



Estrone sulfate (P-Test) in beef cattle as alternative pregnancy detection



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Introduction

- Current pregnancy detection methods include ultrasonography and blood biomarkers. These methods are expensive and labor intensive to the producer.
- Estrone sulfate is an estrogen sulfo-conjugate that originates in the conceptus during gestation in cattle.
- Estrone sulfate can be found in plasma however the ability to measure within urine is currently unknown.
- A simple, cost-efficient method of determining early pregnancy, chute side is lacking within the industry.

Hypothesis and Objectives

Estrone sulfate (P-Test) is a less invasive, successful means to determine pregnancy in cattle.

Determine if the accuracy of estrone sulfate colorimetric urine test is consistent with proven pregnancy detection methods.

Materials and Methods

- Estrus Synchronization:** Cattle were synchronized utilizing the Co-Synch + CIDR protocol with FTAI occurring ~72-74 hrs post CIDR removal. GnRH administered at FTAI.
- EstroTech Patches:** EstroTect Breeding Indicators were applied to females to help determine estrus status at the time of FTAI
 - 1 - no patch present
 - 2 - <25% of patch absent
 - 3 - 50-75% of patch absent
 - 4 - > 75% of patch absent
- Pregnancy Detection:** Pregnancy status was determined via transrectal ultrasonography at 30 and 60 days following FTAI. Blood samples were collected from all beef heifers and cows at the time of pregnancy determination (n = 14, d 30 and d 60 of gestation) by coccygeal venipuncture. Urine samples were collected from all beef heifers and cows on d 60 of gestation.
- Biomarker Analysis:**
 - Plasma progesterone concentrations were measured in duplicate using a commercially available chemiluminescence assay (Immulite 2000 XPI Immunoassay System, Siemens Healthcare, CA, USA).
 - Plasma Pregnancy specific protein b (PSPB) concentrations was examined in duplicate using the IDEXX Laboratories (Westbrook, ME) Bovine Pregnancy Test (BPT).
 - Urine estrone sulfate concentrations were detected using a commercially available colorimetric assay (P-Test) (EMLab Genetics, Arcola, IL, USA).
- Statistical Analysis:** Data were analyzed using PROC FREQ in SAS v. 9.4

Results

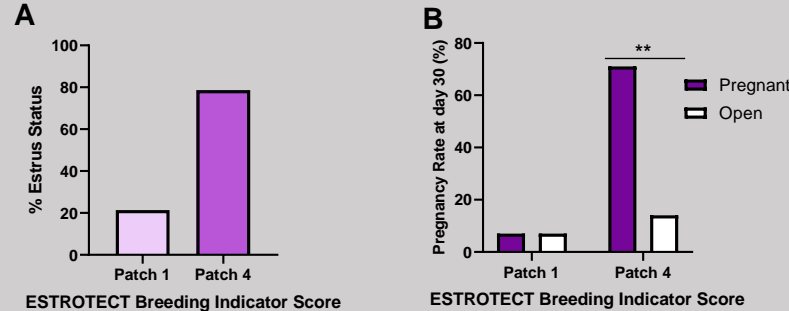


Figure 1. Estrus activity prior to FTAI scores numerically increased and remained elevated on day 30 of gestation. (A) On day 10 of FTAI synchronization protocol, each female was assigned a breeding indicator score based on estrus intensity. A patch score 1 showing little signs of estrus (n = 2) to a patch score 4 presenting signs of standing heat (n = 11). (B) Breeding Indicator activation of a Patch 4 had increased pregnancy rates at d 30 after AI. Analysis represents chi-squared value of pregnancy. ($P < 0.001$; 7% Patch 1 and 72% Patch 4)

Ultrasound

P-Test	Open	Pregnant
Open	14 ^a	7 ^b
Pregnant	36 ^b	43 ^c

^a Using ultrasonography as the standard of detection, urine testing accurately predicted lack of pregnancy in 14% of the herd.
^b Using ultrasonography as the standard of detection, urine testing inaccurately predicted pregnancy status in 43% of the herd.
^c Using ultrasonography as the standard of detection, urine testing accurately predicted pregnancy in 43% of the herd.

Table 1. Frequency analysis of pregnancy detection (% of population) by ultrasonography or urine testing.

PSPB

P-Test	Open	Pregnant
Open	7 ^a	14 ^b
Pregnant	0 ^b	79 ^c

^a Using PSPB as the standard of detection, urine testing accurately predicted lack of pregnancy in 7% of the herd.
^b Using PSPB as the standard of detection, urine testing inaccurately predicted pregnancy status in 14% of the herd.
^c Using PSPB as the standard of detection, urine testing accurately predicted pregnancy in 79% of the herd.

Table 2. Frequency analysis of pregnancy detection (% of population) by PSPB or urine testing.

Progesterone

P-Test	Open	Pregnant
Open	0 ^a	21 ^b
Pregnant	0 ^b	79 ^c

^a Using progesterone as the standard of detection, urine testing accurately predicted no open females in the herd.
^b Using progesterone as the standard of detection, urine testing inaccurately predicted pregnancy status in 21% of the herd.
^c Using progesterone as the standard of detection, urine testing accurately predicted pregnancy in 79% of the herd.

Table 3. Frequency analysis of pregnancy detection (% of population) by progesterone or urine testing.

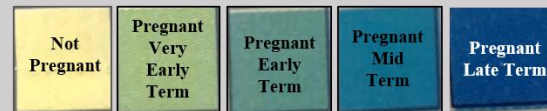


Figure 2. P-Test Colorimetric Scheme

Conclusions

- Estrone sulfate was detected in urine at 60 days post AI, however the stage of gestation did not match the results of the colorimetric system of the P-Test.
- Early pregnancy detection provides information on reproductive soundness and female longevity in the herd.
- Because estrone sulfate may have the potential to monitor embryonic growth and fetal-placental development, a sensitive cost-efficient assay will improve production efficiency.

Implications

Our results indicate that the increase of estrone sulfate in pregnancy is comparable to the increase of biomarkers however, the urine colorimetric P-Test assay doesn't accurately predict pregnancy.

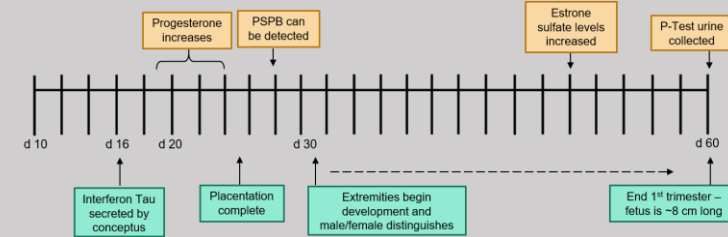


Figure 3. Timeline of critical biological activity and biomarkers of early gestation in cattle.

Future Directions

- Unlocking the correlation of estrone sulfate levels in plasma and urine is key to ensuring a consistent result within the colorimetric system.
- Further investigation into the specific timeframe that would result in the optimum conclusion for a pregnancy detection method.
- Looking into how contaminants can interfere with the P-Test results.

Acknowledgements and References

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