

Differences in Hormone Levels Around Parturition in Hanwoo (*Bos Taurus Coreanae*) Following Artificial Insemination and Embryo Transfer

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Short communication

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Abstract

Hanwoo (*Bos taurus coreanae*) are well adapted to the environment of Korea and exhibit unique genetic traits; however, the perinatal mortality rate of Hanwoo is 2–3%, which imposes an economic burden. The timing of parturition is often predicted subjectively due to insufficient data on hormonal changes around parturition, and few studies have examined hormones in Hanwoo. Therefore, we measured the changes in various hormones around parturition in Hanwoo to seek an objective predictor of parturition time. We measured progesterone, prolactin, and cortisol concentrations daily in jugular vein blood samples beginning 6 days before parturition until 7 days after parturition in 14 female Hanwoo. Five were induced to conceive with artificial insemination and nine were induced to conceive via embryo transfer. Progesterone concentration decreased significantly during parturition in the embryo transfer (n = 9) and total (n = 14) groups, but did not change significantly in the artificial insemination (n = 5) group. Prolactin concentration increased on the day of parturition, but did not differ significantly among the groups. Cortisol remained constant throughout. We concluded that the parturition time can be predicted in Hanwoo using progesterone concentration. This knowledge can reduce perinatal mortality, which will help to improve farm income and animal welfare.

Background

In the last century, reproductive technology has revolutionized cow production. Artificial insemination (AI) was first used successfully in cattle in the early 1900s [1] and has since been used widely to reproduce valuable genetics in the cattle industry [2]. AI breeding also avoids the need to have bulls on each farm, which improves farm safety [1]. In 1951, the first embryo transfer (ET) calf was born following surgical transfer of a 5-day embryo from a slaughterhouse [3]. It was subsequently found that there was no difference in the pregnancy rate following ET [4]. Today, most AI and ET is performed with frozen–thawed semen and embryos [5, 6].

Parturition is the process of giving birth at the end of gestation. It is a critical phase in livestock production. Most calf mortality occurs at birth in more than 60% of producers [7]. Perinatal mortality is defined as death of the fetus or calf before, during, or within 48 h of calving at full term (> 260 days) [8]. Recently, concerns have been raised about high and increasing perinatal mortality in Holstein primipara [9] and the normalization of these losses [10]. In cattle, perinatal mortality has various causes, including chromosome and endocrine abnormalities, malnutrition, vitamin and mineral deficiencies, systemic disease, high fever, transport stress, fetal malformations, multiple births, umbilical cord torsion, and late or no interventions (Korean Rural Development Administration). Thus, reducing the perinatal mortality rate would confer economic benefits [11].

Hormone levels change drastically during pregnancy and parturition in cattle. Many studies have examined hormone changes in Holstein cattle [12, 13]. The progesterone concentration in cattle decreases abruptly after luteolysis [14] and a parallel increase in the estrone sulfate concentration indicates calving within 24 h [15]. Meanwhile, the salivary cortisol concentration in cattle increases only

during the last hour before birth of the calf [16]. A blood progesterone test was evaluated as a diagnostic test to predict the time of calving within a 24 h period in near-term dairy cows [12].

Hanwoo (*Bos taurus coreanae*) is a Korean native cow breed that has adapted to the hot, humid summers and cold, dry winters of Korea for 4,000 years. The miscarriage and stillbirth rate of Hanwoo is 2–3% (Korean Rural Development Administration). Despite the unique genetic and environmental conditions of Hanwoo, few studies have examined their hormone levels during parturition.

The development of methods that can predict calving time before the appearance of imminent signs of birth would enable farmers to implement precise calving-management programs to help reduce undesirable perinatal mortality due to late intervention [17]. In addition to observing hormone changes in bovine parturition, we also investigated whether there are hormone differences at parturition between AI and ET in Hanwoo.

Methods

Animals

The animal experiment was approved by the Institutional Animal Care and Use Committee of the Gyeongsangbuk-do Livestock Research Institute (Gyeongsangbuk-do, Korea) and all applicable national laws and policies regarding the care and use of animals were observed during the experiment. This study measured hormone levels in 14 cows (51.2 ± 3.8 months, 2.2 ± 0.2 parities, 401.4 ± 10.2 kg). During the experiment, the 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 to 5. Animals were excluded if they had abnormalities in the ovaries and uterus detected by transrectal ultrasonography.

Artificial insemination

The AI program was based on an ovulation synchronization protocol [18]. 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 F₂ α on day 7; they were inseminated 18 h after the injection on day 9.

Oocyte *in vitro* maturation (IVM), *in vitro* fertilization (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 2–8 mm in diameter were aspirated using an 18-gauge needle. The COCs, those surrounded by more than three layers of cumulus cells and evenly distributed the cytoplasm were sorted. For IVM, COCs were cultured for 22 h in 450 μ L TCM-199 supplemented with 0.005 AU/mL follicle-stimulating hormone (F2293; Sigma-Aldrich, USA), 10% fetal bovine serum (GIB16000-044; Thermo Fisher Scientific, USA), 1 μ g/mL 17 β -estradiol (E4389; Sigma-

Aldrich, USA), and 100 μ M cysteamine (M6500; Sigma-Aldrich, USA) in a humidified atmosphere of 5% CO₂ at 38.5°C.

Motile spermatozoa were refined by the Percoll gradient method [19]. The spermatozoa were purified from thawed semen straws by density-gradient centrifugation on a Percoll discontinuous gradient (45–90%) at 1500 rpm for 15 min. The Percoll density 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 onto the top of the Percoll gradient solution and the tube was centrifuged. The pellet was washed twice with capacitation-Tyrode's albumin lactate pyruvate (TALP) via centrifugation for 5 min at 1500 rpm. The active, motile spermatozoa from the pellet were added to droplets containing matured oocytes. Oocytes were inseminated on day 0 with $1-2 \times 10^6$ spermatozoa/mL for 18 h in IVF-TALP medium (NO-100; Nutricell, Brazil) under mineral oil in a humidified atmosphere of 5% CO₂ at 38.5°C. The fertilized oocytes were denuded and cultured in two-step chemically defined culture medium (5 days in early stage medium and then 2 days in later stage medium) at 38.5°C in an atmosphere of 5% O₂, 5% CO₂, and 90% N₂ [20].

Embryo transfer and pregnancy diagnosis

A single transgenic bovine embryo was loaded into the central drop of BioLife Transfer & Holding medium (C15C; Agtech, USA) in a 0.25-mL straw with two microdrops side by side, and then sealed. Embryo-loaded straws were transported to Gyeongsangbuk-do Livestock Research Institute in an embryo transporter (TE 100 Compact; WTA Reproduction Technologies, Brazil). One straw was loaded onto a 0.25-mL ET Gun (16301; WTA Reproduction Technologies, Brazil) with minimal contamination. The loaded embryo was transferred to the uterine horn of a recipient by the transcervical method on day 7 (estrus = day 0 = day of fusion) using a non-surgical approach [21]. Surrogates were examined by rectal palpation and ultrasonography 50 days post-estrus to assess embryo survival and pregnancy. Pregnant cattle were checked by rectal palpation and ultrasonography at regular intervals thereafter.

Blood sampling

Blood samples were collected daily beginning 6 days before expected parturition until 7 days after parturition. The samples were collected via jugular venipuncture and stored in EDTA-containing (18.0 mg) collection tubes (Vacutainer 10 mL; Becton Dickinson, New Zealand). After collection, the samples were immediately placed on ice and then transferred to 4°C and stored for 24 h before the plasma was isolated via centrifugation at $1900 \times g$ for 30 min at 4°C. The plasma was stored frozen at -80°C until further analysis.

Enzyme-linked immunosorbent assay (ELISA)

Progesterone, prolactin, and cortisol concentrations were measured using sandwich ELISA assays with the Bovine Progesterone ELISA Kit (NBP2-60122-1; Novus Biologicals, USA), Bovine Prolactin ELISA Kit (OKCD06890; Aviva Systems Biology, USA), and Cortisol Parameter Assay Kit (KGE008B; R&D Systems, USA), respectively, according to the manufacturers' instructions. For ELISA measurement, 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, USA). Hormone levels were quantified by extrapolating the signal into the linear range of a standard curve. SoftMax Pro 7.0.2 (Molecular Devices, USA) was used for data analysis.

Statistical analysis

All results are presented as the mean \pm standard error of the mean. 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 8 (ver. 8.3.0; GraphPad Software, USA).

Results

Progesterone, prolactin, and cortisol level changes around parturition in Hanwoo

To identify the hormone best able to predict parturition time in Hanwoo, we measured progesterone, cortisol, and prolactin levels. The progesterone concentration was high ($> 29.7 \pm 3.5$ ng/mL) before parturition and then decreased significantly on the day of parturition (17.3 ± 1.9 ng/mL, $p < 0.001$) and remained low thereafter ($< 12.1 \pm 1.3$ 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, there were no significant differences over the investigated period. The cortisol level remained constant regardless of parturition ($< 15.6 \pm 0.8$ ng/mL) (Figure 1; Supplementary Table 1).

Comparison of progesterone, prolactin, and cortisol levels between AI and ET surrogate Hanwoo

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 and 2). The progesterone levels did not differ between the AI and ET groups throughout the period (Figure 2A; Supplementary Table 2). The prolactin levels did not differ within the AI and ET groups or AI and ET cows during the period (Figure 2B; Supplementary Table 2). The cortisol levels did not differ within the AI and ET groups, but differed significantly between AI and ET surrogate mothers 3 days after parturition (Figure 2C; Supplementary Table 2, $p < 0.005$).

Discussion

The perinatal mortality rate of Hanwoo in South Korea is 2–3% (Korean Rural Development Administration). Parturition time can be predicted clinically, but this is subjective and imprecise. Therefore, we sought to identify objective indicators of parturition time in Hanwoo by measuring the changes in three hormones around parturition, which is a critical phase in livestock production. Many producers experience most calf mortality at birth [7]; therefore, reducing the perinatal mortality rate would be beneficial.

In the AI group, the blood progesterone level decreased at birth, albeit not significantly. After including the nine animals from the ET group, the decrease was significant, suggesting that too few cows were studied to assess statistical significance. Similarly, the prolactin level did not differ significantly around parturition, due either to the small sample size or relatively large individual differences.

The progesterone concentration measurement range of the NBP2-60122-1 kit (Novus Biologicals, USA) is 0.5–30 ng/mL, which is broader than that of other kits, and this kit detects other progesterone analogues. Further research is needed to determine whether the high progesterone concentration in Hanwoo is a characteristic of the breed or due to the kit used in this experiment.

In cattle, the cortisol concentration increases only during the last few hours before the birth of the calf [16]. However, no increase in cortisol was observed in our study, perhaps because we evaluated serum cortisol at 24-h intervals. Measuring hourly blood cortisol levels might yield results similar to the previous reports.

Conclusions

Veterinarians are frequently asked to examine prepartum cows and determine when parturition will occur. This may be assessed using real-time ultrasound [22], changes in body temperature [23], relaxation of the pelvic ligament [24], or intravaginal devices that are activated when pushed out of the vagina by the amniotic sac [25]. However, the exact time of birth based on these methods is not accurate. Other prediction methods studied include blood 17- β -estradiol levels [26] and electrolyte concentration in mammary secretions [27]. In addition to the above methods, we believe that progesterone concentration can be used as an accurate indicator of parturition in Hanwoo.

Abbreviations

AI: Artificial insemination

ET: Embryo transfer

IVM: In vitro maturation

IVF: In vitro fertilization

IVC: In vitro culture

COC: Cumulus-oocyte complex

TALP: Tyrode's albumin lactate pyruvate

ELISA: Enzyme-linked immunosorbent assay

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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

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Authors' information (Optional)

Not applicable

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Figures

Hormonal changes before & after parturition (n=14)

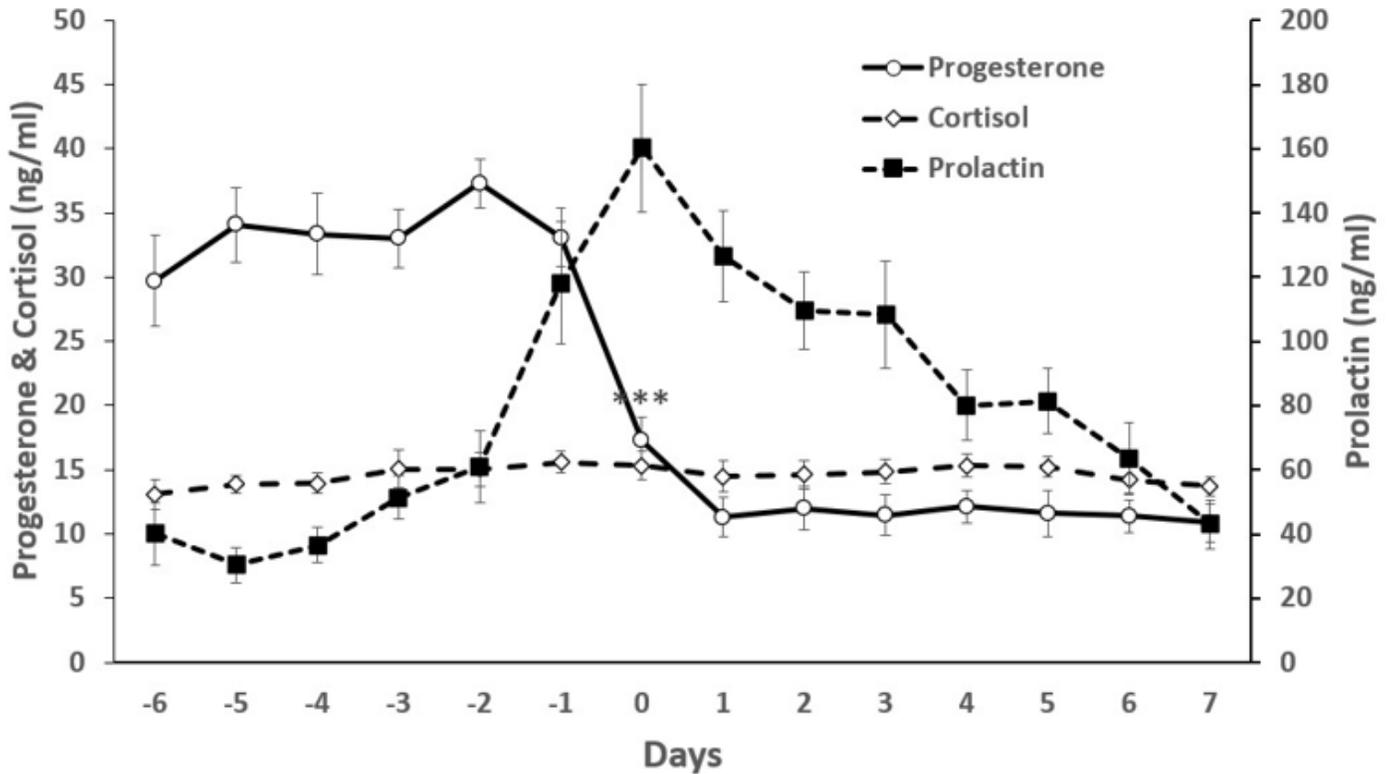


Figure 1

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.

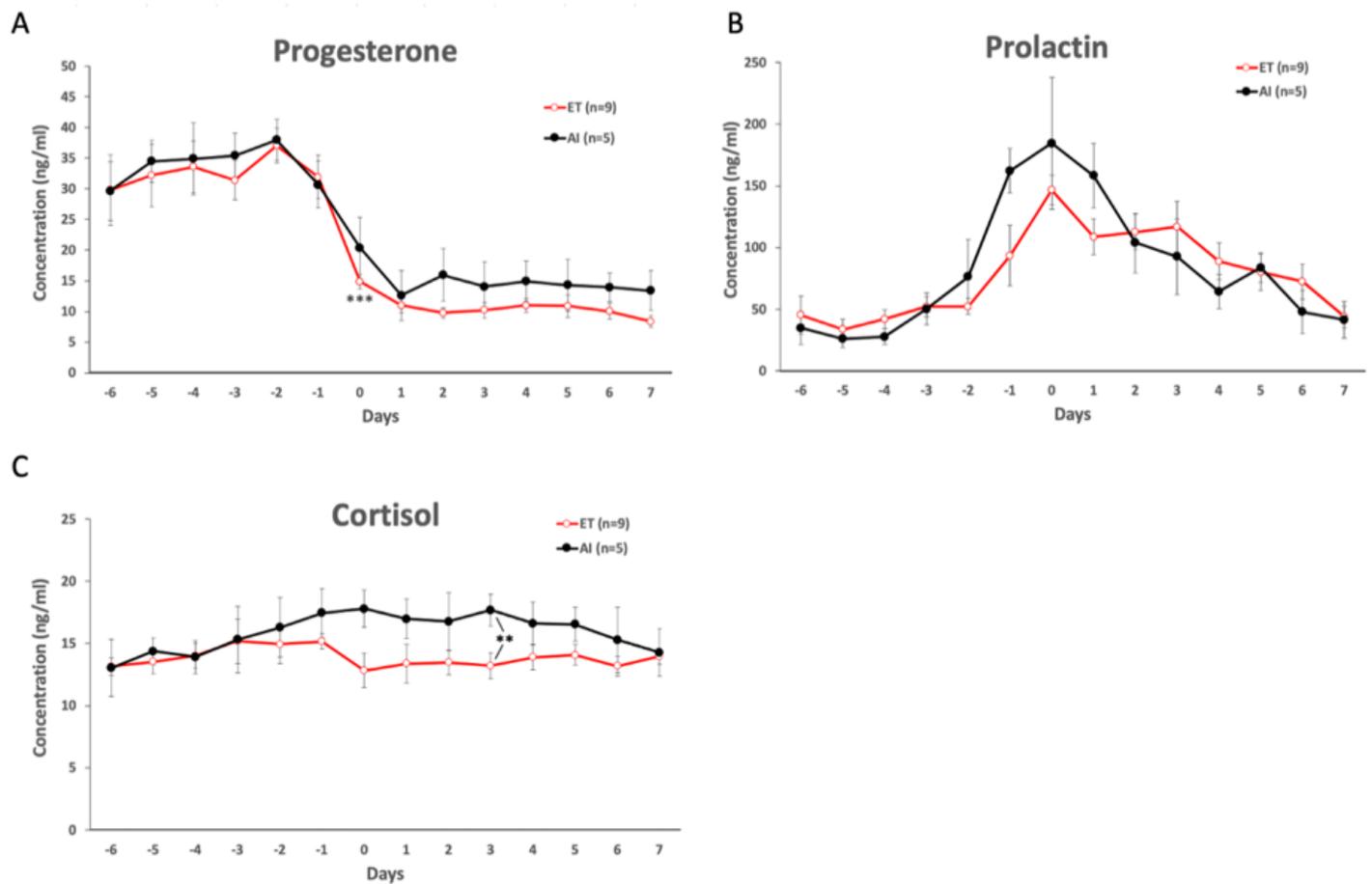


Figure 2

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 ($p < 0.005$).

Supplementary Files

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