International Symposium on World Camel Day, Pakistan, June 22, 2020

Isolation and partial characterization of pregnancy-specific protein B (PSPB) from Iraqi dromedary camels

Prof. Dr. Talal A. Abdulkareem

Livestock Reproductive Physiology
Department of Animal Production
College of Agricultural Engineering Sciences,
University of Baghdad, Iraq

drtalalabdulkareem2013@gmail.com
talal.al@coagri.uobaghdad.edu.iq

International Symposium
on World Camel Day, Pakistan, June 22, 2020

Isolation and partial characterization of pregnancy-specific protein B (PSPB) from Iraqi dromedary camels

Talal A. Abdulkareem1 Yassen T. Abdul-Rahaman2
Hani M. Al-Rawi2 Sajeda M. Eidan1 Chuck W. Passavant3
R. G. Sasser3

1 Department of Animal Production,
College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
2 College of Veterinary Medicine, University of Fallujah, Iraq.
3 BioTracking LLC, Moscow, ID, USA.

drtalalabdulkareem2013@gmail.com
talal.al@coagri.uobaghdad.edu.iq

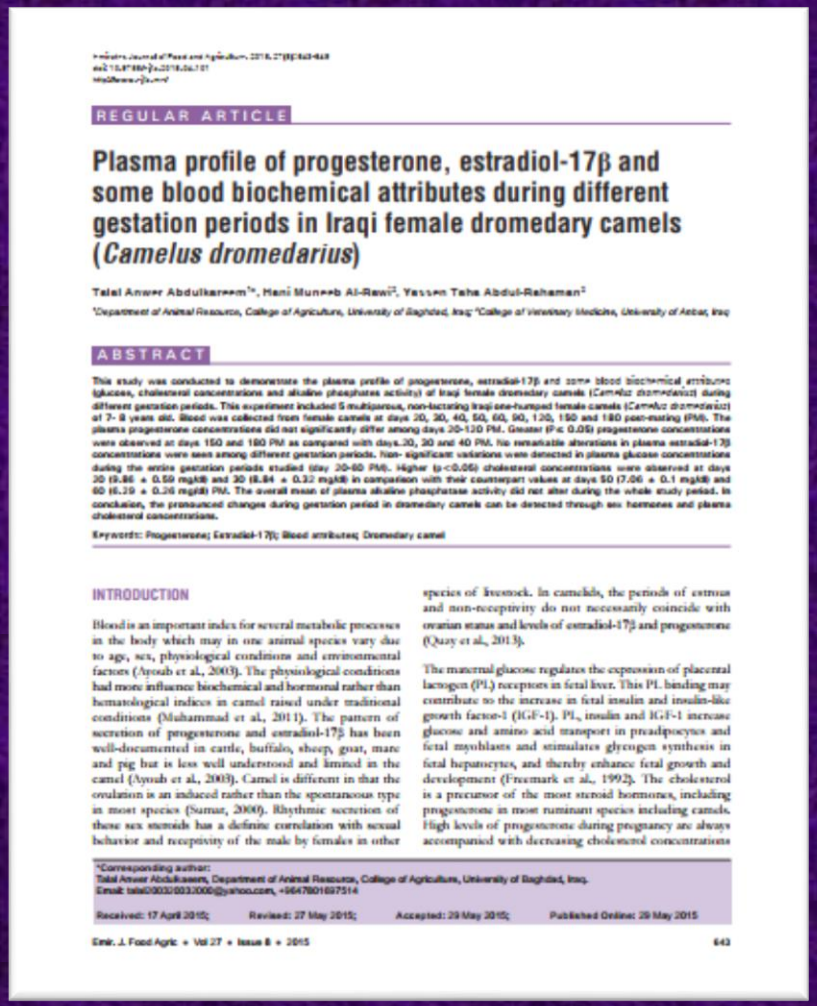
Importance of early pregnancy detection in camel

- Pregnancy detection is an important part of reproductive management of ruminants including a camel.

- Accurate and early detection of pregnant and non-pregnant female camel has become essential for monitoring their fertility due to difficult returning to service on account of less obvious external signs of estrus.

Methods of pregnancy detection in camel

- **Physical changes,** 1) tail cocking, 2) body weight increasing, 3) dark yellow colored urine, 4) urine pH becomes 3.10, 5) specific gravity of 1.038-1.086 and 6) vaginal folds become prominent.
- **Clinical methods:** rectal palpation by 60 days post-mating and ultrasonography.
- **Chemical methods:** Cuboni and barium chloride tests
- **Biological (or immunological) methods,** including 1) gonadotrophins, 2) progesterone and 3) pregnancy-specific protein B (PSPB) assays



REGULAR ARTICLE

Plasma profile of progesterone, estradiol-17β and some blood biochemical attributes during different gestation periods in Iraqi female dromedary camels (*Camelus dromedarius*)

Talal Anwer Abdulkareem[1]*, Hani Muneeb Al-Rawi[2], Yassen Taha Abdul-Rahaman[2]

[1]Department of Animal Resource, College of Agriculture, University of Baghdad, Iraq; [2]College of Veterinary Medicine, University of Anbar, Iraq

ABSTRACT

This study was conducted to demonstrate the plasma profile of progesterone, estradiol-17β and some blood biochemical attributes (glucose, cholesterol concentrations and alkaline phosphatase activity) of Iraqi female dromedary camels (*Camelus dromedarius*) during different gestation periods. This experiment included 5 multiparous, non-lactating Iraqi one-humped female camels (*Camelus dromedarius*) of 7- 8 years old. Blood was collected from female camels at days 20, 30, 40, 50, 60, 90, 120, 150 and 180 post-mating (PM). The plasma progesterone concentrations did not significantly differ among days 20-120 PM. Greater ($P< 0.05$) progesterone concentrations were observed at days 150 and 180 PM as compared with days 20, 30 and 40 PM. No remarkable alterations in plasma estradiol-17β concentrations were seen among different gestation periods. Non- significant variations were detected in plasma glucose concentrations during the entire gestation periods studied (day 20-60 PM). Higher ($p<0.05$) cholesterol concentrations were observed at days 20 (9.46 ± 0.59 mg/dl) and 30 (8.84 ± 0.32 mg/dl) in comparison with their counterpart values at days 50 (7.06 ± 0.1 mg/dl) and 60 (6.29 ± 0.26 mg/dl) PM. The overall mean of plasma alkaline phosphatase activity did not alter during the whole study period. In conclusion, the pronounced changes during gestation period in dromedary camels can be detected through sex hormones and plasma cholesterol concentrations.

Keywords: Progesterone; Estradiol-17β; Blood attributes; Dromedary camel

INTRODUCTION

Blood is an important index for several metabolic processes in the body which may in one animal species vary due to age, sex, physiological conditions and environmental factors (Ayoub et al., 2003). The physiological conditions had more influence biochemical and hormonal rather than hematological indices in camel raised under traditional conditions (Muhammad et al., 2011). The pattern of secretion of progesterone and estradiol-17β has been well-documented in cattle, buffalo, sheep, goat, mare and pig but is less well understood and limited in the camel (Ayoub et al., 2003). Camel is different in that the ovulation is an induced rather than the spontaneous type in most species (Sumar, 2000). Rhythmic secretion of these sex steroids has a definite correlation with sexual behavior and receptivity of the male by females in other species of livestock. In camelids, the periods of estrus and non-receptivity do not necessarily coincide with ovarian status and levels of estradiol-17β and progesterone (Quay et al., 2013).

The maternal glucose regulates the expression of placental lactogen (PL) receptors in fetal liver. This PL binding may contribute to the increase in fetal insulin and insulin-like growth factor-I (IGF-I). PL, insulin and IGF-1 increase glucose and amino acid transport in preadipocytes and fetal myoblasts and stimulates glycogen synthesis in fetal hepatocytes, and thereby enhance fetal growth and development (Freemark et al., 1992). The cholesterol is a precursor of the most steroid hormones, including progesterone in most ruminant species including camels. High levels of progesterone during pregnancy are always accompanied with decreasing cholesterol concentrations

*Corresponding author:
Talal Anwer Abdulkareem, Department of Animal Resource, College of Agriculture, University of Baghdad, Iraq.
Email: talal0003@yahoo.com, +9647801697514

Received: 17 April 2015; Revised: 27 May 2015; Accepted: 29 May 2015; Published Online: 29 May 2015

Abdulkareem, T.A., Abdulrahaman, Y.T., Al-Rawi, H.M.A. 2015. Plasma profile of progesterone, estradiol-17β and some blood biochemical attributes during different gestation periods in Iraqi female dromedary camels (*Camelus dromedarius*). Emir. J. Food Agric., 27: 643-649.

Pregnancy-specific Protein B (PSPB)

- Pregnancy-specific protein B (PSPB) is a protein that was first isolated from bovine extra-embryonic membranes (Butler et al., 1982) and was found in the serum of pregnant cattle (Sasser et al., 1986).

- The gene for PSPB was cloned, and PSPB is known as the aspartic acid proteinase family of proteins (Xie et al., 1991 and Lynch et al., 1992).

- The PSPB, by immunohistochemical methods, was found in the binucleate cells of the ruminant trophectoderm

Pregnancy-specific Protein B (PSPB)

- PSPB molecules were also successfully purified and characterized from sheep, goats, elk and moose, American bison, water buffalo and fallow deer.

- PSPB has never been isolated and characterized in camelid family.

However, immunohistochemical study have revealed specific and cellular PAG localization in alpaca (Majewska *et al.*, 2011).

Reproduction in Domestic Animals

Reprod Dom Anim 46, 455–462 (2011); doi: 10.1111/j.1439-0531.2010.01693.x
ISSN 0936-6768

Early Pregnancy Detection of Iraqi Riverine Buffalo (*Bubalus bubalis*) Using the BioPRYN Enzyme-Linked Immunosorbent Assay for PSPB and the Progesterone Assay

TA Abdulkareem[1], SAM Al-Sharifi[2], MA Ishak[3], SM Eidan[1], MA Alnimr[3], CW Passavant[4], JR Branen[4] and RG Sasser[4]

[1]*Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad;* [2]*Former Minister of Agriculture, Baghdad, Iraq*
[3]*Department of Animal Production, Faculty of Agriculture, University of Jordan;* [4]*BioTracking LLC, Moscow, ID, USA*

Contents

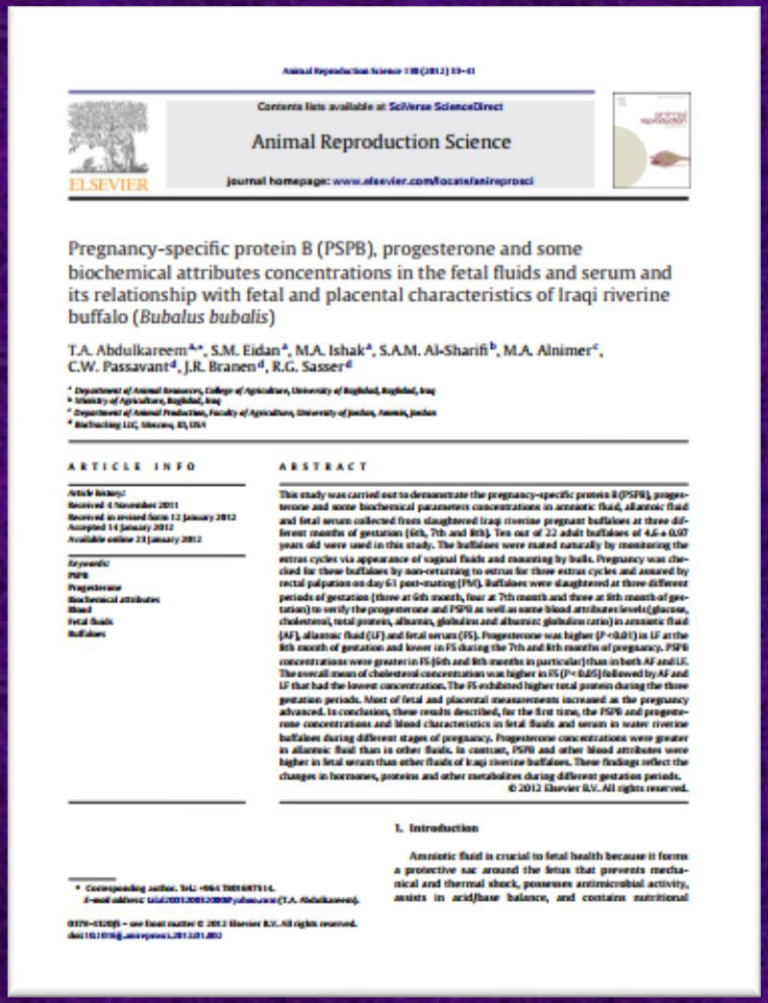
This study was undertaken to detect pregnancy in Iraqi riverine buffalo (*Bubalus bubalis*) using three different methods (rectal palpation, plasma progesterone concentration and detection of the presence of pregnancy-specific protein B (PSPB) with the BioPRYN® enzyme-linked immunosorbent assay (ELISA) test. The aim of the study was to identify the most sensitive, early and accurate method for detecting pregnancy. Twenty-two female riverine buffalo that were 6.0 ± 0.93 years old were used. Four blood samples per buffalo were taken via jugular venipuncture at days 22–24, 32–34, 42–44 and 58–61 post-mating (PM) to measure the progesterone concentration (ng/ml) and to detect the presence of plasma PSPB. The rectal palpation method was employed to evaluate all buffalo on days 42–44 and 58–61 PM. The BioPRYN® test differed ($p < 0.01$) from the other tests with earlier accuracy for detecting pregnant and non-pregnant buffalo. Eighty-eight percent of pregnant and 76.9% of non-pregnant buffalo were distinguished early (days 22–24 PM) using BioPRYN® and plasma PSPB-ELISA level (2.09 ± 0.12 ng/ml) in relation to 66.7% and 53.9% detected using the progesterone assay at similar days (4.30 ± 0.40 ng/ml). In conclusion, these results described, for the first time, the early and accurate pregnancy detection of water riverine buffalo using BioPRYN® technology and provided the plasma levels of PSPB using an ELISA test. These findings will improve the reproductive and productive efficiency of Iraqi riverine buffalo by adapting the recent management and reproductive strategies in Iraq and in the world.

Introduction

Pregnancy detection is an important part of reproductive management of ruminants including buffalo. Accurate and early detection of pregnant and non-pregnant buffalo cows has become essential for monitoring the fertility of buffalo. Early pregnancy detection is also one of the key methods for decreasing of calving intervals for dairy cattle (Gábor et al. 2007). Late pregnancy detection insures loss of an additional 18–24 days if the buffalo cow is not pregnant and no oestrous synchronization is applied (Karen et al. 2007). It was shown that the earlier the pregnancy detection was performed, the more profitable it was to owners (Oltenacu et al. 1990). Romano (2004) reported that the daily cost for a cow that persisted open past day 100 (after calving) was between $2.50 and because of poor oestrous expression in buffalo (Pawshe et al. 1994). Measurement of progesterone concentration in blood or milk by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) at days 20–24 post-mating (PM) has been used to specify non-pregnant buffalo cows with an accuracy of 80–90%. However, the accuracy of the test for selecting pregnant buffalo cows was low and ranged from 57.1% to 75% (Singh and Pathiyandy 1980; Perera et al. 1981; Gupta and Prakash 1990; Ghoneim et al. 1994 and Kaul and Prakash 1994). Rectal palpation is a simple method for pregnancy detection; however, this method is only accurate from days 45 PM onwards in buffalo (Arthur et al. 1996). In addition, palpation of fluctuation within the uterus, identification of the amniotic vesicle and slipping of the chorioallantoic membranes may increase the incidence of foetal mortality in dairy cattle (2–12%; Franco et al. 1987).

Placental aspartic acid proteinases were first discovered by Butler et al. (1982) when they reported the finding of pregnancy-specific protein B (PSPB). The PSPB, by immunohistochemical methods, was found in the binucleated cells of the ruminant trophectoderm (Eckblad et al. 1985; Sasser and Ruder 1987; and Zoli et al. 1992a). A RIA was developed for the measurement of PSPB and was used to find the protein in the circulation of cattle (Sasser et al. 1986). This was the first establishment of a protein marker for pregnancy in cattle. Plasma PSPB/pregnancy-associated glycoprotein (PAG) measurements have been used to characterize the embryonic mortality in cattle (Humblot et al. 1988a; Gábor et al. 2007; Lopez-Gatius et al. 2007 and Eidan 2008). Bovine PSPB was characterized as a glycoprotein showing relative molecular mass (Mr) between 47 and 53 kDa and presenting different isoelectric points (pI) from 4.0 to 4.4 (Sasser et al. 1989). The pI were closely related to the amounts of sialic acid of each isoform. Nine years after the work of Butler et al. (1982), Zoli et al. (1991), using the methods of Butler et al. 1982 and with testing of isolates by Sasser's laboratory, isolated the same protein and gave it the name PAG designated as PAG-1-67 because its Mr was 67 kDa. Four isoforms (pI: 4.4, 4.6, 5.2 and 5.4) were detected in the initial

Abdulkareem, T.A.; Al-Sharifi, S.A.M.; Ishak, M.A.; Eidan, S.M.; Alnimr, M.A.; Passavant, C.W.; Branen, J.R. And Sasser, R.G 2011. Early pregnancy diagnosis of Iraqi riverine buffaloes using BioPRYN enzyme-linked immunosorbent assay for PSPB and progesterone assay. Reprod. Dom. Anim., 46: 455 – 462.



Animal Reproduction Science 130 (2012) 33–41

Contents lists available at SciVerse ScienceDirect

Animal Reproduction Science

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

Pregnancy-specific protein B (PSPB), progesterone and some biochemical attributes concentrations in the fetal fluids and serum and its relationship with fetal and placental characteristics of Iraqi riverine buffalo (*Bubalus bubalis*)

T.A. Abdulkareem[a,*], S.M. Eidan[a], M.A. Ishak[a], S.A.M. Al-Sharifi[b], M.A. Alnimer[c], C.W. Passavant[d], J.R. Branen[d], R.G. Sasser[d]

[a] Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad, Iraq
[b] Ministry of Agriculture, Baghdad, Iraq
[c] Department of Animal Production, Faculty of Agriculture, University of Jordan, Amman, Jordan
[d] BioTracking LLC, Moscow, ID, USA

ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 12 January 2012
Accepted 14 January 2012
Available online 23 January 2012

Keywords:
PSPB
Progesterone
Biochemical attributes
Blood
Fetal fluids
Buffaloes

ABSTRACT

This study was carried out to demonstrate the pregnancy-specific protein B (PSPB), progesterone and some biochemical parameters concentrations in amniotic fluid, allantoic fluid and fetal serum collected from slaughtered Iraqi riverine pregnant buffaloes at three different months of gestation (6th, 7th and 8th). Ten out of 22 adult buffaloes of 4.6 ± 0.07 years old were used in this study. The buffaloes were mated naturally by monitoring the estrus cycles via appearance of vaginal fluids and mounting by bulls. Pregnancy was checked for these buffaloes by non-returning to estrus for three estrus cycles and assured by rectal palpation on day 61 post-mating (PM). Buffaloes were slaughtered at three different periods of gestation (three at 6th month, four at 7th month and three at 8th month of gestation) to verify the progesterone and PSPB as well as some blood attributes levels (glucose, cholesterol, total proteins, albumin, globulins and albumin/globulin ratio) in amniotic fluid (AF), allantoic fluid (LF) and fetal serum (FS). Progesterone was higher ($P < 0.01$) in LF at the 8th month of gestation and lower in FS during the 7th and 8th months of pregnancy. PSPB concentrations were greater in FS (6th and 8th months in particular) than in both AF and LF. The overall mean of cholesterol concentration was higher in FS ($P < 0.05$) followed by AF and LF that had the lowest concentration. The FS exhibited higher total protein during the three gestation periods. Most of fetal and placental measurements increased as the pregnancy advanced. In conclusion, these results described, for the first time, the PSPB and progesterone concentrations and blood characteristics in fetal fluids and serum in water riverine buffaloes during different stages of pregnancy. Progesterone concentrations were greater in allantoic fluid than in other fluids. In contrast, PSPB and other blood attributes were higher in fetal serum than other fluids of Iraqi riverine buffaloes. These findings reflect the changes in hormones, proteins and other metabolites during different gestation periods.

1. Introduction

Amniotic fluid is crucial to fetal health because it forms a protective sac around the fetus that prevents mechanical and thermal shock, possesses antimicrobial activity, assists in acid/base balance, and contains nutritional

* Corresponding author. Tel.: +964 7801687514.
E-mail address: tala2001200@yahoo.com (T.A. Abdulkareem).

0378-4320/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2012.01.002

Abdulkareem, T.A.; Eidan, S.M.; Ishak, M.A.; Al-Sharifi, S.A.M.; Alnimer, M.A.; Passavant, C.W.; Branen, J.R., Sasser, R.G 2012. Pregnancy- specific protein B (PSPB), progesterone and some biochemical attributes concentrations in the fetal fluids and serum and its relationship with fetal and placental characteristics of Iraqi riverine buffalo (Bubalus bubalis) Anim. Reprod. Sci., 130: 33- 41.

- **Three** adult Iraqi female camels of used in this study. Females were naturally mated with the fertile male. Pregnancy checked for these females using rectal palpation at days 60 and 90 PM.

- **Caesarean section** performed by a specialist veterinarian surgeon to each female at months 5.5, 6 and 7.5 of gestation period.

Photo 1.The experimental Iraqi dromedary camels

* The separated placenta was washed by phosphate buffer saline (PBS) of pH 7.4 and their total weight measured using precise balance.

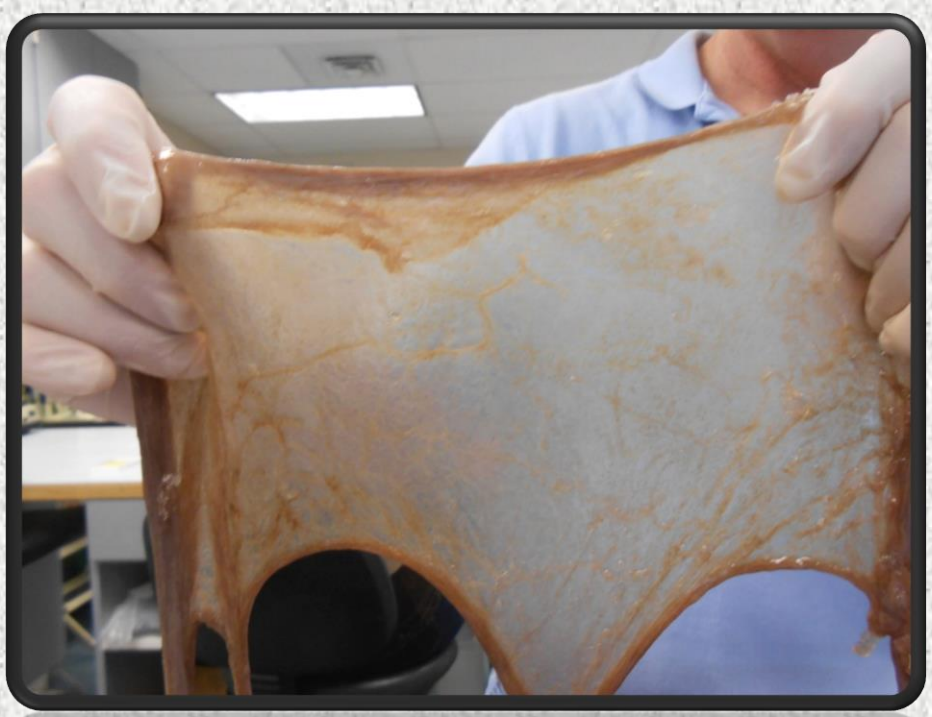
Isolation, purification and partial characterization procedures carried out at the laboratories of Biotracking LLC, Idaho, USA, in collaboration of Dr. C. W. Passavant, and his team.

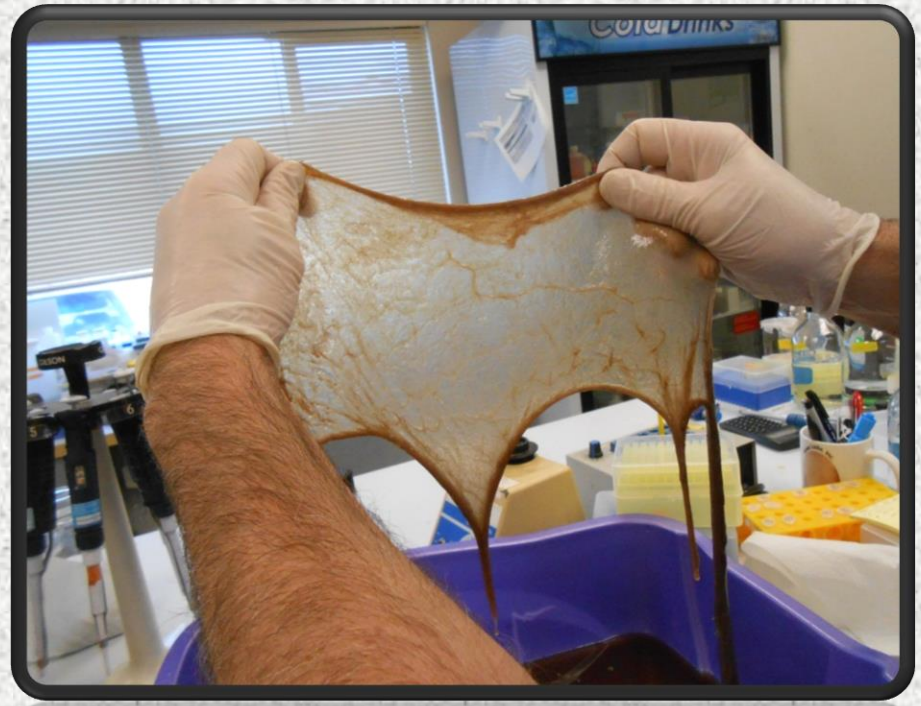
Before transporting to Biotracking, samples were gamma irradiated at Foreign Animal Disease Diagnostic Laboratory (FADDL) that belong to Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA) for samples sterilization.

Weighting the placenta

Washing the placenta with PBS

Beginning of isolation and purification processes at BioTracking, USA





Twenty three consecutive trials were carried out to isolate, purify and partially characterize camel PSPB

Evaluation of several α-bovine antibodies for potential use in detecting camelid PSPB

- A series of polyclonal α-bovine antibodies to PSPB were serially diluted from 1:10K to 1:80K for use as coating antibodies.

- Rabbit anti-bovine PSPB (MIX-2) and rabbit anti-sheep and goat (48-16 and 42-4) were also coated at these dilutions for use as controls. Pregnant cow serum was used to test for their response relative to the MIX-2, 48-16 and 42-4 antibodies.

Isolation and purification of camelid PSPB- tissue processing.

- Microtiter wells were coated with bovine **MIX-2**, and **48-16,** ovine **42-2,** chicken-IgY **4970-10th** and sheep α-moose PSPB (wildlife) to follow the protein during purification process with other **anti-PSPB antibodies** .

- All plates were treated following the standard protocol using **monoclonal antibodies** from one set of mice cloned screened **(MAb-45)** or **biotin-B6** as secondary antibodies.

Results

- **MIX-2/ MAb-45 ELISA can be used to follow camelid PSPB (cPSPB) throughout the purification procedure, and the current anti-bovine antibodies can be used to successfully monitor the location of camelid PSPB during the purification steps.**

- **The camel PSPB had isoelectric points ranged from 4.1 to 5.4 and molecular mass of 15 kDa, 25 kDa, 50 kDa, 55 kDa and 65kDa. The major band was at 55 kDa and 65 kDa.**

Results

- This study describes for the first time the partially production of polyclonal antisera raised against PSPB molecules isolated from female camels placenta. .

- Proteins issued from two different fractions (DEAE 45-70 mM NaCl, Sephadex G75 and DEAE 70-90 mM NaCl) were used to immunize rabbits for the production of antibodies.

Take Home Message

- Isolation, purification and partial characterization of **PSPB** from camel placenta were done for the first time .

- Production of antibodies against **cPSPB** for sensitive and specific **RIA** or **ELISA** assay in female camelid family **is warranted**

- Early pregnancy detection of dromedary female camels by quantification of **plasma PSPB** concentrations **is also warranted.**


Thank you for your attention