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Differences in hormone levels around parturition in Hanwoo cattle (*Bos taurus coreanae*) following artificial insemination and embryo transfer

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Abstract

Background: With unique genetic traits, Hanwoo cattle (*Bos taurus coreanae*) are well-adapted to the Korean environment. However, their perinatal mortality rate is 2%–3%, which imposes an economic burden.

Objective: Due to insufficient data on hormonal changes around parturition, the timing of parturition is often predicted subjectively; few studies have examined hormones in Hanwoo cattle. We measured the changes in various hormones around parturition, to seek an objective predictor of parturition time.

Methods: In 14 female Hanwoo cattle, we measured progesterone, prolactin and cortisol concentrations daily in jugular vein blood samples, beginning 6 days before parturition until 7 days after parturition. Conception was induced in five animals using artificial insemination. Nine animals received embryo transfer.

Results: During parturition, the concentration of progesterone decreased significantly in the embryo transfer group ($n = 9$) and in the total population ($n = 14$); it did not change significantly in the artificial insemination group ($n = 5$). Prolactin concentration increased on the day of parturition but did not differ significantly among the groups. Cortisol remained constant throughout the study course.

Conclusion: We concluded that parturition time can be predicted in Hanwoo cattle using progesterone concentration. This knowledge can reduce perinatal mortality, which would help to improve farm income and animal welfare.

KEYWORDS
Hanwoo cattle (Bos taurus coreanae), parturition, perinatal mortality, progesterone

1 | INTRODUCTION

Reproductive technology has revolutionised cattle production over the last century. Artificial insemination (AI) of cattle was first performed successfully in the early 1900s (Moore & Hasler, 2017). It has since been used widely within the cattle industry to reproduce valuable genetics (Bo & Baruselli, 2014). AI avoids the need to keep bulls on each farm, which improves farm safety (Moore & Hasler, 2017). The first successful embryo transfer (ET) was performed in 1951 (Willett et al., 1951). There was found to be no difference in pregnancy rates following ET (Hasler, 2001). Today, most AI and ET procedures are performed using frozen–thawed semen and embryos, respectively (Hasler, 2014; Vishwanath, 2003).

Parturition is the process of giving birth at the end of gestation—a critical phase in livestock production. Most calf mortality occurs at birth, in more than 60% of producers (Spicer et al., 1994). Perinatal mortality is defined as death of the fetus or calf before, during or within 48 h of calving at full-term (>260 days) (Mee, 2008). Recently, concerns have been raised about high and increasing perinatal mortality in Holstein primipara (Hansen et al., 2004), and the normalisation of these losses (Mee, 2013). Perinatal mortality in cattle has various causes, including chromosome and endocrine abnormalities, malnutrition, vitamin and mineral deficiencies, systemic disease, high fever, transport stress, foetal malformations, multiple births, umbilical cord torsion and late or no intervention (Korean Rural Development Administration). Reducing perinatal mortality would confer economic benefits (Chassagne et al., 1999).

Progesterone from the corpus luteum is critical for the establishment and maintenance of pregnancy. In ruminants, it plays a major role in regulating endometrial secretions essential for stimulating and mediating changes in conceptus growth and differentiation in early pregnancy (Lonergan et al., 2016).

Prolactin is a protein secreted from the pituitary gland that stimulates the mammary glands to produce milk (lactation). Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands and preparation for milk production, which begins when progesterone levels fall, by the end of pregnancy. Prolactin also plays an important role in maternal behaviour (Lucas et al., 1998).

Cortisol is a steroid hormone produced mainly by the adrenal cortex of the adrenal gland. It plays a key role in the body's stress response (van der Valk et al., 2018). In foetal lambs, cortisol increase after about Day 130 promotes maturation of the lungs by about Day 135 (Mescher et al., 1975). The timing of cortisol elevation in foetal sheep varies; on average, it is 11.8 days before the onset of labour (Magyar et al., 1980). In several livestock species (e.g. cattle, sheep, goats and pigs), a surge of foetal cortisol late in gestation triggers the onset of parturition by removing the progesterone block of cervical dilation and myometrial contraction (Ishimoto & Jaffe, 2011).

Hormone levels change drastically during pregnancy and parturition in cattle. Many studies have examined hormonal changes in Holstein cattle (Kindahl et al., 2002; Matsas et al., 1992). Progesterone levels in cattle decrease abruptly after luteolysis (Streyl et al., 2011). A parallel increase in the estrone sulphate level indicates calving within 24 h (Shah et al., 2006). The salivary cortisol concentration in cattle increases during the last hour before birth (Nagel et al., 2020). A blood progesterone test has been evaluated as a diagnostic test for predicting the time of calving in near-term dairy cows, within a 24-h period (Matsas et al., 1992).

Hanwoo cattle (Bos taurus coreanae) is a 4000-year-old native Korean cattle breed, adapted to hot, humid summers and cold, dry winters. It has a miscarriage/stillbirth rate of 2%–3% (Korean Rural Development Administration). Despite their unique genetics and environmental conditions, few studies of Hanwoo cattle have examined their hormone levels during parturition.

Methods to predict calving before the appearance of imminent signs of birth would enable farmers to implement precise calving-management programs that could help to reduce perinatal mortality due to late intervention (Titler et al., 2015). We observe hormone changes in parturition of Hanwoo cattle, and also investigate whether differences exist in hormone levels between those that receiving AI or ET.

2 | MATERIAL AND METHODS

2.1 | Animals

This study was approved by the Institutional Animal Care and Use Committee of the Gyeongsangbuk-do Livestock Research Institute. All applicable national laws and policies regarding the care and use of animals were observed during the experiment. We measured hormone levels in 14 cows (age, 51.2 ± 3.8 months; parity, 2.2 ± 0.2; weight, 401.4 ± 10.2 kg). During the experiment, surrogate mothers were housed in a stanchion barn with sufficient space and were given feed according to the Korean feeding standards. Rice straw, mineral blocks and water were available ad libitum. At the beginning of the experiment, the cows had a mean body condition score of approximately 2.3 ± 0.03 on the Korea Animal Improvement Association scale of 1–5. Animals were excluded if transrectal ultrasonography detected any abnormalities in the ovaries or uterus.

2.2 | Artificial insemination

The AI program was based on an ovulation synchronisation protocol (Pursley & Martins, 2011). Cows in the AI group ($n = 5$) were treated with 0.021 mg intramuscular gonadotropin-releasing hormone, on Days 0 and 9, and 25 mg intramuscular prostaglandin F2α on Day 7. The cows were inseminated 18 h after their injection on Day 9.

2.3 | Oocyte in vitro maturation (IVM), in vitro fertilisation (IVF) and in vitro culture (IVC) of embryos

Ovaries were obtained from a local abattoir and maintained in saline at 35°C during transport to the laboratory. Cumulus-oocyte complexes

(COCs), from follicles of 2–8 mm diameter, were aspirated using an 18-gauge needle. COCs surrounded by more than three layers of cumulus cells and evenly distributed in the cytoplasm were isolated. For IVM, COCs were cultured for 22 h in 450 μl tissue culture medium 199 supplemented with 0.005 AU/ml of follicle-stimulating hormone (F2293; Sigma-Aldrich, St. Louis, MO, USA), 10% foetal bovine serum (GIB16000-044; Thermo Fisher Scientific, Waltham, MA, USA), 1 μg/ml 17β-estradiol (E4389; Sigma-Aldrich) and 100 μM cysteamine (M6500; Sigma-Aldrich), in a humidified atmosphere with 5% CO_2 at 38.5°C.

Motile spermatozoa were refined using a Percoll gradient method (Machado et al., 2009). Semen was purified from thawed straws using density-gradient centrifugation (Percoll discontinuous gradient [45%–90%] at 1500 rpm for 15 min). The gradient was prepared by layering 1 ml of 45% Percoll solution onto 1 ml of 90% Percoll solution in a 15-ml conical tube. The thawed semen was layered on top of the Percoll gradient solution, then the tube was centrifuged. The pellet was washed twice by capacitation: centrifugation with Tyrode's albumin lactate pyruvate (TALP) for 5 min at 1500 rpm. Active, motile spermatozoa from the pellet were then added to droplets containing matured oocytes. Oocytes were inseminated under mineral oil on Day 0, with 1–2 × 10^6 spermatozoa per ml, for 18 h in an IVF-TALP medium (NO-100; Nutricell, Campinas, Brazil) in a humidified atmosphere with 5% CO_2 at 38.5°C. Fertilised oocytes were denuded and cultured in a two-step chemically-defined culture medium (5 days in early-stage medium, then 2 days in later-stage medium) at 38.5°C in an atmosphere with 5% O_2, 5% CO_2 and 90% N_2 (Lim et al., 2007).

2.4 | Embryo transfer and pregnancy diagnosis

A single transgenic bovine embryo was loaded into the central drop of BioLife Transfer and Holding medium (C15C; Agtech, Piscataway, NJ, USA) in a 0.25-ml straw with two microdrops side-by-side, then sealed. Embryo-loaded straws were transported to Gyeongsangbuk-do Livestock Research Institute in an embryo transporter (TE 100 Compact; WTA Reproduction Technologies, Cravinhos, Brazil). One straw was loaded into a 0.25-ml ET Gun (16301; WTA Reproduction Technologies) with minimal contamination. The loaded embryo was transferred to the uterine horn of a recipient via the transcervical method on Day 7 (estrus = Day 0 = day of fusion) using a nonsurgical method (Baruselli et al., 2011). At 50 days post estrus, surrogates were examined via rectal palpation and ultrasonography to assess embryo survival and pregnancy. Pregnant cattle were monitored by rectal palpation and ultrasonography at regular intervals thereafter.

2.5 | Blood sampling

Blood samples were collected daily from 6 days before expected parturition until 7 days after parturition. The samples were collected via jugular venipuncture and stored in ethylenediaminetetraacetic acid-containing (18.0 mg) collection tubes (Vacutainer 10 ml; Becton Dickinson, Auckland, New Zealand). After collection, samples were immediately placed on ice, then transferred to storage at 4°C for 24 h before isolating plasma via centrifugation at 1900 × g for 30 min at 4°C. The plasma was stored frozen at −80°C until further analysis.

2.6 | Enzyme-linked immunosorbent assay (ELISA)

Progesterone, prolactin and cortisol concentrations were measured via sandwich ELISA assays using the Bovine Progesterone ELISA Kit (NBP2-60122-1; Novus Biologicals, Littleton, CO, USA), Bovine Prolactin ELISA Kit (OKCD06890; Aviva Systems Biology, San Diego, CA, USA) and Cortisol Parameter Assay Kit (KGE008B; R&D Systems, Minneapolis, MN, USA), respectively, according to the manufacturers' instructions. Progesterone, prolactin and cortisol were diluted 1, 2 and 30 times, respectively. The sample recovery rate was 80%–120% in all tests. Signals were obtained using a SpectraMax 190 microplate reader (Molecular Devices, San Jose, CA, USA). Hormone levels were quantified by extrapolating the signal into the linear range of a standard curve. SoftMax Pro version 7.0.2 (Molecular Devices) was used for data analysis.

2.7 | Statistical analysis

All results are presented as means ± standard error. Statistical significance was estimated using analysis of variance, followed by Tukey's multiple correction, if not stated otherwise. All statistical analyses were performed using GraphPad Prism version 8.3.0 (GraphPad Software, San Diego, CA, USA).

3 | RESULTS

3.1 | Progesterone-, prolactin- and cortisol-level changes around parturition

To identify the hormone best able to predict parturition time in Hanwoo cattle, we measured progesterone, cortisol and prolactin levels. The progesterone concentration was high before parturition (> 29.7 ± 3.5 ng/ml), then decreased significantly on the day of parturition (17.3 ± 1.9 ng/ml; $p < 0.001$); it remained low thereafter (< 12.1 ± 1.3 ng/ml). Prolactin began to increase 5 days before parturition, peaked on the day of parturition (160.1 ± 20.0 ng/ml) and gradually decreased thereafter; however, the difference was not significant. Cortisol remained constant, regardless of parturition (< 15.6 ± 0.8 ng/ml) (Figure 1; Supplementary Table S1).

3.2 | Comparison of progesterone, prolactin and cortisol levels between AI and ET

Progesterone, prolactin and cortisol were measured before and after parturition to examine hormone differences at parturition according to whether AI or ET was performed. Overall, the progesterone level changed markedly on the day of parturition, but the difference on the day of parturition was significant only in the ET group (Figures 1

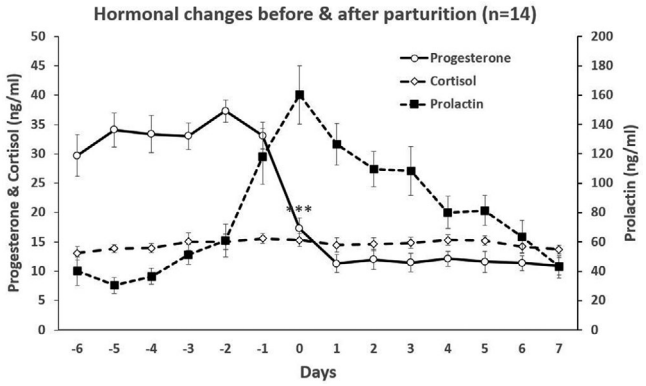

FIGURE 1 Parturition-related hormone changes around parturition in Hanwoo. Only the progesterone levels fell significantly on the day of parturition ($p < 0.001$). The prolactin levels began increasing 5 days before parturition, peaked on the day of parturition and decreased thereafter, with no significant differences over the period. Cortisol levels remained stable

and 2). Progesterone levels did not differ between the AI and ET groups throughout the period (Figure 2a; Supplementary Table S2). Prolactin levels did not differ within or between the AI and ET groups during the period (Figure 2b; Supplementary Table S2). Cortisol levels did not differ within the AI and ET groups, but differed significantly between the AI and ET groups 3 days after parturition ($p < 0.005$) (Figure 2c; Supplementary Table S2).

4 | DISCUSSION

In South Korea, the perinatal mortality rate of Hanwoo cattle is 2%–3% (Korean Rural Development Administration). The time of parturition can be predicted clinically but it is subjective and imprecise. We sought to identify objective indicators of parturition time in Hanwoo cattle by measuring changes around parturition in the levels of three hormones. Parturition is a critical phase in livestock production. Many producers experience most calf mortality at birth (Spicer et al., 1994). Thus, reducing perinatal mortality would be beneficial.

In the AI group, blood progesterone levels decreased at birth, albeit not significantly. The decrease was significant after we included the animals from the ET group. This suggested that there were too few cows for obtaining statistical significance. Similarly, prolactin levels did not differ significantly around parturition. This might be due to the small sample size or to relatively large individual differences.

Hiew et al. indicated that progesterone concentrations in Holstein cows 24 h before and on the day of calving were 4.6 and 2.0 ng/ml, respectively (Hiew et al., 2020). We measured much higher levels of 33.1 ± 2.3 and 17.3 ± 1.9 ng/ml, respectively. However, we found a similar decrease in progesterone before and after delivery. We inferred

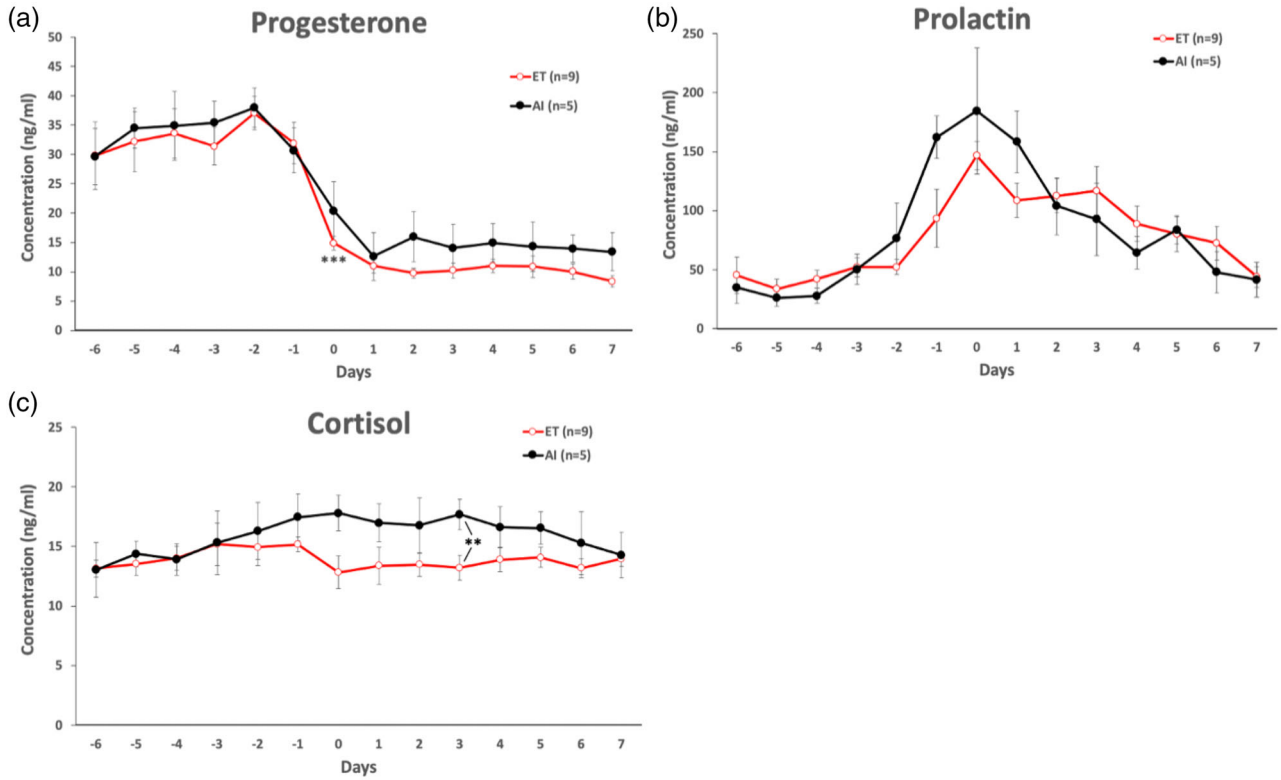

FIGURE 2 Hormone changes around parturition in ET ($n = 9$) and AI ($n = 5$) Hanwoo. All hormone levels were determined from 6 days before parturition to 7 days afterwards. (a) Progesterone levels fell significantly only in ET Hanwoo ($p < 0.001$). (b) Prolactin levels did not differ within or between groups. (c) Cortisol levels differed significantly only between ET and AI surrogate mothers 3 days after parturition ($p < 0.005$)

that progesterone concentration could be an indicator of parturition time.

The range of progesterone concentration measurement using the NBP2-60122-1 kit is 0.5–30 ng/ml, which is broader than that of other kits; the kit also detects other progesterone analogues. Whether the high progesterone concentration we found in Hanwoo cattle is characteristic of the breed or is an effect of the kit is a matter for further research.

Cortisol levels increase in cattle only during the last few hours before birth (Nagel et al., 2020). We observed no increase in cortisol, perhaps because we evaluated serum cortisol at 24-h intervals only. Hourly measurements of blood cortisol might yield results similar to previous reports.

Veterinarians are often asked to examine prepartum cows to determine when parturition will occur. Their assessments may include real-time ultrasound (Wright et al., 1988), changes in body temperature (Burfeind et al., 2011), relaxation of the pelvic ligament (Dufty, 1971) and intravaginal devices that activate when pushed out of the vagina by the amniotic sac (Palombi et al., 2013). However, prediction of the exact time of birth using these methods is not accurate. Other prediction methods that have been studied include blood 17-β-estradiol levels (Shah et al., 2007) and electrolyte concentrations in mammary secretions (Bleul et al., 2006). We believe that progesterone concentration can additionally be used as an accurate indicator of parturition in Hanwoo cattle.

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CONFLICT OF INTEREST

We confirm that this manuscript has not been published in whole or in part and is not being considered for publication elsewhere. There are no any conflict of interest for all authors.

AUTHOR CONTRIBUTIONS

JY, SY and JM: Conceptualisation; DK and JY: methodology; SY and JM analyse the data with software; JY, SY, DK and JM validation; JY and SY formal analysis; DK, SH, JH, JK and DJ investigation; GJ data curation; JY and JM funding acquisition; JM writing—original draft preparation; JY, SY and JM writing—review and editing; JM visualisation; WL and JM supervision. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

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SUPPORTING INFORMATION

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