USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

DETERMINATION OF PREGNANCY WASTAGE AND ITS FINANCIAL IMPLICATION IN COWS SLAUGHTERED AT THE SOKOTO MODERN ABATTOIR USING PREGNANCY SPECIFIC PROTEIN B

A DISSERTATION

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DEDICATION

This dissertation is dedicated to my aged father and maternal grandmother whom by the special grace of Allah remain alive till this moment and to my beloved mother and wife who are my sources of inspiration.

CERTIFICATION

This dissertation by ABUBAKAR, Musa Imam (Adm. No: 16/210617001) has met the requirements for the award of the degree of Master of Science (Animal Production) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

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LIST OF ABBREVIATIONS

CBN – Central Bank of Nigeria

ECF – Early Conception Factor

ELISA – Enzyme Linked Immunosorbent Assay

FAO – Food and Agriculture Organization

GDP – Gross Domestic Product

ICM – Inner Cell Mass

OD – Optical Density

PAG - Pregnancy Associated Glycoprotein

PGF2α – ProstaglandinF2α

PSP 60 – Pregnancy Serum Protein 60

PSPA- Pregnancy Specific Protein A

PSPB - Pregnancy Specific Protein B

RIA – Radioimmunoassay

RPM – Revolution Per Minute

SMA - Sokoto Modern Abattoir

TMB – Tetramethybenzidine

ABSTRACT

Pregnancy wastage due to slaughter of pregnant animals is a common practice in Nigeria. This study was design to determine pregnancy wastage and its financial loss in cows slaughtered at the Sokoto Modern Abattoir using Pregnancy Specific Protein B (PSPB). Cows presented for slaughter at the Sokoto Modern Abattoir were selected using convenient sampling method, by collecting 5mls of blood into non-heparinized tube for PSPB assay. The uteri of sampled animals were examined post slaughter for the presence or absence of foetus. Recovered foetuses were aged using crown-rump length to estimate the stage of pregnancy. In addition, the financial loss from the slaughter of pregnant cow was also determined. Out of the 361 cows examined, 72 (19.9%) were diagnosed pregnant using PSPB. This was higher than the 32 (8.9%) diagnosed by post slaughter inspection method. In addition PSPB was more sensitive (93.8%) than post slaughter inspection (41.7%). Most of the wastages 22 (68.8%) recorded using the post slaughter inspection methods were in the second trimester. The estimated financial loss associated with pregnancy wastage using PSPB was ₦4,156,333. This is higher than the ₦1,847,664 obtained using post slaughter inspection method. It was concluded that PSPB test used in this study revealed a higher incidence of pregnancy wastage and subsequent higher financial loss compared to the post slaughter inspection. PSPB also proved to be more sensitive in detecting pregnancy of cows at slaughter than post slaughter inspection. It was therefore concluded that PSPB test should be included in the routine ante mortem screening for pregnant cows.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The indiscriminate slaughter of pregnant animals is a regular phenomenon in many Nigerian abattoirs, leading to wastage of food animals. It cut across all domestic animals ranging from small ruminants (Muhammad *et al.,* 2007; Addass *et al.*, 2010; Bokko, 2011), large ruminants (Abdulkadir *et al.*, 2008; Alhaji, 2011), camels (Bello *et al.,* 2008) to pigs (Amuta *et al.,* 2018). This practice has been reported in many part of the country like Maiduguri (Chaudhari and Bokko, 2000; Bokko, 2011), Sokoto (Garba *et al.*, 1992), Kaduna (Ngbede *et al.*, 2012), Makurdi (Odeh *et al.*, 2015), Lafia (Hassan *et al.,* 2016), Niger (Alhaji *et al.*, 2017), Ibadan (Ogunbodede and Oladele, 2016) Abeokuta (Raimi *et al.*, 2017), Ebonyi (Nwakpu and Osakwe, 2007) and Akwa-Ibom (Akpabio and Babalola, 2014). This problem has also been reported in some African countries like Ghana (Atawalna *et al.,* 2013), Ethiopia (Simenew *et al.,* 2011), and Zambia (Zulu *et al.,* 2013). In cattle, the prevalence rate of 1.5% (Ibironke, 2010) to 10.24% (Odeh *et al.*, 2015) have been reported, 23.99% in camels (Bello *et al.*, 2008), 9.0% in pigs (Amuta *et al.*, 2018), 22.78% in sheep and 17.88% in goats (Bokko, 2011). The mean average of the Nigeria's cattle population was put at 13.9 million as at 1990, 90% of which are concentrated in the northern region of the country (Blench, 1999).

Pregnancy-Specific Protein B (PSPB) also known as Pregnancy Associated Glycoprotein (PAG) and Pregnancy Serum Protein 60 (PSP-60) (Sousa *et al.*, 2008) were initially described as placental antigens found in maternal circulation after

implantation (Sousa *et al*., 2006). PSPB was first discovered in ruminants although they are also produced in other mammalian species (Sousa *et al*., 2008). These proteins are synthesized in the mono- and binucleate cells of the ruminant's trophectoderm (Sousa *et al*., 2006) and released into circulation where their concentrations are used to determine the status of pregnancy and some obstetric diseases (Karen *et al*., 2003a; Adeyeye *et al.*, 2016). Their concentrations are particularly important to clinicians and researchers studying the pathophysiology of pregnancy in ruminants (Breukelman *et al*., 2005). PSPB reflect the trophoblastic secretory functions of the developing foetus (Sousa *et al*., 1999).

1.2 Research Problem

The high rate of pregnancy wastage is unfavourable to cattle population which leads to a decrease in the overall animal protein availability for human health below the recognized global average of 41.9kg/person/year by the Food and Agriculture Organization (FAO, 2013). In addition, the practice depletes the national herd size and thereby affecting the national food security and the Gross Domestic Product (GDP) of the country. This has been attributed to the financial losses associated with pregnancy wastage in Nigeria, estimated to be about $1,757,926.10 (₦615,274,107) annually in cattle (Alhaji *et al.*, 2017).

1.3 Justification

Studies on bovine foetal wastage have been based on post slaughter inspection of bovine uterus. This involves gross recovery of foetus after slaughter which is capable of detecting pregnancy wastage at the second and third trimesters of pregnancy. Another method used in detecting pregnancy wastage is the flushing of the uterus immediately after slaughter but this is also bound to have limitation in detecting pregnancy wastage

accurately. Determination of PSPB in cows at slaughter will aid in detection of early pregnancy and subsequent isolation of pregnant cows, thereby ensuring that the pregnancy is not wasted thus enhancing the productivity of the cattle industry. In addition, information gathered from the study will provide a better picture of the scale of pregnancy wastage in cattle slaughtered at the Sokoto modern abattoir and its estimated financial loss.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study is to determine the level of pregnancy wastage and its financial implication in cows slaughtered at the Sokoto Modern Abattoir.

1.4.2 Objectives

The specific objectives of the study are to:

1) determine pregnancy wastage in cows slaughtered at the Sokoto modern abattoir using PSPB and post slaughter inspection method
2) compare the number of pregnancy wastage obtained using PSPB and post slaughter inspection method
3) determine the stage of pregnancy at which the wastage occurs and its financial implication

1.5 Research Questions

1) What is the level of pregnancy wastage in cows slaughtered at Sokoto modern abattoir using the PSPB and post slaughter inspection method?

2) Is there any difference between the use of PSPB and post slaughter inspection method in determining the level of pregnancy wastage in cows slaughtered at the Sokoto modern abattoir?

3) What is the estimated financial loss incurred due to pregnancy wastage in cows slaughtered at the Sokoto modern abattoir?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Significance of Cattle

Cattle production play a vital role in Nigerian agricultural system and it accounts for more than 50% of the country's meat supply (RIM, 1992). It provides draught power for land cultivation and transportation, convert resources (such as crop residue) that otherwise would have been wasted into valuable animal products (meat and milk) as well as providing manure (Ahamefule *et al*., 2007). They also serve as a source of income through local sale and export of animal products and bye-products (such as meat, milk, skin, hide, fat, bone, horn, hooves, blood and rumen contents), thus contributing greatly to the foreign exchange earnings of the country. Livestock in developing countries provides the teeming populace with better nutrition, income, employment opportunities, draft power, and well balanced agricultural system (Kubkomawa, 2017).

The economic, social and cultural importance of cattle have been well established, the cattle industry provides a means of livelihood for the pastoralist and those involved in the livestock value chain (Okunmadewa, 1999). It creates employment and serves as a source of income for the dealers/ sellers of live animals, the butchers, transporters, and all those involved in the processing and marketing of the products and bye-products of the animal (Lawal-Adebowale, 2012). Cattle being a major source and mostly consumed animal protein in Nigeria, it contribute significantly to the national food supply and family nutrition, which makes the animal a highly valued livestock in Nigeria where they are principally kept for meat, hide, milk and traction (Tukur and Maigandi, 1999; Swanepoel *et al.*, 2010; Tibi and Aphunu, 2010).

In Nigeria, Cattle production contributes about 12.7% to the agricultural Gross Domestic Product (GDP) and about 4% to the overall GDP (CBN, 1999; Mbanasor, 2000; Ifeanyi and Olayode, 2008) and it also function as a means of foreign exchange earner through export of products and bye-products of cattle (Payne and Wilson, 1999). Apart from their important role as a source of food, they raise the social status of the owners, enhance gender equality through ownership by women and children and play a major role in some cultural and religious functions where they are used as gift or as bride price in traditional rites (Payne and Wilson, 1999; Tibi and Aphunu, 2010; Waters-Bayer and Letty, 2010). It also function as a mobile bank, insurance against crop failure, and as a means of wealth and asset accumulation by the resource poor households thereby serving as the first step out of poverty by rural dwellers (Payne and Wilson, 1999; Kubkomawa, 2017).

Cattle are widely used as draught animals in Nigeria and in different part of the world where small holder farmers cannot afford costly mechanized farm power. They are used for cultivation, transportation, water lifting, and powering food processing equipment where they have improved crop production significantly (Jahake, 1992; Payne and Wilson, 1999; Kubkomawa *et al*., 2011; Babayemi *et al*., 2014). When incorporated into farming, they improve soil fertility by providing manure, reduce cost of weed control and utilizes crop residues as feed that otherwise would have been wasted (Ahamefule *et al*., 2007).

The bye-products of cattle represent 66% of its live weight and serve as a good source of raw materials for many industries and constitute about 11.4% of the gross income realized from cattle (Liu, 2009). Globally, cattle are principally utilized for meat, although other bye-products such as skin, hide, fat, bone, horn, hooves, blood and

rumen contents are used as raw materials by medical, pharmaceutical, leather, cosmetics and feed industries (Ghandi, 2009, Liu, 2009; Babayemi *et al*., 2014).

2.2 Breed and Distribution of Cattle in Nigeria

Indigenous African cattle can be broadly divided into four groups; humpless *Bos taurus*, humped *Bos indicus*, Sanga (African humpless *Bos Taurus* x humped *Bos indicus*) and Zenga (Sanga x Zebu (*Bos indicus*) back cross) (Mwai *et al*., 2015). Majority of the cattle breeds found in Nigeria belong to the Zebu (*Bos indicus*) breeds which are the dominating breed of cattle types in Africa and the Nigerian breeds include; White Fulani (Bunaji), Red Bororo (Rahaji), Sokoto Gudali, Adamawa Gudali, Azaouak and Wadara. Other breeds indigenous to Nigeria that belong to the *Bos taurus* include; Keteku, N'dama, and Kuri (Lawal-Adebowale, 2012; Mwai *et al*., 2015).

2.2.1 White Fulani (Bunaji)

The White Fulani is the most numerous cattle breed found in Nigeria and also has a wide distribution in several West Africa countries (Tawah and Rege, 1996). The Bunaji is characterized by a white coloured coat on a black skin, black ears, eyes, muzzle, hooves, horn tips, tip of the tail and a medium horn (Blench, 1999; AGTR, 2011a). In Nigeria, the white Fulani represents about 32.7% of Nigerian National herd (RIM, 1992) and can be found anywhere between Lagos and Sokoto, Katsina and Kano states spreading across the middle belt. They were observed to be significantly absent in Borno, where the Rahaji and Wadara predominate (Blench, 1999). They are used for meat, milk and draught, although they are mainly kept for milk by traditional pastoralist (AGTR, 2011a)

2.2.2 Sokoto Gudali

Gudali is a Hausa word for "Short-horned and Short-legged animals", (AGTR, 2011b). In West Central Africa they are referred to as Fulbe of Peuhl Zebu (AGTR, 2011b). The Sokoto gudali has a uniform cream, light grey or dun colour, the dewlap and skin folds are highly developed and horns almost absent (Blench, 1999). They are stereotypically found in North-western Nigeria, although it is now distributed widely throughout the country (Blench, 1999). It represents 31.6% of the National cattle herd (RIM, 1992). They are known for their meat and milk and also have a good reputation of high quality beef (AGTR, 2011b).

2.2.3 Adamawa Gudali

The Adamawa Gudali looks like the Bunaji in conformation, but characterized by a medium to large body size with a medium-length horns, they are usually blotched or with a white, black, red or brown coat. It has thick crescent-shaped horns, a short head and muzzle and a characteristics pendulous hump that distinguishes it from Bunaji. It represents about 2% of the National herd (RIM, 1992) and are mostly restricted to the Adamawa region of Nigeria as the name implied (Blench, 1999)

2.2.4 Red Bororo (Rahaji)

The Rahaji is characterized by its deep burgundy-coloured coat, pendulous ear and long, thick horns (Blench, 1999). It constitutes about 22% of the national cattle herd making it the 3rd most numerous cattle in Nigeria (RIM, 1992). The red bororo is adapted to arid and semi-arid regions and are rarely found beyond Kaduna in the wet season, except for some isolated population on the Mambila plateau in the North East (Blench, 1999)

2.2.5 Wadara

The Wadara constitutes about 6.6% of the Nigerian cattle herd. They are medium-sized and are usually dark red, black, blotched, or brown in colour. They are characterized by having short horn and a small erect hump. Wadara cattle are widely distributed around Borno and are sometimes referred to as "our cattle" by the Koyam and related pastoralists. Wadara are frequently referred to as Shuwa, which derived its name from the Shuwa Arabs that also herd them (Blench, 1999).

2.2.6 Azaouak

The Azaouak is lightly built with medium-length horns and are reported to be native to the Azawak-valley, in North East Nigeria with distribution along the North West border. They are commonly described as red in Niger, although those found in Nigeria are principally light fawn colour and can also appear white, brown, blotched, and black. It represents an estimate of about 0.7% of the Nigerian National cattle herd (Blench, 1999)

2.2.7 Kuri

The Kuri (*Bos Taurus longiforms*) belong to the Bos Taurus group, it is a humpless longhorn cow and has distinctive, inflated, spongy horns unknown in any other breeds of African cattle with a mean height of 1.5 meters and a weight of about 550kg (Blench, 1999; AGTR, 2011c). They are vastly white in colour with pigmented mucous membranes, other variant with red, red pied, light, grey or dun dark or black spots reddish brown, greyish red or speckles markings can also be seen (AGTR, 2011c). They are excellent swimmers and are adapted to hot and humid climate that characterized their original habitat. They are particularly found within the region of former Lake Chad and along its eastern shores (Blench, 1999; AGTR, 2011c). In Nigeria, Kuri are not restricted to the Lake Chad area but can also be found on its shores and along the Yobe

valley, as far west as Gashagar (Blench, 1999). The Kuris has a high potential for milk and are mainly used for milk production and to a little extent serve the purpose of meat production (Tawah *et al.,* 1997).

2.2.8 N'dama

The N'dama breed is characterised by a medium-sized compact body with lyre-shaped black tipped horns and no hump (Blench, 1999). The male possess a small dewlap with a fairly large head. N'dama breed are dual purpose animals, used for both milk and meat production. They are indigenous to Senegambia and adjacent parts in the west of West Africa (Starkey, 1984; Blench, 1999). They were first brought to Nigeria in 1939 for experiment to improve resistance of local herd to trypanosomiasis through cross breeding (Blench, 1999).

2.2.9 Muturu

Also referred to as the West African dwarf shorthorn or the Nigerian shorthorn, the muturu is a small-bodied cattle that is blocky in conformation with short, fine-boned limbs. It has a compact body, humpless, with a straight back and a broad head, a slightly dished face and very short horns. Muturu breeds of cattle found in South Central Nigeria are generally black, fawn, black and white, black with white patches, or white with brown or black spots of varying degrees, likewise those on Jos Plateau are also black and white though are distinctively larger than the lowland cattle. In the Northern part of Nigeria, muturu has lighter shades with varying colour of brown, red and tawny (Blench, 1999; Adebambo, 2001). The Muturus are rarely milked because their milk production is hardly sufficient for their calves. They are however milked for medicinal purposes by the Koma people of the old Gongola State (Adebambo, 2001).

2.2.10 Keteku

Keteku are the crosses of Muturu x Zebu (Borgu Keteku) or Muturu x N'dama (Lagos Keteku) (Blench, 1999). The Borgu Keteku also referred to as Kataku, Katari, Borgawa and Kaiama. It is a trypanotolerant stabilized crosses of Muturu x Zebu (Gates, 1952), which combines the features of Muturu and Bunaji with white, grey and black types predominantly and more occasionally red and brown. They are larger and taller in the North than they are in the South and are characterised with a longer horn than Muturu and smaller hump and shorter legs than Bunaji. In Nigeria, Keteku in Herds are restricted to a narrow band along the Benin republic border in the regions usually known as Borgu (Blench, 1999).

2.3 Reproduction/ Pregnancy in Cattle

Successful pregnancy is dependent on a series of carefully planned events in the reproduction cycle (Simintiras and Forde, 2017). Following fertilization of the female gamete (oocyte) by the male gamete (spermatozoa), a zygote is formed which is a single celled embryo and that commence the embryonic period. The embryonic period is defined as the portion of gestation from conception to the end of differentiation stage, approximately 42 days of the gestation period (Committee on Bovine Reproductive Nomenclature, 1972) The zygote undergoes series of cell division and the first division occur in the oviduct to give rise to embryonic cells called blastomeres (Senger, 2003). Due to the peristaltic contractions and ciliary current in the uterine tube, the embryo is propelled and arrive the uterus around 3 - 4 days. It undergoes further division between days 4 – 7 to form a mass of cell called morula (16 – 32 cell stage), the morula is transformed into a hollow ball of cells called blastocyst (Noakes *et al.*, 2001; Schatten and Constantinescu, 2007). Around 9 – 11 days, the blastocyst hatches from the zona pellucida and form a hatched blastocyst which undergo massive growth, elongation and

attaches to the uterus around day 12 in cows (Noakes *et al.,* 2001; Des Coteaux *et al.,* 2010). At this stage the blastocyst produce a specific protein called interferons which serve as a signal for the maternal recognition of pregnancy in cow, these interferons act on the endometrial cells of the uterus to inhibit the production of oxytocin receptor, by so doing, the production of PGF2α through oxytocin will be prevented (Northey and French, 1980). This will result in the low concentration of PGF2α and thus prevent luteolysis of the corpus luteum and thereby maintain pregnancy. The blastocyst produces maximum amounts of interferons between day 16 and 19 of gestation and continues secretion until day 38 of gestation (Godkin *et al.,* 1980; Northey and French, 1980; Schatten and Constantinescu, 2007). At the blastocyst stage, the embryo consist of an Inner cell mass (ICM) and outer sphere of cells and the trophoblast surrounding a fluid filled cavity (Blastocoelic cavity) (Schatten and Constantinescu, 2007). The trophobalst cells component of the blastocyst contribute exclusively to the development of extra embryonic membrane while the inner cell mass would develop into the embryo/foetus and it also contributes to the components of yolk sac, amnion, and allantois and this stage the embryo begins to form extra embryonic membranes (Schatten and Constantinescu, 2007).

The mesoderm begins to develop between the primitive endoderm and the embryo, which in most species arise from ingression of embryonic ectoderm through the primitive streak of the embryonic disc (Schatten and Constantinescu, 2007). The mesoderm continues to grow, pushes against the trophectoderm and fuse with it, this mesoderm/trophoblast combination form the chorion or somatopleure. The chorion form amnionic folds by an upward fold, these folds continue to grow upward around the embryo and form the amnion. The amnion compartment is filled with fluid to protect the embryo by mechanical shocks. The amnion cavity is rectally palpable between days

30 and 45 as a turgid balloon in the uterus. The mesodermal tissue together with the yolk sac endoderm soon develops a blood supply, thus establishing the first efficient maternal foetal exchange system called the yolk sac placenta (Schatten and Constantinescu, 2007).

The allantois is formed later on during the development, is a diverticulum arising from the primitive gut which contains liquid waste from the embryo (Senger, 2003). During pregnancy the allantois becomes filled with fluid and grows as a projection into the exocoelom and presses against the chorion to from a new membrane called the allantochorion. The allantochorion will provide the surface for attachment to the endometrium. Up until day 35 of gestation, the ends of the allantochorion membrane degenerate leading to shorting of the allantois (Des Côteaux *et al.*, 2010). The placenta consists of a foetal component derived from the chorion and a maternal component from the uterine endometrium. Attachments of the embryo to the uterine endometrium provide the embryo adequate nutrition and protection during development.

Ruminants have a cotyledonary placenta. A placentome is a placental unit of trophoblastic origin consisting of blood vessels and connective tissue (Senger, 2003). The placentome consist of a foetal cotyledon and a maternal caruncle originating from the caruncular region of the uterus. The placentomes form specific zones for metabolic exchange. At about 25 days of pregnancy in cattle, the chorion initiates attachment to the caruncles of the uterus. During this formation, villi from the chorion protrude and attaches into crypts in the caruncle, finishing around day 40. The border between the chorion and the endometrium consist of the chorion and the endometrial epithelium with partial erosions filled with binucleate giant cells. The binucleate giant cells are formed around 18 – 20 days and originate from trophoblast cells. The cells migrate from the chorion epithelium to the endometrial epithelium. Around 20 % of the placenta consists

of the binucleate cells (Senger, 2003). The cells help the conceptus to exchange molecules from the foetal to the maternal placenta. The binucleate giant cells secrete pregnancy specific protein B and are important in producing progesterone and estrogens (Senger, 2003).

2.3.1 Foetal development

The first somites from the embryo are formed around day 19 of gestation. At this time, the embryo is 3 - 4 mm long, the neural tube close and the first heartbeats can be detected. On day 22, the optic vesicles, hepatic primordium and the mesonephros are formed (Des Côteaux *et al.,* 2010). The limbs originate at day 25 – 26 of gestation, at day 26 the embryo curves and its 7 – 8 mm long. Up to day 50 it grows by an average of 1.1 mm/day (Pierson and Ginther, 1984). Around day 35, folds around the eyes are visible, the neck is clearly developed and digits are recognizable on all four limbs. On day 40 of gestation, the genital tubercle is located on the medium line between the posterior limbs. At day 45, the foetus loses its rudimentary embryonic shape and the face, neck, limbs and tail elongate, rudimentary eyelids and ear pinnae are seen. At day 40 of gestation, the jejunum and ileum can be differentiated but the differentiation of the large intestines comes later starting at day 130 of gestation. The mesonephros and the liver fill almost the entire abdomen during the first few months of gestation. Around day 60 of gestation, the stomach divided into the 4 compartments. At this stage of gestation the omasum is the largest part of the stomach, after 6 months the abomasum starts growing faster and will be the largest part at birth. The mesonephros atrophies around day 70 of gestation and approximately at day 90 the kidneys possess the ‘normal’ lobular shape (Des Côteaux *et al.,* 2010). The bones start to ossify somewhere between day 50 and 100.

In the third month of gestation, hair follicles near the eyes and lips are formed. In the fourth month the claws are cornified and there is dental development. In the fifth month testicles descent and in the sixth month hair is formed at the eyelashes, ears and the end of the tail. In the seventh month hair is formed all over the body and the eyelids are open. In the eight month a full coat of short hair is formed and in the ninth month a hair tuft arises at the end of the tail (Des Côteaux *et al.,* 2010).

2.4 Pregnancy Diagnosis in Cattle

Pregnancy diagnosis (Cyesiognosis) is the act of accurately identifying or detecting pregnant animal from non-pregnant animal. An ideal pregnancy test should be highly sensitive (i.e. correctly identify pregnant animals), highly specific (i.e. correctly identify non-pregnant animals), inexpensive, simple to conduct and should have the ability to determine pregnancy status as at when used (Fricke *et al.,* 2016).

The methods of pregnancy diagnosis have been classified into 2; (Direct and Indirect) methods of pregnancy diagnosis.

2.4.1 Direct methods of pregnancy diagnosis

The direct methods of pregnancy diagnosis involve direct detection of tissues and/or pregnancy associated fluids manually or with the aid of electronic instruments (Fricke *et al.,* 2016). Currently, the methods being used for the direct detection of pregnancy are transrectal or per-rectal palpation and ultrasonography.

2.4.1.1 Transrectal or Per-Rectal palpation

Transrectal or per-rectal palpation of the uterus was first described in 1800s by Cowie (1948) making it the oldest and most widely used method for early diagnosis of

pregnancy in large dairy animals (Balhara *et al.,* 2013; Fricke *et al.,* 2016). The growth of the conceptus in any of the uterine horns leads to changes in the size, tenseness, location and morphology of the uterine horn, which form the basis of pregnancy diagnosis. During early pregnancy the uterus lies in the pelvic cavity in heifers and just ahead of the pelvic brim in pleuriparous large sized cows. As it grows in size, its growth is forward so it starts descending into the abdominal cavity (approx. 3½ - 4 months). At approximately 4½ - 5 months it reaches the abdominal floor and at this time only cervix is palpable within the pelvic cavity which is also drawn forward. The growth is then forward and then again upward. The entire uterus or the foetus is therefore barely palpable during the 4 - 6½ months period and diagnosis has to be dependent on other features of pregnancy (placentomes or fremitus). After this period the foetal parts are usually palpable and clinicians find no difficulty in commenting whether the animal is pregnant or not. During early pregnancy (day 30 - 60) clinicians have to depend on finding of the foetal membrane slip or the palpation of the amniotic vesicle (Purohit, 2010).

The definite signs of pregnancy in the cow as determined by rectal palpation are; palpation of enlarged uterine horn containing the placental fluids, palpation of the amniotic vesicle, slipping of the foetal membranes, palpation or ballottement of the foetus, palpation of the placentomes, and palpation of enlarged thin walled “whirring” uterine arteries (Purohit, 2010). Transrectal palpation is the cheapest means of pregnancy diagnosis (Balhara *et al.,* 2013), it is however associated with increased chances of iatrogenic pregnancy loss (Franco *et al.,* 1987).

2.4.1.2 Ultrasonography

Ultrasound is a minimally invasive accurate and efficient technique for early pregnancy diagnosis, which minimizes the risk of iatrogenic pregnancy loss associated with rectal palpation (Balhara *et al.,* 2013). Transrectal ultrasonography as a method of pregnancy diagnosis is able to detect pregnancy as early as 28 days after insemination (Franco *et al.,* 1987) which is earlier than the 35 days obtainable in using the per-rectal palpation methods. The first visible sign of pregnancy can be detected at day 21 when the foetal heart can be visualized, giving information about the viability of the pregnancy (Curran *et al.,* 1986).

Transrectal ultrasonography has an additional benefit of giving further information on the ovarian structures, twining, foetal age, sex, viability and allow thorough examination of the reproductive health of the animal (Fricke, 2002; Sharma *et al.,* 2011). As accurate as this method is, it is limited in its reduced sensitivity (accuracy in identifying pregnant animals) when used before 30 days after insemination and its high cost (Fricke, 2002; Giordano and Fricke, 2012).

2.4.2 Indirect methods of pregnancy diagnosis

Indirect method of pregnancy detection in the early stages utilizes qualitative or quantitative measures of hormone, enzymes or pregnancy biomarkers in maternal circulation to determine the presence or absence of a viable pregnancy (Fricke *et al.,* 2016). Reliable indirect methods of pregnancy diagnosis in cows include; plasma/milk progesterone, detection of Estrone Sulphate, Early pregnancy factor and Pregnancy Associated Glycoprotein (Balhara *et al.,* 2013; Fricke *et al.,* 2016).

2.4.2.1 Progesterone test

Progesterone is produced primarily by the corpus luteum formed on the ovary following ovulation. In normal cycling cow, the corpus luteum is lysed by prostaglandin produced by the uterus if pregnancy is not achieved and the progesterone level decline (Purohit, 2010). In essence, low progesterone concentration in maternal blood at 18 – 24 days post breeding indicates that the animal is not pregnant while high concentration of progesterone gives a clue that the animal is likely pregnant (Purohit, 2010). Quantification of progesterone level in blood or milk can be achieved in the laboratory using Radioimmunoassay (RIA) or Enzyme Linked Immunosorbent Assay (ELISA) methods (Fricke *et al.,* 2016) which are available commercially. Progesterone test is the easiest proven method of detecting non pregnant animal due to its high specificity of about 98% (Liang *et al.,* 1980; Pennington *et al.,* 1985; Waldman, 1993) and a non-invasive method when testing for milk progesterone. However, the sensitivity (ability to accurately detect pregnant animal) is reported to be low around 75% due to false results that may be obtained following early embryonic death and non-return to oestrus by non-pregnant cow (Purohit, 2010). The high cost of the progesterone test kit limit its use.

2.4.2.2 Estrone Sulphate detection

Estrone sulphate (E_1S) is a conjugated steroid product of estrone, predominantly present in the bovine placentomes (Eley *et al.,* 1979). It is one of the major oestrogens in the milk of pregnant and lactating cows (Noakes *et al.,* 2001). E_1S is the main estrone present in allantoic and amniotic fluids of the foetus and maternal peripheral plasma of cows with measurable quantities detectable by day 52 and its concentrations increase from day 60 and plateau around day 150 after insemination till the end of gestation (Heap and Hamon, 1979; Robertson and King, 1979). However, reliable pregnancy detection is possible only after day 100 of gestation and therefore this test can only

detect late pregnancy (Hamon *et al.,* 1981). During gestation, the concentration increases gradually so that after day 105 it is present in the milk of all pregnant animals, whereas in non-pregnant individuals it is low or undetectable (Noakes *et al.,* 2001). The identification of estrone sulphate in the milk of a cow at 105 days of gestation, or later, is a very reliable method of pregnancy diagnosis (Hamon *et al.,* 1981). The detection of estrogens depends on the availability of suitable laboratory and availability of commercial assay kits. Laboratories evaluating concentrations of estrogens in urine or serum are usually equipped with radioimmunoassay, enzyme immuno-assay or other more precise and specific diagnostic modalities for assay of steroids in urine, serum, faeces (Bamberg *et al.,* 1984) or other body fluids (Purohit, 2010). Evaluation of steroids like estrogen from faeces is especially helpful for zoo and feral species where faeces are the most easily collected specimens (Bamberg *et al.,* 1991; Herman *et al.,* 1987). Commercial kits have been developed for pregnancy detection in mares by using on farm kits like Wee-Foal-Checker® or Equitest ES® which require urine or serum as the test material. These commercially available tests are recommended to be performed only after 120 days of gestation and specially suggested for miniature horses and donkeys in which pregnancy diagnosis by rectal palpation or ultrasonography is extremely difficult (Purohit, 2010). Concentration of estrone sulphate in the maternal peripheral circulation can be used as an indicator of placental functions and estimation of foetal numbers as higher concentration indicates presence of more than one foetus (Hirako *et al.,* 2002; Balhara *et al.,* 2013). Its use as an ideal pregnancy biomarker is however limited by the fact that its concentration in plasma and milk can be influenced by many factors such as genetic makeup, weight, parity and environment (Lobago *et al.,* 2009).

2.4.2.3 Early Pregnancy Factor

Early pregnancy factor or early conception factor is an immunosuppressive protein that appears as early as 6 to 48 hours of breeding which functions to supress the maternal immune response thereby allowing the pregnancy to progress (Shaw and Morton, 1980). It is a 10.84 kDa protein associated with pregnancy (Noakes *et al.,* 2001; Cavanagh, 1996). It was first identified in pregnant mice by Morton *et al.* (1987), later in sheep and cattle (Nancarrow *et al.,* 1981) and in large number of other domestic species using the rosette inhibition bioassay (Noakes *et al.,* 2001). With the assay, early pregnancy factor was detected in the sera of pregnant mammals within 6 to 24 hours of fertilization (Morton *et al.,* 1976) and disappeared within 24 to 48 hours after death or removal of embryo (Morton *et al.,* 1987).

Early pregnancy factor has reportedly been detected in the sera of most pregnant mammals including human (Qin and Zheng, 1987; Smart *et al.,* 1982), Mice (Morton *et al.,* 1976), Sheep (Morton *et al.,* 1979; Wilson *et al.,* 1983), Cows (Cordoba *et al.,* 2001; Gandy *et al.,* 2001; Whisnant *et al.,* 2001), Pigs (Grewal *et al.,* 1985), Mares (Ohnuma *et al.,* 1996) and some wild mammals (Cruz *et al.,* 2001).

A commercially available test kit (ECF test, Concepto Diagnostics Knoxwille.TN) has been developed and marketed in the US. Its reliability has however been reported to be poor (Adams and Jardon, 1999; Des Coteaux *et al.,* 2002). Another limiting factor is that the early pregnancy factor is not strictly pregnancy specific because it can be secreted from non-placental sources such as tumour and transformed cells (Cavanagh, 1996).

2.4.2.4 Pregnancy Associated Glycoproteins (PAGs)

Pregnancy associated glycoproteins are known to be produced by specialized trophoblastic giant cells in ruminant species such as cattle, buffalo, sheep and goats by the ruminant placenta from the time it attaches up to parturition (Humblot *et al.,* 1990; Karen *et al.,* 2003b; Wooding *et al.,* 2005; Karen *et al.,* 2007). Two pregnancy specific proteins (PSP) A and B have been isolated from bovine foetal membrane extracts (Butler *et al.,* 1982). Of these PSP-A was identified as a-fetoprotein and PSP-B was found to be specific to the placenta. Pregnancy Specific Protein B (PSPB) is the first member of the Pregnancy associated glycoprotein (PAG) family to be discovered (Butler *et al.,* 1982). Sasser *et al.* (1989) also isolated similar protein from the blood of pregnant cows, which was also described as Pregnancy Specific Protein B (PSPB). PSPB and other PAGs have been used for pregnancy detection in dairy cattle as early as 21 days following Artificial Insemination (Piechotta *et al.,* 2011) and it is recommended for routine pregnancy detection from Day 30 after AI or mating in dairy and in beef cows, and from Day 28 in heifers using the ELISA test kit for PSPB (BioPRYN®, Biotracking LLC, Moscow, ID, USA) (Sousa *et al.,* 2006). Ruminants blood based pregnancy-specific assay as first described by Sasser *et al.* (1986) was initially done on the basis of radioimmunoassay, where blood concentrations of these PAGs were measured by radioimmunoassay (Zoli *et al.,* 1992; Humblot *et al.,* 1988a; Humblot *et al.,* 1988b, Szenci *et al.,* 1998).

Recently, a "sand wich" type of Enzyme Linked Immunosorbent Assay (ELISA) was made available for the detection of PSPB concentrations in bovine sera/plasma (Sousa *et al.,* 2006). This system as described by Sousa *et al.* (2006) " consist of a mixture of monoclonal antibodies raised against semi-purified PAG molecules produced in early pregnancy (Day 24, 34 and 80) was coated in the wells. A polyclonal rabbit antiserum

raised against PAG purified from mid-pregnancy cotyledons (Day 150) was used to bind the immobilized PAG, this complex being revealed by use of an alkaline phosphatase-conjuged anti-rabbit antibody". This ELISA has been made available commercially under the trade name BioPRYN® from Biotracking LLC, Moscow, ID, USA. BioPRYN® is an acronym for "Biotracking Pregnancy Yes/No "and it's a kit developed and applied commercially to detect the presence of pregnancy specific protein B in the blood of pregnant animals (Howard *et al.,* 2007). This kit has been evaluated and found to be practical, reliable, and safe for pregnancy detection especially in the early stages of gestation (Howard *et al.,* 2007). The BioPRYN test has been designed to be specie-specific and that has given rise to the availability of several BioPRYN test such BioPRYN test for cattle, bison, buffalo, goats and sheep and $BioPRYN_{WILD}$ for wild ruminants such as elk, deer, and moose.

Serum PSPB and PAG concentrations increases progressively with pregnancy from day 30, and its concentration in maternal circulation remain less than 160 ng/ml in early and mid-gestation and it increases exponentially reaching peak value of about 1000 to 5000 ng/ml few days before calving, then decline thereafter (Sasser *et al.,* 1986; Zoli *et al.,* 1992). Concentrations decrease steadily in the postpartum period reaching undetectable levels only by day 100 postpartum (Zoli *et al.,* 1992). Though, the half-life of PSPB in maternal circulation following parturition ranges from 4.3 – 7.0 days and 2.7 – 7.0 days after induction of embryonic mortality (Semambo *et al.,* 1992; Szenci *et al.,* 2003).

In the small ruminants (ewe and goats), PAG can also be used as an indicator of pregnancy, where it can be detected at days 22 – 26 following breeding (Ranilla *et al.,* 1994; Sousa *et al.,* 1999). Though the pattern or characteristics of flow differs from that of cattle (Sousa *et al.,* 2008), its concentration increases rapidly from week 3 to 4 reaching a higher level of about (20 ng/ml) during the first month of pregnancy and

remain elevated until parturition with no pre-partum exponential increase observed (Sousa *et al.,* 2008). After parturition, the concentration of PAG decline faster (4 weeks) in ewes than in cows (Ranilla *et al.,* 1994).

The sensitivity and specificity of PSPB based on RIA is known to be 92.0% and 82.6 to 91.9% from 29 to 30 days post insemination (Szenci *et al.,* 1998) and increases as pregnancy progress. More recently simple ELISA techniques have been developed that detect the PAG molecule in the serum of cows (Breed *et al.,* 2009; Green *et al.,* 2005; Green *et al.,* 2009; Silva *et al.,* 2007). The limitations to the wide use of this test is non-availability of the protein in milk or urine (Purohit, 2010), assay variability (depending on the antibody used and specific pregnancy associated glycoprotein(s) detected), protracted half-life following pregnancy loss, parturition and effect of variables (such as milk yield) other than pregnancy factor (Whitlock and Maxwell, 2008).

2.4.2.5 Visual or management method of pregnancy diagnosis

Another method of pregnancy diagnosis in cows is the visual or management method of detecting animals not returning to oestrus. When an animal is bred and does not return to oestrus, it is assumed that the animal is pregnant. During pregnancy, the conceptus inhibits regression of the corpus luteum and thus prevents the animal from cycling (Purohit, 2010). This method is the easiest and cheapest method for detecting non-pregnant animal early after breeding (Fricke *et al.,* 2016) but its reliability depends on the efficiency and accuracy of oestrus detection (Noakes *et al.,*2001). Other limitations to the use of this method include; persistence of corpus luteum due to reasons other than pregnancy, seasonality in the breeding pattern of some species of animals, anoestrus and rare occurrence of gestational oestrus in cattle and buffaloes, difficulty in oestrus detection and silent oestrus in buffaloes, reduced intensity and duration of oestrus

expression in recent generation of dairy cow, and prolonged inter-oestrus intervals in some cows. (Dransfield *et al.*, 1998; Lucy, 2001; Sartori *et al.*, 2004; Purohit, 2010).

Apart from non-return to oestrus, other visual methods of pregnancy diagnosis in late pregnancy include increase in abdominal mass, development of the udder specially in dairy heifers (4months onwards), slight vaginal discharge (from 4-5months onward in dairy cows) and movements of the foetus visible externally (especially in fed cows on the right side of abdomen 6 months onwards). However, the accuracy of these visual diagnostic methods is always low and should be used as a supplement to other methods of pregnancy diagnosis (Purohit, 2010).

2.5 Pregnancy Wastage

Pregnancy wastage is the loss of viable pregnancy through the slaughter of pregnant animals, the unethical practice of slaughtering pregnant animals for meat has become a regular phenomenon in Nigerian abattoirs leading to the wastage of conceptus in food animals. This practice is one of the most counter-productive practices man has ever adopted (Abassa, 1995). The destruction of foetuses due to the slaughter of pregnant animals is forbidden by law in nearly all countries of the world (Economic Commission for Africa, 1988) through the animal legislations formulated to protect animals from unnecessary suffering and harm.

Population increase and relative increase in wealth has resulted in high demand for meat as a source of protein. In a bid to provide meat for consumption of the human populace, there is unethical practice of slaughtering young and pregnant female animals routinely in some Nigerian abattoirs (Taiwo *et al.*, 2006; Muhammed *et al.*, 2008; Idahor *et al.*, 2009). The increasing human population and demand for animal protein is an indication that more food animals for the purpose of meat will be needed, but the destruction of

domestic animals in the form of pregnancy wastage resulting from slaughter of pregnant domestic animals like cattle, goat and sheep is a hindrance to achieving this much needed increase and will no doubt worsen the already insufficient supply of animal protein to the populace (Ngbede *et al.,* 2012).

The net effect of the continuous pregnancy wastage will results in wastage of scarce protein available to consumers and a decrease in the livestock growth capacity of the country through loss of potential herd replacement animal wasted in pregnancy by slaughtering pregnant animals (Cadmus and Adesokan, 2010).

2.5.1 Prevalence of pregnancy wastage

Pregnancy wastage has been reported to account for about 20 - 25% of the fall in livestock production in sub-Saharan Africa (Chaudhari and Bokko, 2000). The magnitude of pregnancy losses due to the slaughter of reproductively active dams has been reported in all domestic animals slaughtered in Nigerian abattoirs. In cattle a prevalence rate of varying degrees has been reported in several abattoirs located in different part of the country, Garba *et al.* (1992) reported a rate of 9.8% in Sokoto abattoir, 3.9% (Abdulkadir *et al.,* 2008) and 10.24% (Odeh *et al.,* 2015) were reported in Makurdi, 9.15% in Ebonyi abattoir (Nwakpu and Osakwe, 2007), 8.2% in some selected abattoir in Ogun state (Fayemi *et al.,* 2008), 1.5 - 2.1% in Oko-Oba abattoir, Lagos (Ibironke, 2010), 4.6% in five municipal abattoir located in Niger State (Alhaji *et al.,* 2017). Studies on other domestic animals revealed a prevalence rate of 23.99% in camels (Bello *et al.,* 2008) slaughtered in Sokoto modern abattoir, 9.0% in pigs slaughtered in Makurdi (Amuta *et al.,* 2018), 22.78% in sheep and 17.88% in goats slaughtered in selected slaughter points and municipal abattoirs in the North Eastern Nigeria (Bokko, 2011).

Pregnancy wastages is mostly determined based on the presence or absence of foetal material after slaughter of the animals, with little or no consideration been given to the loss of conceptus in the early stages of pregnancy where little or no signs of pregnancy may be observed grossly. Due to this limitation, Hamman *et al*. (1997) suggested that retrograde flushing of embryo from the uterus immediately after slaughter to recover pre-implanted or implanted embryos would perhaps reveal the true picture of pregnancy wastages. This procedure has been performed in camels slaughtered in Sokoto abattoir by Bello *et al.* (2008) and 12 embryo at various stages of organogenesis were recovered from the 53 uteri flushed giving an incidence of 23.99% of pregnancy wastage in camels.

2.5.2 Economic implication of pregnancy wastage

The high rate of pregnancy wastage is a threat to the growth of cattle population in Nigeria. This leads to a significant loss of protein due to the growing human population and revenue loss from the livestock industry (Alhaji *et al.,* 2017). The continuous practice of slaughtering pregnant animals is uneconomical and will have a serious implications and negative impact on the socio-economic well-being of those involved in the livestock value chain through meat shortages and reduced income for the farmers (Abdulkadir *et al.,* 2008; Alhaji *et al.,* 2017) and loss of future productive herd (Atwalna *et al.,* 2013). In Nigeria, the associated economic loss from pregnancy wastage is massive and limits the active contribution of livestock to our Gross Domestic Product (GDP) of the Country, (Okorie-Kanu *et al.,* 2018).

Substantial amount of pregnancy wastage has been recorded in many abattoirs across the country, resulting in massive waste annually. Ngbede *et al.* (2012) reported annual financial loss of about ($236,590.278 - $337,986.11) associated with bovine pregnancy

wastage in an abattoir in Kaduna, North-west Nigeria. Another report by Alhaji *et al.* (2017) on bovine foetal wastage in some abattoirs in Niger state indicated that a total amount of $8,789,630.50 was estimated to be lost over a period 4 years with an annual average of $1,757,926.10.

Economic implications were most likely underestimated by these studies because estimations were based on the conventional post slaughter inspection method involving recovery of foetus after slaughter. Little or no consideration has been given to the financial loss associated with conceptus loss in early stages of pregnancy where no gross sign of pregnancy is observed.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Sokoto modern abattoir located in Sokoto, the capital of Sokoto State. Sokoto state is geographically located in the North-western geo-political zone of Nigeria between 11°30' to 13°50' East and 4° to 6°40' North covering an area of 26,648.48 km^2. There are 23 local government areas in the state and the state is bordered in the North by Niger Republic, Zamfara State to the East and Kebbi State to the South and West. Sokoto state falls within the Sudan Savannah vegetation zone which is suitable for cultivation of grains, cash crops and animal husbandry. The state is a major livestock producer and is estimated to have cattle population of 1.18 million (FDLPCS, 2002; MOCIT, 2002).

3.2 Study Design

Cows slaughtered at the Sokoto modern abattoir from September to November 2018 were selected using convenient sampling method. They were identified according to age and breed based on the methods described by Hassan and Nwannenna (2009). About 5mls of blood was collected at slaughter into non-heparinized tube, labelled appropriately and transported on ice pack to the Central Research Laboratory, Faculty of Veterinary Medicine, City Campus, Usmanu Danfodiyo University, Sokoto. Uteri of sampled animals were examined after slaughter to ascertain the presence or absence of foetus.

3.2.1 Sample size

The sample size was determined using the methods of Thrusfield (2007), as described below;

$$n = \frac{1.96^{2} Pexp(1 - Pexp)}{d^{2}}$$

Where n= sample size

P_{exp}= expected prevalence

d= desired absolute precision

Using the prevalence of 10.2% obtained by Odeh *et al.* (2015), n was calculated as 141

However, 361 cows were sampled to increase precision.

3.2.2 Stage of pregnancy determination

This was determined by estimating the gestational age of the foetus using the crown-rump length as described by Richardson (1980) below:

X = 2.5 (Y +21)

Where;

X = the developmental age in days

Y = the crown-rump length

2.5 = constant for cattle

21 = Mathematical constant

The stage of pregnancy was determined from foetal age using an average gestation length of 283 days. The sex of the foetus was determined by observing the external genitalia.

3.2.3 Assay for Pregnancy Specific Protein B (PSPB)

The blood collected from each cow was centrifuged at 5000 RPM for 5 minutes and the serum harvested was stored at -20°c until use. Pregnancy Specific Protein B was assayed using BioPRYN® ELISA test kits manufactured by BioTracking LLC, Moscow, ID, USA. The ELISA kit can detect pregnancy in cattle at or greater than 28 days post breeding and 73 days or 90 days (In the BioPRYN $_{QK}$ kit) since last calf and the assay can be completed in a minimum assay time of 6.5 hours or 2.5 hours (In the BioPRYN $_{QK}$ kit). This antigen-capture or "sand wich", ELISA detects PSPB in bovine sera/plasma. Serum PSPB binds to antibodies coated in the wells and is detected by secondary binding of a labelled antibody. Binding of the labelled antibody conjugate is detected by the addition of the Enhancer and 3, '3, 5,'5 –tetramethylbenzidine (TMB) and is quantified by the subsequent colour development. A strong colour indicates binding and substrate reactivity of the labelled antibody conjugate to the bound PSPB, and is a positive indication of pregnancy. Weak colour development indicates little or no binding of the labelled antibody conjugate due to the absence of PSPB in the sample, and is a negative indication of pregnancy. Standards of known concentrations of PSPB are run with each assay and were used to determine the optical density (OD) values for assigning Pregnant / Not Pregnant ranges.

The assay was done as described by the manufacturer briefly:

The plate grid was prepared for proper recording and identification of samples as entered on the plate, all reagents were mixed thoroughly and warmed with the plates at

room temperature. 50µl of the detector solution was added to each well of the PSPB antibody coated plate, then 100 µl of standards (Hi and Lo) provided was added to designated wells A1- DI. About 150 µl of serum samples were loaded to an uncoated transfer plate, 100 µl of which were harvested and transferred simultaneously to the coated plates using multichannel pipette. The plates were sealed with aluminium foil, covered and incubated for 1 to 2 hours at room temperature. After the incubation, contents of the plates were discarded and washed four times with distilled water and blot dried each time. 100 µl of enhancer solution was added to each well and discarded after incubation for 30±5 minutes at room temperature. The plate was washed again with distilled water and blot dried each time as before. 100 µl of TMB (3, '3, 5,'5 - tetramethylbenzidine) was added to each well, sealed with aluminium foil, mixed gently and incubated for 15 minutes at room temperature. Stop solution was added to the plate and read within 30 minutes on ELISA plate reader to determine the optical density (OD) of the sample and the standard.

The validity of the test was determined by calculating for the sensitivity and specificity of the diagnostic test. The sensitivity indicates the accuracy of the BioPRYN® test kit in detecting pregnant cows while the specificity indicates the accuracy of the test kit in detecting non-pregnant cows. It was determined using the following:

a. True positive: cow was pregnant at slaughter and tested positive for PSPB
b. False positive: cow had no foetus at slaughter but tested positive for PSPB
c. True negative: cow had no foetus at slaughter and was negative for PSPB
d. False negative: cow had foetus at slaughter but tested negative for PSPB

The sensitivity was therefore expressed as, $= \frac{a}{a+d} \times 100$

While the specificity is expressed as, $= \frac{c}{c+b} \times 100$

3.2.4 Estimation of financial loss

The financial losses associated with the wasted pregnancies were estimated using the method of Amuta *et al.* (2018) as described below;

a. Number of Pregnancy wasted
b. Expected calf mortality rate
c. Price of a mature cow = Average weight (kg) at 2 years x price per kg
d. Total production cost = Feed cost + Veterinary expenses + Labour Cost + Miscellaneous expenses
e. Financial loss = (a – b) x (c – d)

The expected calf mortality rate used was adopted from the report of Olafadehan (2015) where a rate of 5.51% was reported.

3.3 Data Management and Statistical Analysis

Data generated using PSPB to determine the level of pregnancy wastage was subjected to descriptive statistics and presented in table as appropriate. Kappa's statistics was used to measure agreement between use of Pregnancy Specific Protein B (PSPB) test kit and the post slaughter inspection method at a significant level of 5% and 95% confidence interval. The stage of pregnancy, sex of recovered foetus and associated financial loss were subjected to descriptive statistics and presented in tables as appropriate.

CHAPTER FOUR

4.0 RESULTS

4.1 Diagnosis of Pregnancy in Cows Slaughtered at the Sokoto Modern Abattoir using Pregnancy Specific Protein B and Post Slaughter Inspection Method

The cows sampled were between 2 years and 8 years old. The breeds were Bunaji, Rahaji, Bokoloji and their crosses. Of the 361 cows examined, 72 (19.9%) were diagnosed to be pregnant using Pregnancy Specific Protein B and 32 (8.9%) were diagnosed to be pregnant using post slaughter inspection as shown in Table 4.1.

Table 4.1: Diagnosis of pregnancy in cows slaughtered at the Sokoto modern abattoir using pregnancy specific protein B and post slaughter inspection method

Diagnostic method	Number pregnant cows	Prevalence (%)
PSPB	72	19.9
Post slaughter inspection	32	8.9

KEY:

PSPB: Pregnancy Specific Protein B

4.2 Comparative Analysis of Pregnancy Specific Protein B and Post Slaughter Inspection Method in Detecting Pregnancy of Cows at Slaughter

The comparative analysis of Pregnancy Specific Protein B with post slaughter inspection method in detecting pregnancy of cows at slaughter is presented in Table 4.2.

Table 4.2: Comparative analysis of pregnancy specific protein B and post slaughter inspection method in detecting pregnancy of cows at slaughter

		Post Slaughter Inspection		
		Negative	Positive	Total
PSPB	Negative	287 (79.5%)	2 (0.6%)	289 (80.1%)
	Positive	42 (11.6%)	30 (8.3%)	72 (19.9%)
Total		329 (91.1%)	32 (8.9%)	361

KEY:

PSPB: Pregnancy Specific Protein B

4.3 Sensitivity and Specificity of Pregnancy Specific Protein B and Post Slaughter Inspection Method

The sensitivity of the PSPB test is 93.8% while that of post slaughter inspection is 41.7% and the specificity of PSPB is 87.2% while that of post slaughter inspection is 99.3%. Kappa value of 0.52 was obtained as presented in Table 4.3

Table 4.3: Sensitivity and specificity of pregnancy specific protein B and post slaughter inspection method

	PSPB	Post slaughter inspection
Sensitivity	93.8%	41.7%
Specificity	87.2%	99.3%
Kappa Value		0.52

KEY:

PSPB: Pregnancy Specific Protein B

4.4 Stage of Pregnancy of Cows Slaughtered and Sex of Recovered Foetus at Sokoto Modern Abattoir

Based on post slaughter inspection method, the stage of pregnancy of cows slaughtered and the sex of recovered foetus are presented in Table 4.4. A total number of 32 pregnancies were recorded, out of this, 22 pregnancies were observed in the second trimester while 5 each in the first and third trimesters. 18 of the recovered foetus were females and 14 were males.

Table 4.4: Stage of pregnancy of cows slaughtered and sex of recovered foetus at Sokoto modern abattoir

Stage of Pregnancy	Number of Foetus	Sex of Foetus	
		Male	Female
1st Trimester	5	3	2
2nd Trimester	22	9	13
3rd Trimester	5	2	3
Total	32	14	18

4.5 Financial Loss Associated with Wasted Pregnancy Detected by Pregnancy Specific Protein B and Post Slaughter Inspection Method

The possible financial loss associated with the wasted pregnancy detected by both PSPB and post slaughter inspection for an estimated period of 2 years was calculated to be ₦4,156,633 and ₦1,847,664 respectively as shown in Table 4.5.

Table 4.5: Financial loss associated with wasted pregnancy detected by pregnancy specific protein B and post slaughter inspection method

Diagnostic Method	Number of wasted pregnancy detected (a)	Estimated Calf Mortality rate of 5.51% (b)	Average Price of a Mature Cow at 2 years (c)	Total Production Cost (d)	Estimated Financial Loss (e)
PSPB	72	3.97	₦120,000	₦58,900	₦4,156,633
Post Slaughter	32	1.76	₦120,000	₦58,900	₦1,847,664
Difference					₦2,308,969

KEY:

PSPB: Pregnancy Specific Protein B

CHAPTER FIVE

5.0 DISCUSSION

In this study, the rate of pregnancy wastage in cows detected using Pregnancy Specific Protein B was 19.9%. This is higher than the 9.8% earlier reported from the Sokoto modern abattoir (Garba *et al.,* 1992). It is also higher than 6.7% reported in Zaria (Salami *et al.,* 2010), 10.2% reported in Makurdi (Odeh *et al.,* 2015) and 4.6% reported in Niger state (Alhaji *et al.,* 2017). The high prevalence may be attributed to the difference in the method of detecting pregnancy in the slaughtered cows. The present study used PSPB which is a more sensitive method than the post slaughter inspection explored in earlier studies. PSPB is capable of detecting early pregnancy at embryonic stage, unlike the post slaughter inspection that relies on the presence or absence of foetus. This is an indication that more pregnant cows may have been slaughtered compare to what was captured by those earlier reports. This is probably why Hamman *et al.* (1997) suggested that retrograde flushing of embryo from the uterus immediately after slaughter to recover pre-implanted or implanted embryos would perhaps reveal the true picture of pregnancy wastages.

A prevalence rate of 8.9% was obtained using the post slaughter inspection method. This is lower than the 19.9% obtained using PSPB method. This further buttress the earlier point, that PSPB detects more pregnancies than post slaughter inspection. The prevalence obtained by post slaughter inspection is similar with the finding in earlier studies (Garba *et al.,* 1992; Nwakpu and Osakwe, 2007; Fayemi *et al.,* 2008). PSPB test was more sensitive in detecting pregnancy than post slaughter inspection in this study, although post slaughter inspection show more specificity than PSPB. The low sensitivity of the post slaughter inspection method compared to PSPB suggests that it is

less accurate in detecting pregnancy status of slaughtered cows which is a limitation of this method of pregnancy wastage estimation.

In this study post slaughter inspection revealed that the highest rates of pregnancy wastage occurred in the second trimester compared to the first and third trimesters. This is in agreement with the findings of Ogunbodede and Oladele (2016) in cows slaughtered at Bodija central abattoir and Raimi *et al.* (2017) in Lafenwa abattoir, Abeokuta that reported higher rates of pregnancy wastage in second and third trimesters when pregnancy are relatively easier to diagnose than those of the first trimester. Suggesting that, the main reason for slaughtering pregnant cows at this stage may be due to diseases, poverty, festivities and feed shortages. However about 42 wasted pregnancies detected by PSPB had no foetus at post slaughter, probably due to the fact that they are in their embryo stage of pregnancy when organogenesis have not commenced and therefore may be classified as wastages encountered in the first trimester. This may mean that more wastage actually occurred in the first trimester than other stages. This is in agreement with the findings of Ngbede *et al.* (2012), Odeh *et al.* (2015) and Alhaji *et al.* (2017) that reported more pregnancy wastage in the first trimester than other stages. This may be due to the fact that ante mortem examination relies majorly on visual assessment of pregnancy which may not be apparent during this early stage and hence may be passed as not pregnant and slaughtered. It may also be attributed to the lack of controlled breeding and breeding records thereby unable to separate pregnant from non-pregnant cows, leading to passage for slaughter.

A female to male sex ratio of 1.3: 1.0 was observed among the recovered foetuses in this study using the post slaughter inspection method, suggesting that more females were wasted in pregnancies than males. This is in agreement with the findings of Ogunbodede and Oladele (2016) in cows slaughtered at the Bodija Central abattoir,

Ibadan and Bello *et al.* (2008) in camel cows slaughtered at the Sokoto modern abattoir. Considering the importance of females in reproduction and their unique position as essential reproductive vessels with the fact that they outlived the males producing for a longer period (Umaru *et al.,* 2009), this would lead to decrease reproductive efficiency and propagation of livestock population (Amuta *et al.,* 2018).

The economic loss associated with wasted pregnancies in this study was estimated to be ₦ 4,156, 633 using the PSPB detection method but was ₦ 1,847,664 using the post slaughter inspection method. This gives a difference of ₦ 2,308,969 between the two methods. The differences highlighted between these two methods shows that financial loss associated with pregnancy wastage using the PSPB detection method is higher compared to the post slaughter inspection method. The financial loss obtained in this study using either the PSPB and post slaughter inspection method is lower than the reports of Alhaji *et al.* (2017) in Niger state and Ngbede *et al.* (2012) in Kaduna. The variation in the financial loss may be due to the differences in the methods used to estimate financial loss. In this study consideration was given to cost of production, calf mortality rate and other expenses associated with raising a calf till maturity, compared to Alhaji *et al.* (2017) and Ngbede *et al.* (2012) that estimated financial loss by multiplying average calf birth weight with cost per live birth weight of cattle and multiplying number of wasted foetus with average cost of an adult cattle respectively. Another reason for the variation is the duration of the study. This study lasted for 3 months while theirs involves 5 – 6 years retrospective survey. These financial losses associated with pregnancy wastage could have been an additional income to the farmers as well as an addition to the GDP of the nation at large. These wastages therefore amount to a colossal loss to the livestock industry and to the country in general, thus

reducing the quantity of available animal protein to the exponentially increasing human population.

5.1 Conclusion and Recommendations

5.1.1 Conclusion

The PSPB test used revealed a high incidence of pregnancy wastage and a subsequent high financial loss associated with pregnancy wastage, compared to the post slaughter inspection method. It also proved to be sensitive in detecting pregnancy of cows at slaughter. There is loss of future productive herds in wasted pregnancies, which represent a significant loss to the livestock industry as well as reduction to the national herd size.

5.1.2 Recommendations

It is therefore recommended that;

1) PSPB test should be included in the routine ante mortem screening for pregnant cows. PSPB detection method on abattoir will aid in detection of early pregnancy and subsequent isolation of any pregnant cow. This will minimize the error associated with visual assessment of pregnancy and reduce the risk of pregnancy loss associated with the rectal palpation method of pregnancy detection.
2) Use of PSPB test in routine pregnancy diagnosis on abattoir and on farm will aid in early and accurate detection of pregnancy and thus increase productivity of the livestock industry

3) Existing law such as the Meat Inspection Act of 1968 prohibiting the slaughter of pregnant animals for meat in Nigeria should be strengthened.
4) Training and retraining of abattoir staff on how to recognize and isolate pregnant animals before passing for slaughter.
5) Enlightenment of farmers/cattle dealers at the Sokoto modern abattoir about the cruelty and huge economic loss associated with these counter-productive practices will go a long way in reducing the menace of slaughtering pregnant cows in the abattoir.
6) The use of PSPB should be included into the routine livestock extension services in order to help farmers avoid pregnancy wastage from slaughter of pregnant cows.
7) Further investigation of the rate of pregnancy wastage should be done in other livestock species slaughtered at the abattoir using PSPB

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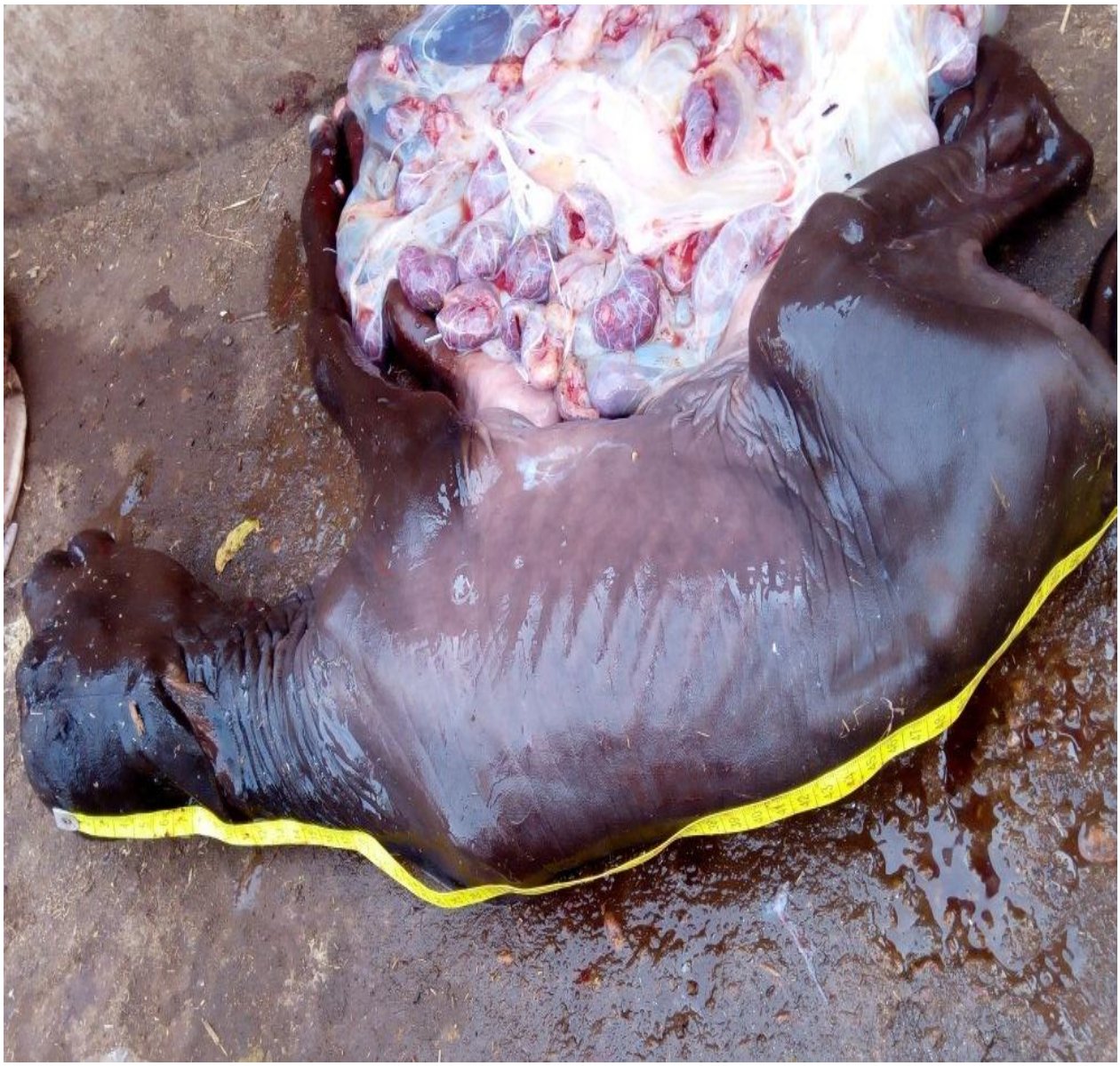
APPENDIX I

Age estimation of calf foetus in the first trimester using crown-rump length

APPENDIX II

Age estimation of calf foetus in the second trimester using crown-rump length

APPENDIX III

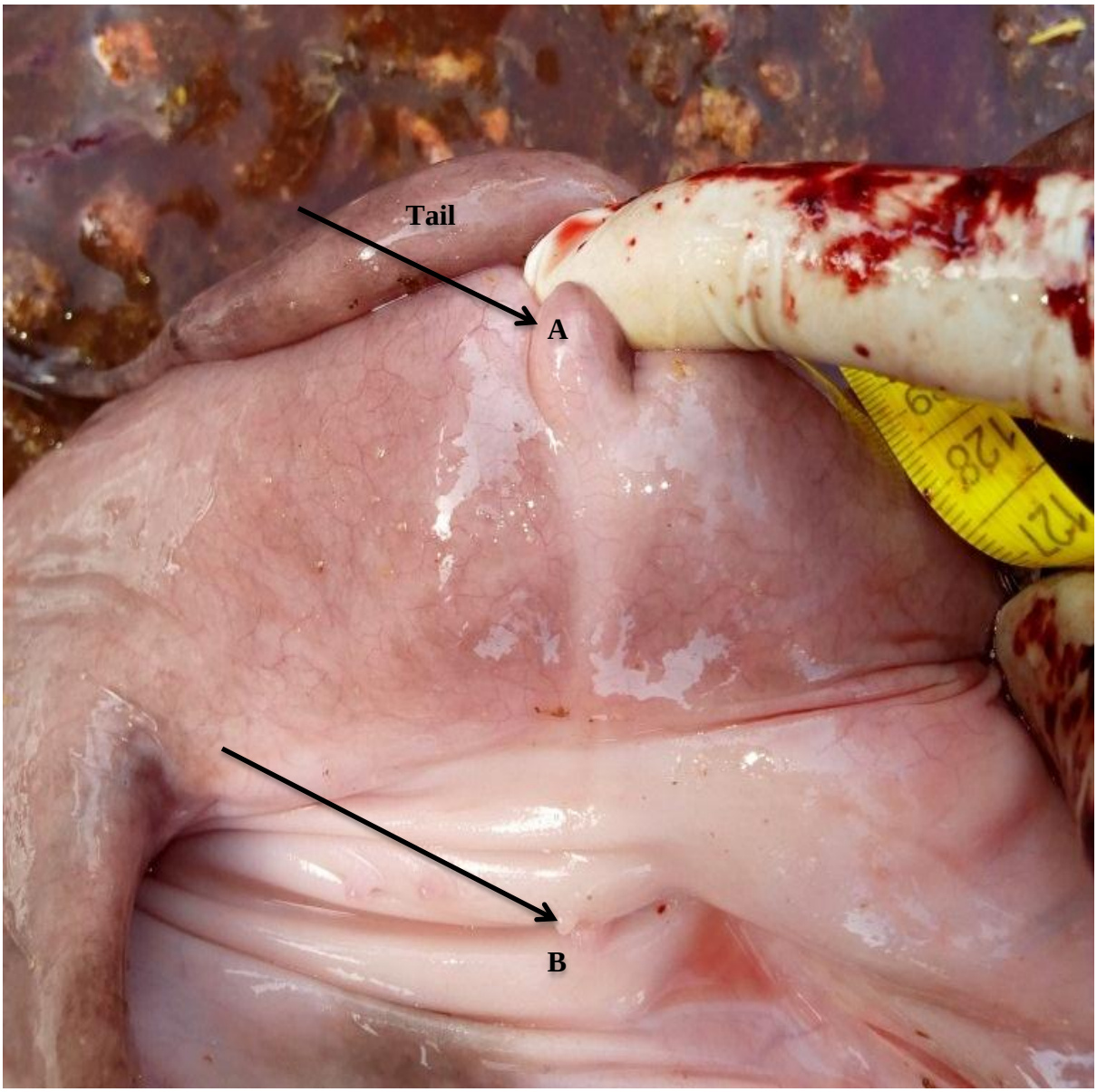


Age estimation of calf foetus in the third trimester using crown-rump length

APPENDIX IV

Male foetus in the second trimester identified by the presence testicles (arrow)

APPENDIX V



Female foetus in the second trimester identified by the presence of the vagina slit (arrow A) and teats (arrow B)