014, Jain et al. 2012), and biological active substance assays (Okuda et al. 2004, Katagiri & Moriyoshi 2013). These studies also give us a clue to developing new methods of diagnosing uterine functions. On the other hand, Katagiri & Takahashi (2004) indicated that the concentration of epidermal growth factor (EGF) on endometrial biopsy samples could be a potential diagnostic index, as the concentrations of EGF on endometrial biopsy samples in repeat breeders were significantly lower than those in control cows on days 3 and 14 after estrous. Therefore, the concentration of EGF in the endometrium may be a useful diagnostic index for repeat breeder cows.

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