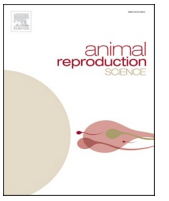
Animal Reproduction Science 272 (2025) 107660

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci



Diagnosing american bison pregnancy during its two last trimesters: Comparison between rectal palpation associated with ultrasonography and hormonal assays in serum or in feces

Vincent Frisée [a], Goulven Rigaux [b], Patrice Dufour [c], Flore Brutinel [d], Philippe Bossaert [a], Sophie Egyptien [d], Olimpia Barbato [e], Frédéric Farnir [f], Caroline Le Goff [c], Stéfan Deleuze [d], Etienne Cavalier [c], Jérôme Ponthier [d,*]

[a] Production Animals Department, FARAH Sustainable Livestock Production, Liège University, Belgium
[b] Pan Anima, Tournai, Belgium
[c] Clinical Chemistry, University Hospital (CHU), Liège University, Belgium
[d] Theriogenology unit, FARAH Comparative Veterinary Medicine, Liège University, Belgium
[e] Veterinary Medicine Department, Perugia University, Perugia, Italy
[f] Biostatistics and bioinformatics applied to Veterinary Sciences, FARAH Sustainable Livestock Production, Liège University, Belgium

ARTICLE INFO

Keywords:
Bison bison
Pregnancy diagnosis
Pregnancy associated glycoproteins
Estrogens
Liquid-chromatography and mass spectrometry
Placenta
Serum
Feces

ABSTRACT

The study aimed to propose sensitive, specific and easy pregnancy diagnosis for American Bison. Trans-rectal Palpation and Ultrasonography (TRPUS), concentration of Pregnancy Associated Glycoproteins (PAG), Progesterone (P4), Estrone (E1), Estrone-Sulfate (E1S) in serum and P4 and E1S in feces were compared. Immunoassay was used for PAG, whereas steroids were assayed using Liquid Chromatography coupled to Mass Spectrometry. Pregnancy and its period were retrospectively assessed after calving. Animals included were distributed as follows: 40 were non-pregnant, 27 were in their second and 15 in their third trimester of gestation. *Fisher's* test was used to determine sensitivity and specificity of TRPUS. Thresholds leading to best sensitivities and specificities for hormonal assays were determined using Receiver Operating Characteristic Curves. Although TRPUS requires contention, palpation skills, it was conclusive in 70.63 % of cases and good sensitivity (97.50 %) and decent specificity (83.33 %) were observed. Concentrations of PAG, P4, E1S and E1 in serum were higher in Pregnant animals (P), with thresholds giving decent specificity and sensitivity (lower sensitivity for E1 and E1S: 92.11 %; lower specificity for P4: 93.48 %). In feces, P4 and E1S were higher in P: pregnancy diagnosis using E1S assay in feces showed 100 % of sensitivity and specificity. In conclusion, assaying E1S in feces during the two last trimesters of American Bisons pregnancy is a sensitive and specific gestation diagnosis. Pregnancy diagnosis using TRPUS can be useful in ranches but requires contention and skill adaptation. Serum hormonal assays can help evaluating the pregnancy period but also require contention.

* Correspondence to: Equine Clinic – University of Liège – Quartier Vallée 2, 5D, Avenue de Cureghem, B41, B-, Liège 4000, Belgium.
E-mail address: Jerome.Ponthier@uliege.be (J. Ponthier).

https://doi.org/10.1016/j.anireprosci.2024.107660
Received 1 March 2024; Received in revised form 18 October 2024; Accepted 28 November 2024
Available online 29 November 2024

1. Introduction

Although American Bison (*Bison bison bison* or *Bison bison athabascae*) were nearly extinct until recently, they are now re-colonizing their natural environment and bred in natural parks or for meat production. Easy and non-invasive pregnancy diagnosis technique is required to sort pregnant and non-pregnant females before culling or to assess fertility in wild groups. American Bison are however often extensively bred and simple manipulations like blood sampling or Trans-Rectal Palpation and Ultrasonography (TRPUS) are time-consuming, dangerous for animals and operators, because of bisons' wild comportment (McCorkell et al., 2013b; Love et al., 2017), thus requiring heavy restraint methods associated with important workforce. Moreover, uterine body is a little bit longer and pelvis narrower compared to cows, making diagnosis by trans-rectal palpation more difficult (Haigh et al., 1991).

Pregnancy diagnosis using semi-quantitative BIO-PRYN Enzyme-Linked Immuno-Sorbent Assay (ELISA) for Pregnancy-Specific Protein-B (PSPB), one of the Pregnancy Associated Glycoproteins (PAG) (Haigh et al., 1991) has recently been validated in Bison serum and showed good specificity and sensitivity, even in early gestation (Love et al., 2017), but the evolution of the PSPB concentration during pregnancy was not reported (Love et al., 2017). In a previous study (Frisee et al., 2022), we assayed PAG in American Bison using a dedicated Radio-Immuno Assay (RIA) and observed an increase of PAG after three months of pregnancy. For steroids, Liquid Chromatography coupled to Mass Spectrometry (LC-MS/MS) has become the gold standard as it improves sensitivity, specificity and reproducibility (Conley, 2016; Conley and Ball, 2019). We previously validated Estrone (E1), Estrone-Sulfate (E1S) and Progesterone (P4) assays in serum of American Bison using LC-MS/MS (Dufour et al., 2021). In a previous study, E1, E1S and PAG were demonstrated as potential pregnancy markers whereas P4 was not (Frisee et al., 2022), but cut-off values, sensitivity and specificity of these assays used as potential pregnancy diagnosis were not reported.

To address the concerns about contention, animals and operators security while sampling blood in American Bison, urinary and fecal sexual steroids have been tested in early studies using immunoassays for total estrogens, with no information about the type of estrogen produced (Kirkpatrick et al., 1991, 1992). Results observed were promising, but the small numbers of animals were all at the same gestational period (third month of pregnancy) (Kirkpatrick et al., 1991, 1992), requiring new studies to use this methodology during the nine months pregnancy. In our previous report, fecal concentrations of P4 and E1S were assayed using a LC-MS/MS dedicated method (Dufour et al., 2024). Despite the heterogeneity of steroids repartition in feces and the evolution of their concentrations during 20°C storage, a good correlation between serum and feces concentrations was observed for E1S, whereas a poor correlation was observed for P4 (Dufour et al., 2024). The use of theses assays in feces for a pregnancy diagnosis still requires to determine cut-off values, sensitivity and specificity.

The present study aimed to compare physical diagnosis (TRPUS), hormonal assays of PAG, P4, E1 and E1S in serum and of P4 and E1S in feces to determine the most sensitive, specific and easy pregnancy diagnosis for American Bisons. The ability of the different methods to assess the trimester of pregnancy was also evaluated.

2. Material and methods

2.1. Animals and sampling

In the Belgian Ardennes (±50°N), two ranches are extensively breeding wild American Bison (*Bison bison)* imported from Montana (USA) since 1998 and 2004, respectively. The first ranch herd is divided in two separate meadows of 19 and 21 ha, each welcoming two sexually mature American Bison bulls and respectively 25 and 26 sexually mature cows. In the second ranch, ten sexually mature American Bison cows are roaming freely with one sexually mature bull in a 15 ha meadow. In both ranches, animals are not used to human presence and to manipulations, but the whole herd is monitored every morning from a tractor or a quad-bike, to detect events as dystocia, retained placentas or abortion. Once a year, animals are gathered and immobilized for mandatory diseases screening in a specific handling/chute system. Between January and February 2019, and between February and March 2020, this opportunity was used to collect blood in dry-tubes by venipuncture under the tail of all matures cows. Blood samples were centrifuged at 20°C (1000 × g) and stored frozen (-80°C) until assays. Feces (± 50 mL) were also collected by rectal palpation before TRPUS and stored frozen (-20°C).

Calving days of the American Bison cows were recorded for the year following the sampling procedures. Fertilization day of cows that calved was retrospectively obtained by subtracting 276 days of the calving day (Kirkpatrick et al., 1992; Vervaecke and Schwarzenberger, 2006; Frisee et al., 2022). Gestation day on sampling day was then determined by subtracting the fertilization day from the sampling day. Other events, such as abortion, disease, culling or late birth (more than 276 days after sampling) were reported by the breeders during the following year. American Bison's samples were divided in four classes: Non-Pregnant, when no calving was observed within the next nine and a half months (NP), first trimester of pregnancy, from day one to day 92 (1TP), second trimester of pregnancy, from day 93 to day 183 (2TP), third trimester of pregnancy, from day 184 to the end (3TP).

2.2. Trans-rectal palpation and ultrasonography (TRPUS)

Trans-rectal palpation was performed before ultrasonography (US) using EBmax RKU10 with a 5Mhz multifrequency transrectal probe (Echomedic, Lahamaide, Belgium) by three operators that are skilled bovine practitioners: the total procedure was limited to three minutes but could be shortened when the animal was becoming violent. Positive pregnancy diagnosis was established when the uterus was enlarged at palpation and cotyledons or parts of the fetus were observed during US. If the uterus was small and there were no cotyledons or no liquid in the uterine lumen, the cow was categorized as non-pregnant. In the case of discordant information after

three minutes or when the animal was becoming violent, pregnancy diagnosis by TRPUS was considered as non-conclusive.

2.3. Assays in serum and in feces

Dedicated RIA previously described (Frisee et al., 2022) was used to assay PAG in serum using: the minimum detection limit (MDL), was 0.1 ng/mL and the intra- and inter-assay coefficients were 2.9 % and 7.3 %, respectively. Assays of P4, E1 and E1S were performed in serum using the validated LC-MS/MS method described by our team (Dufour et al., 2021; Frisee et al., 2022). For P4, E1 and E1S, Lower Limit Of Quantification (LLOQ), defined as the lowest concentration in the validation standards that reported Relative Standard Deviation and Relative Bias lower than 15 %, were established at respectively 0.1 ng/mL, 2.0 pg/mL and 0.5 ng/mL (Dufour et al., 2021).

Assays of P4 and E1S were performed in feces using the pre-analytical method previously described and validated (Dufour et al., 2024). Hormones' concentrations were then determined using our LC-MS/MS validated method (Dufour et al., 2024) without replicate: the LLOQ of the method were set at 20 pg/g and 4 ng/g of matter for E1S and P4, respectively.

2.4. Statistics

Graphpad Prism was used (version 9.0 for Mac OSX, Graphpad Inc., San Diego, USA) and statistical significance was established at $p < 0.05$ for this double-blind prospective study. Normal distribution of values was tested using *Kolmogorov-Smirnov test*. Results are expressed as mean ± standard deviation or median and 25 and 75 quartiles if they are not normally distributed.

For TRPUS, *Fisher exact test* was used to determine significance of contingency table's distribution and subsequent sensitivity (Se), specificity (Sp) and Likelihood Ratio (LR) were determined. Unclear pregnancy diagnose were also studied by this way, assuming the equal chance of non-conclusive TRPUS to lead to a pregnancy or a non-pregnancy diagnosis as null hypothesis.

As hormonal concentrations in serum and in feces were not normally distributed, they were log-normalized, and, as *Leven's test* showed differences in observed variances of Pregnant (P) and NP groups for all hormonal assays, *Welch corrected t test* was used. Receiver Operating Characteristic (ROC) (Detilleux et al., 1999; Ponthier et al., 2010) were then performed to determine the best threshold to discriminate P and NP animals for each hormone assayed in serum or in feces. For each ROC analysis, *Fisher's* test was used to determine significance of contingency table and of the subsequents Se, Sp and LR. For serum assays of P4 and E1S, a combined model was created to determine the thresholds of these hormone to achieve best Se and Sp.

For P4 and E1S assays in feces, correlation between day of pregnancy and hormone concentration was tested using *Spearman* non-parametric test. To diagnose the trimester of pregnancy, *Welch corrected t test* was performed on log-normalized data of 2 and 3TP for concentrations of E1 and E1S in serum and of P4 and E1S in feces. The ROC procedure was then used to determine the best threshold to discriminate 2 and 3TP using LR.

3. Results

3.1. General results

Among the 52 pregnancies observed in this study, three were in the 1TP group and were excluded from this study because of the low amount of data available in this period. Five pregnant animals at the sampling time aborted later and two were culled: as the calving days were unknown, the trimesters of pregnancy were impossible to determine, and these animals were excluded from the present study. Respectively, 27 and 15 observed pregnancies were in 2TP and 3TP. Forty American Bison cows were included in NP group.

3.2. Trans-rectal palpation and ultrasonography for pregnancy diagnosis in American Bison

According to the operators involved in the present study, TRPUS technique in American Bison did not really differs from domestic

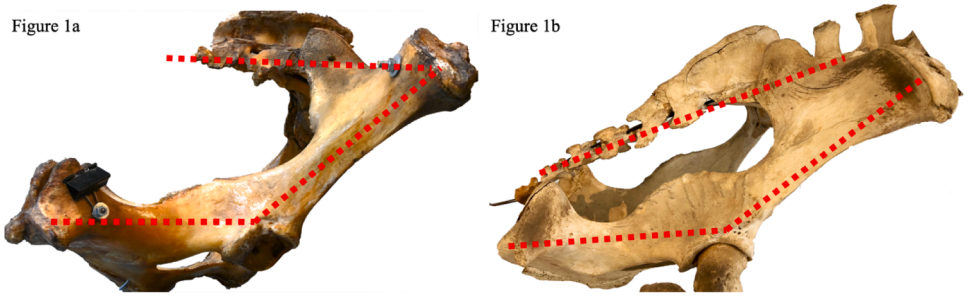


Fig. 1. Pelvis anatomy of Bovine and American Bison cows, Fig. 1a is a picture of a dairy cow pelvis.Fig. 1b is a picture of an American Bison cow. Main lines of the pelvis are red dotted. Angle between pelvic floor and ilium is more closed in cattle (145°) than in American Bison cows (155°). Pictures credits: Vincent Frisée and Jérôme Ponthier.

cows. The uterine body is a little bit longer and the bison pelvic floor is not horizontal, as it is observed in the cow, but presents an ascending slope parallel to the sacrum. Our observations on available anatomic pieces estimated angulation between ilium and pelvic floor of 155° versus 145° for cattle (See Fig. 1). This conformation explains the narrower and longer pelvis of American Bison, followed by a pronounced angulation with the abdomen reported by the TRPUS operators of this study. Among the 82 American Bison presented for pregnancy diagnosis, TRPUS were not realized on one animal that was too stressed and violent, kicking through the specific handling/chute system. A conclusion about gestational status could be given in 70.73 % of performed TRPUS, with a high Se (100 %; see Table 1) and decent Sp (81.82 %). Among the 24 remaining unconclusive cases, 16 turned out to be in the NP group. As these data did not differ from a random distribution, no deduction can be drawn from the cases of non-conclusive TRPUS, that are not clearly associated with NP animals. In these unclear cases, duration of the procedure and stress of the animal were the main limitations. These concerns also explain why TRPUS did not enable the operators to give a gestational age, as it would require increasing procedures duration.

3.3. Hormonal assays in serum and feces for pregnancy diagnosis in American Bison

In the serum, PAG, P4, E1 and E1S were all significantly different between NP and P animals. The thresholds with the higher LR determined by the ROC procedures were selected. Table 1 is summarizing these results. The lowest Se (92.11 %) of the studied pregnancy diagnosis means using serum was observed for E1 and E1S assay in serum, whereas the lowest Sp (93.48 %) was reached by P4 assay in serum. Using a combined model with P4 or E1S to diagnose pregnancy, Se and Sp reached 100 % and 95.65 %, respectively with combined threshold of P4 and E1S above 3.7 ng/mL and 1.2 ng/mL. Between 2 and 3TP, differences could be observed in E1 and E1S serum concentrations (respectively, $P = 0.0401$ and $P = 0.0004$). Use of ROC curve led to observe the best Se of 66.67 % (95 % interval: 41.72–84.82 %) and Sp of 65.22 % (95 % interval: 44.89–81.19 %) for E1 threshold at 30.80 pg/mL, whereas these values were respectively 84.62 % (95 % interval: 57.77–97.27 %) and 73.33 % (95 % interval: 48.05–89.10 %) for E1S threshold at 5.55 ng/mL.

Feces could easily be collected on animals, including the cow that did not tolerate TRPUS. Determination of P4 concentration in feces for pregnancy diagnosis in American Bison has decent Se and Sp, but clearly below those observed for E1S assay in feces (see Table 1). In our population, assay of E1S in feces showed perfect combined Se and Sp: E1S concentrations in feces of NP American Bison cows were all below the 20 pg/g LLOQ value, whereas the minimal value observed on a pregnant cow (in 2TP, 146 days of pregnancy) was 57.96 pg/g.

The concentration of P4 in feces was not correlated to the day of pregnancy (Fig. 2), whereas E1S was moderately correlated (*Spearman test values:* $P < 0.0001$ and $r = 0.6748$; Fig. 3). There was however no difference in E1S fecal concentrations between 2 and 3TP, meaning that it cannot be yet used to determine the trimester of pregnancy.

4. Discussion

To the best of our knowledge, this study gathers the highest number of American Bison cows ($n = 82$) undergoing pregnancy diagnosis with physical and hormonal methods. A pregnancy diagnosis method for wild animals should be highly sensitive to avoid the risk of culling pregnant animals while maintaining a high specificity, but it also has to be easy to perform on field without risks for animals and operators. Pregnancy diagnosis by TRPUS was risky for operators and animals, requiring heavy restraint that can be stressful for American Bison (McCorkell et al., 2013a, 2013b), and conclusive in only ± 70 % of cases. When conclusive, TRPUS have a very high Se and a satisfying Sp. Differences in pelvis conformation between cattle and American Bison were observed by skilled bovine practitioners involved in this study: it was disruptive for the first TRPUS, but the operators rapidly adapted to this

Table 1
Comparison of the different diagnosis means evaluated.

	P value	Threshold	Sensitivity *(95 % interval)*	Specificity *(95 % interval)*	Likelihood Ratio
TRPUS	<0.0001		**100.00 %** *(89.57–100.00 %)*	**81.82 %** *(61.48–92.69 %)*	5.50
Serum PAG	<0.0001	4.15 ng/mL	**92.68 %** *(80.57–97.48 %)*	**97.62 %** *(87.68–99.88 %)*	38.93
Serum P4	<0.0001	3.40 ng/mL	**100.00 %** *(90.82–100.00 %)*	**93.48 %** *(82.50–97.76 %)*	15.33
Serum E1	0.0003	3.90 pg/mL	**92.11 %** *(79.20–97.28 %)*	**97.83 %** *(88.66–99.89 %)*	42.37
Serum E1S	0.0007	0.55 ng/mL	**92.11 %** *(79.20–97.28 %)*	**97.83 %** *(88.66–99.89 %)*	42.37
Feces P4	0.0391	11.60 ng/g	**93.55 %** *(79.28–98.85 %)*	**97.37 %** *(86.51–99.87 %)*	35.55
Feces E1S	<0.0001	38.98 pg/g	**100.00 %** *(88.97–100.00 %)*	**100.00 %** *(90.82–100.00 %)*	Undetermined

TRPUS: Trans-Rectal Palpation and Ultra-Sonography; PAG: Pregnancy Associated Glycoproteins concentration; P4: Progesterone concentration; E1: Estrone concentration; E1S: Estrone-Sulfate Concentration.

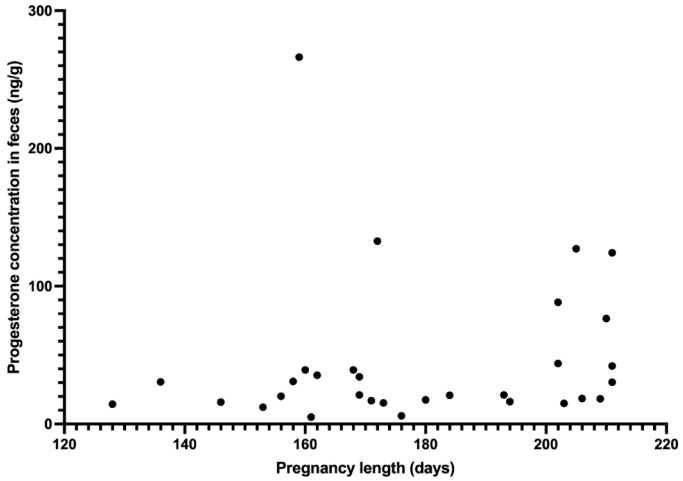


Fig. 2. Evolution of Progesterone concentration (in ng/g of matter) in American Bison cows' feces during the end of pregnancy, Progesterone concentration in feces (in ng/g of matter) assayed by Liquid Chromatography coupled to Mass Spectrometry evolution during American Bison pregnancy in days. Each dot represents the progesterone concentration (ng/g) observed in a different pregnant American Bison cow for its day of pregnancy at sampling (Spearman test values: P = 0.1413; r = 0.2703).

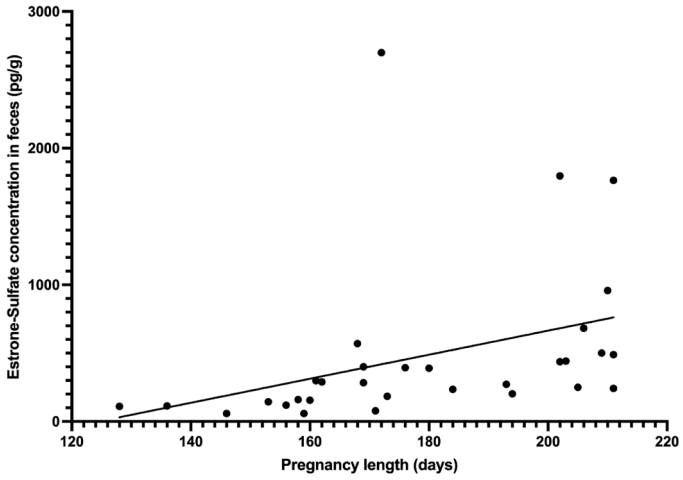


Fig. 3. Evolution of Estrone-Sulfate concentration (in pg/g of matter) in American Bison cows' feces during the end of pregnancy, Estrone-Sulfate concentration in feces (in pg/g of matter) assayed by Liquid Chromatography coupled to Mass Spectrometry evolution during American Bison pregnancy in days. Each dot represents the progesterone concentration (ng/g) observed in a different pregnant American Bison cow for its day of pregnancy at sampling (Spearman test values: P < 0;0001; r = 0.6748).

conformation. On the other hand, ultrasonographic pictures were not different, with cotyledons and fetus most like what is observed in cattle. Nevertheless, the bovine practitioners involved in this study were not able to give a gestational age to the fetus, partially because of the anatomical and developmental differences, but mainly due to the increased examination duration leading to risks for the animal and the operators.

Blood sampling also requires heavy contention, but it can be performed during the mandatory diseases screening (e.g.: brucellosis) and enables assay of PAG and steroids. In this study, assaying P4 in serum for pregnancy diagnosis showed a high Se and satisfying Sp. In our previous study describing P4 evolution during American Bison gestation (Frisee et al., 2022), its assay was unreliable to determine pregnancy period. A couple of factors may explain the absence of evolution during pregnancy and tune down our Se and Sp results. Our sampling sessions were performed in January or February, when most of American Bison cows were in anestrus (Kirkpatrick et al., 1992; Vervaecke and Schwarzenberger, 2006; Frisee et al., 2022), whereas diestrus animals could be more frequently present during other seasons, leading to confusions with pregnant ones. On the other hand, stress is known to induce P4 production in bovine and could also influence these results (Whitlock et al., 2012).

Thus, PAG, that are more specific pregnancy markers were assayed in serum, leading to a satisfying Se, in the range of what has been reported with BIO-PRYN ELISA for PSPB in late American Bison pregnancy, and with a high Sp, but slightly below what has been observed in this previous report (Love et al., 2017). In our study, three pregnant cows at approximatively 6 months of pregnancy had

PAG serum concentrations below the threshold fixed by the ROC procedures, leading to lower our Se. The low Sp observed in this study and in the report using BIO-PRYN ELISA for PSPB (Love et al., 2017) could be explained by the sampling of previously pregnant animals, as PAG could return to basal level up to one month, as it has been observed in water buffaloes (Barbato et al., 2017). Assay of PAG in serum is unsuitable in American Bison to determine if animals were in 2 or 3TP, due to constant PAG production in late pregnancy (Frisee et al., 2022), also observed in buffaloes (Barbato et al., 2017), unlike what is observed in cattle (Shahin et al., 2014).

On the other hand, the increase in E1S serum concentration during American Bison pregnancy is consistent with previous reports in cattle, goats and buffaloes (Hung and Prakash, 1990; Isobe et al., 2003; Singh et al., 2019), and is of interest to determine the gestational period when American Bison cows lose their seasonality in captive conditions (Frisee et al., 2022). In our previous study, E1 and E1S assay in serum both showed differences between 2 and 3TP (Frisee et al., 2022), and the ROC procedure performed in the present study led to observe acceptable Se and Sp to determine trimester of pregnancy with E1S, but not with E1. As an evidence, diagnosing American Bison pregnancy using E1 and E1S assay in serum led to satisfying Se and excellent Sp, that can be increased when used together with P4, similar to results of previous study in American Bison late pregnancy using BIO-PRYN ELISA for PSPB (Love et al., 2017). The placental production of E1S has long been described in ruminants (Tsang, 1974; Hung and Prakash, 1990; Isobe et al., 2003; Singh et al., 2019) and explains the interest for this hormone to detect gestation, as it is pregnancy specific and quickly decreases after the calving (Shah et al., 2007). Serum sampling on wild or semi-wild animals remains however a challenge and alternative methods were investigated.

Collection of American Bison urine and feces for pregnancy diagnosis have been described in early reports (Kirkpatrick et al., 1991, 1992). For our study, feces were preferred as they seem to be easier to collect on animals or to identify in pasture than urine. With a methodology respecting our previous concerns about homogeneity and stability of steroids in American Bison feces (Dufour et al., 2024), P4 and E1S were assayed in feces and evaluated as a potential pregnancy diagnosis. Although P4 concentration in feces showed a low correlation with its serum concentrations in our validation study of this assay (Dufour et al., 2024), use of P4 assay in feces as a pregnancy diagnosis for American Bison led to satisfying Se and high Sp. Progesterone is known to be modified in pregnant cows after nutritional challenge (Shemesh, 1990; Izhar et al., 1992; Shemesh et al., 1994) and stress can increase its adrenal production, even in bulls (Hollenstein et al., 2006). This could partially explain the observed absence of correlation between P4 concentration in feces and the day of pregnancy. Moreover, some pregnant American Bisons cows could be confused with ones in diestrus using single P4 concentration assay in feces, (Frisee et al., 2022). On the other hand, chronically stressed American Bison bulls could be considered as pregnant animals, as P4 and its metabolites concentrations in captured wild elks' feces were higher than in non-pregnant animals (Watson et al., 2023). On the contrary, assay of E1S in feces of pregnant and non-pregnant cows included in this study led to statistically perfect pregnancy diagnosis, as suggested in preliminary studies (Kirkpatrick et al., 1992). This method has higher Se and Sp than all others gestation detection means proposed in this study, but also than BIO-PRYN ELISA for PSPB assay in serum of American Bison in late pregnancy (Love et al., 2017). Some limitations have to be mentioned regarding our previous study validating this assay in feces (Dufour et al., 2024). In the present report, two pregnant animals showing feces E1S concentration below the LLOQ were excluded: one cow in 1TP and the other one with unknown gestational period, as she aborted after sampling. Using these two cows in the current set of data would lead to slightly lowered sensitivity (97.06 %), but specificity would remain perfect, with best performances than those observed with previously described assays (Love et al., 2017). An E1S concentration in feces below LLOQ can thus be interpreted in different ways: NP animal, American Bison cow in early pregnancy or male. To resolve these unknowns, E1S concentration in feces concentration should be investigated in 1TP to determine the onset of its excretion and after the calving to determine time required to return below the LLOQ. Moreover, assay of androgens in American Bison feces should be developed using LC-MS/MS to identify bulls. When compared to E1S assay in serum, the only drawback with its assay in feces for American Bison pregnancy diagnosis would be that it cannot determine the trimester of pregnancy. Metabolism of estrogens inside the feces involving microbiological flora could explain the unparallel evolution of E1S in serum and feces (Dufour et al., 2024). A moderate correlation between E1S concentration in feces and day of pregnancy was however observed in the present data. It can thus be assumed that increasing the number of sampled feces of pregnant American Bison cows assayed for E1S in feces could help addressing this concern.

A pregnancy diagnosis available on feces is an opportunity for fertility investigation in wild herd of American Bison. Feces collection can be performed without any contention, just observing the herd and waiting for it to move, before safely accessing the samples. Moreover, E1S assay for pregnancy diagnosis in American Bison feces can be linked to fecal DNA analysis (Forgacs et al., 2016, 2019) to identify the pregnant or non-pregnant animals and potentially sort them before exportation or culling. Developing multiplex LC-MS/MS assays including P4, E1S, E1, estradiol, testosterone and corticosteroids in feces could also lead to a better comprehension of American Bison cyclicity, pregnancy and levels of stress. This methodology could also be extrapolated and validated for other wild ruminants in the future.

The first limitation is that NP cows could be pregnant at the time of sampling and abortion could occur later, without being observed by the owners. This concern seems to be unlikely, as owners observed five abortions, what is consistent with a normal abortion rate and confirms their ability to record such events. Moreover, the data of TRPUS closely match with the hormonal assays results. The other limitation is the low number of American Bison cows in early pregnancy observed between the end of January and in the beginning of February, despite seasonality loss was observed in Belgium in our previous paper (Frisee et al., 2022), that led to exclude cows in 1TP from this study. An increase of total estrogens concentration in feces was observed as early as three months by the preliminary studies about pregnant American Bison cows (Kirkpatrick et al., 1992). Previous reports using BIO-PRYN ELISA for PSPB also showed satisfying sensitivity and excellent specificity at two months of pregnancy (Love et al., 2017). Further studies should be designed to complete the description of American Bison early pregnancy's endocrinology. Moreover, these studies could also lead to determine what is the earlier and easier diagnosis available in American Bison between TRPUS, serum or feces assays.

5. Conclusion

For wild American Bison, assaying E1S in feces is a nearly perfect pregnancy diagnosis during its last six months and could be linked to individual recognition methods using PCR methods available on feces. Increasing the number of sampled feces in future studies could improve the ability to determine the pregnancy period. On the other hand, in safari parks or in ranches breeding American Bison where the herd is gathered for sanitary screening, TRPUS are still of interest as it is nearly costless and gives satisfying results when it leads to a conclusion. The TRPUS method remains however dangerous for animals and operators and requires to adapt their skills as a heavy restraint. When TRPUS are not feasible or not conclusive, blood collection can help to diagnose the pregnancy, and to estimate gestational age, in case of seasonality loss. Future experiments should focus on the first trimester of American Bison pregnancy to understand its physiology, but also to determine the onsets of hormone production in order to describe early and easy pregnancy diagnosis methods. Such a methodology using matrices easy to collect could also be of interest for gestation detection in other wild ruminants.

Funding source

Clinical incomes of the Theriogenology unit of the Veterinary Medicine Faculty of Liège University financed this research.

Ethical statement

Ethical review and approval were not required for the animal study because the procedures were performed at the request of the owner. The study was realized based on this clinical situation, required by the owner of the animals.

CRediT authorship contribution statement

Vincent Frisée: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jérôme Ponthier:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Goulven Rigaux:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Conceptualization. **Patrice Dufour:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Flore Brutinel:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation. **Philippe Bossaert:** Writing – review & editing, Visualization, Investigation. **Sophie Egyptien:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Olimpia Barbato:** Writing – review & editing, Visualization, Validation, Methodology. **Frédéric Farnir:** Writing – review & editing, Software, Data curation. **Caroline Le Goff:** Writing – review & editing, Visualization, Validation, Methodology, Data curation. **Stéfan Deleuze:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Etienne Cavalier:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of Competing Interest

All authors of the paper entitled “Diagnosing American Bison pregnancy during its two last trimesters: comparison between rectal palpation associated with ultrasonography, assay of Pregnancy Associated Glycoproteins and of steroids in feces or serum” have no conflict of interest to declare.

Acknowledgements

Authors would like to thank Candice Leroy, Laurent Naveau and all the team of the Bison Ranch, located in Orchimont, Vresse Sur Semois, Belgium and M. Jérémie Van Ael from Lignières, Belgium. Authors also would like to thank Prof. J.-F. Beckers, Dr. Marta David, Dr. Coralie Lagamme and Dr. Margot Stipulanti for their help at different steps of the protocol. Authors would also like to thank Dr. Anne-Françoise Rousseau for her careful reviewing of the paper.

References

Barbato, O., Menchetti, L., Sousa, N.M., Malfatti, A., Brecchia, G., Canali, C., Beckers, J.F., Barile, V.L., 2017. Pregnancy-associated glycoproteins (PAGs) concentrations in water buffaloes (Bubalus bubalis) during gestation and the postpartum period. Theriogenology 97, 73–77.

Conley, A.J., 2016. Review of the reproductive endocrinology of the pregnant and parturient mare. Theriogenology 86, 355–365.

Conley, A.J., Ball, B.A., 2019. Steroids in the establishment and maintenance of pregnancy and at parturition in the mare. Reproduction 158, R197–R208.

Detilleux, J., Arendt, J., Lomba, F., Leroy, P., 1999. Methods for estimating areas under receiver-operating characteristic curves: illustration with somatic-cell scores in subclinical intramammary infections. Prev. Vet. Med. 41, 75–88.

Dufour, P., Courtois, J., Seynaeve, Y., Peeters, S., Le Goff, C., Cavalier, E., Ponthier, J., 2021. Development and validation of a liquid chromatography coupled to mass spectrometer (LC-MS) method for the simultaneous quantification of estrone-3-sulfate, progesterone, estrone and estradiol in serum of mares and American bisons. Res. Vet. Sci. 136, 343–350.

Dufour, P., Frisee, V., Rigaux, G., Brutinel, F., Egyptien, S., Bossaert, P., Deleersnyder, J., Deleuze, S., Peeters, S., Le Goff, C., Ponthier, J., Cavalier, E., 2024. Analysis of progesterone and estrone-sulfate in feces of American Bison using liquid chromatography coupled to mass spectrometry: technical validation and correlation with blood levels. Domest. Anim. Endocrinol. 86, 106819.
Forgacs, D., Wallen, R.L., Boedeker, A.L., Derr, J.N., 2019. Evaluation of fecal samples as a valid source of DNA by comparing paired blood and fecal samples from American bison(Bison bison). BMC Genet 20, 22.
Forgacs, D., Wallen, R.L., Dobson, L.K., Derr, J.N., 2016. Mitochondrial genome analysis reveals historical lineages in yellowstone bison. PLoS One 11, e0166081.
Frisee, V., Rigaux, G., Dufour, P., Barbato, O., Brutinel, F., Egyptien, S., Bossaert, P., Deleuze, S., Cavalier, E., Ponthier, J., 2022. American Bison (Bison bison) reproductive endocrinology: serum Pregnancy Associated Glycoproteins (PAG), Progesterone, Estrone and Estrone-Sulfate in non pregnant animals and during gestation. Domest. Anim. Endocrinol. 78, 106684.
Haigh, J.C., Gates, C., Ruder, A., Sasser, R., 1991. Diagnosis of pregnancy in wood bison using a bovine assay for pregnancy-specific protein B. Theriogenology 36, 749–754.
Hollenstein, K., Janett, F., Bleul, U., Hassig, M., Kahn, W., Thun, R., 2006. Influence of estradiol on adrenal activity in ovariectomized cows during acute stress. Anim. Reprod. Sci. 93, 292–302.
Hung, N.N., Prakash, B.S., 1990. Influence of gestation on blood plasma concentrations of oestrone and oestrone sulphate in Karan Swiss cows and Murrah buffaloes. Br. Vet. J. 146, 449–456.
Isobe, N., Nakao, T., Uehara, O., Yamashiro, H., Kubota, H., 2003. Plasma concentration of estrone sulfate during pregnancy in different breeds of Japanese beef cattle. J. Reprod. Dev. 49, 369–374.
Izhar, M., Pasmanik, M., Shemesh, M., 1992. Bovine placental progesterone synthesis. Comp. first Second trimesters Gestation. Biol. Reprod. 46, 846–852.
Kirkpatrick, J.F., Bancroft, K., Kincy, V., 1992. Pregnancy and ovulation detection in bison (Bison bison) assessed by means of urinary and fecal steroids. J. Wildl. Dis. 28, 590–597.
Kirkpatrick, J.F., Kincy, V., Bancroft, K., Shideler, S.E., Lasley, B.L., 1991. Oestrous cycle of the North American bison (Bison bison) characterized by urinary pregnanediol-3-glucuronide. J. Reprod. Fertil. 93, 541–547.
Love, D.M., Mefford, M.T., Ramer, J.C., 2017. Validation of the BioPRYN enzyme-linked immunosorbent assay for detection of pregnancy-specific protein-B (PSPB) and diagnosis of pregnancy in American bison (Bison bison). Reprod. Domest. Anim. 52, 791–797.
McCorkell, R.B., Woodbury, M.R., Adams, G.P., 2013b. Serial ovarian ultrasonography in wild-caught wood bison (Bison bison athabascae). Theriogenology 80, 552–556.
McCorkell, R., Wynne-Edwards, K., Galbraith, J., Schaefer, A., Caulkett, N., Boysen, S., Pajor, E., of, U.C., 2013a. Transport versus on-farm slaughter of bison: physiological stress, animal welfare, and avoidable trim losses. Can. Vet. J. 54, 769–774.
Ponthier, J., Franck, T., Detilleux, J., Mottart, E., Serteyn, D., Deleuze, S., 2010. Association between myeloperoxidase concentration in equine frozen semen and post-thawing parameters. Reprod. Domest. Anim. 45, 811-816.
Shah, K.D., Nakao, T., Kubota, H., Maeda, T., 2007. Peripartum changes in plasma estrone sulfate and estradiol-17beta profiles associated with and without the retention of fetal membranes in holstein-friesian cattle. J. Reprod. Dev. 53, 279–288.
Shahin, M., Friedrich, M., Gauly, M., Holtz, W., 2014. Pregnancy-associated glycoprotein (PAG) profile of Holstein-Friesian cows as compared to dual-purpose and beef cows. Reprod. Domest. Anim. 49, 618–620.
Shemesh, M., 1990. Production and regulation of progesterone in bovine corpus luteum and placenta in mid and late gestation: a personal review. Reprod. Fertil. Dev. 2, 129–135.
Shemesh, M., Harel-Markowitz, E., Gurevich, M., Shore, L.S., 1994. Staurosporine stimulates progesterone production by bovine placental cells. Biol. Reprod. 51, 146–151.
Singh, S.P., Ramachandran, N., Sharma, N., Goel, A.K., Gururaj, K., Kharche, S.D., 2019. Temporal changes in plasma profile of pregnancy-associated glycoprotein, progesterone and estrone sulfate associated with fetal number during early- and mid-pregnancy in goats. Anim. Reprod. Sci. 205, 115–125.
Tsang, C.P., 1974. Changes in plasma levels of estrone sulfate and estrone in the pregnant ewe around parturition. Steroids 23, 855–868.
Vervaecke, H., Schwarzenberger, F., 2006. Endocrine and behavioral observations during transition of non-breeding into breeding season in female American bison (Bison bison). Theriogenology 66, 1107–1114.
Watson, E.M., Kurth, K.A., Metts, D.L., Moorey, S.E., Miller, B.F., Gerhold, R.W., Muller, L.I., 2023. Evaluating the efficacy of noninvasive fecal sampling for pregnancy detection in Elk (Cervus Canadensis). J. Wildl. Dis. 59, 61–70.
Whitlock, B.K., Coffman, E.A., Coetzee, J.F., Daniel, J.A., 2012. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology 78, 737–746.