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**VALIDATION OF TRANS-RECTAL PALPATION WITH SERUM  
PROGESTERONE ASSAY FOR PREGNANCY DIAGNOSIS IN MUTURU  
COWS**

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**ABSTRACT**

*In a study to validate the use of trans-rectal palpation for pregnancy diagnosis in Muturu cows with serum progesterone assay, 9 pregnant cows out of a cattle herd of 21 animals (19 females and 2 males) were selected for the study. The pregnancy status of each animal was determined using trans-rectal palpation while serum progesterone concentration was determined using the enzyme linked immunosorbent assay method. Eleven days after, the same procedures were repeated. Pregnancy was confirmed with trans-rectal palpation using either palpation of amniotic vesicle, membrane slip, cotyledon or foetal parts, whereas a sustained high level of serum progesterone over 11 days interval confirmed presence by progesterone assay. The accuracy of the two methods was evaluated based on the calving record of the animals after 9 months. For trans-rectal palpation, there were 7 pregnant cows during the first palpation but 6 during the second palpation, whereas progesterone assay confirmed 7 cows to be pregnant with plasma progesterone ranging between 5.48 and 33.55 ng/ml and 10.51 and 28.75 ng/ml during the first and second assays respectively. About 8 months after, only 6 cows had calved. A cow confirmed to be pregnant by both techniques had embryo loss since it did not calve nine months after. The overall calving rate in the selected 9 animals for this study was 66.7% whereas it was 36.8% for the whole herd within the period of study. The percentage efficiency of using trans-rectal palpation for pregnancy diagnosis was 60.0% during the first palpation and 100% during the second palpation giving an average of 80.0%. On the other hand, percentage efficiency using the serum progesterone assay was 88.9% based on sustained high level of serum progesterone over an 11 days period. The results of the present study further validate the efficacy of trans-rectal palpation in diagnosing pregnancy in Muturu cow especially when it is repeated after an interval.*

**Keywords:** Muturu cow, pregnancy diagnosis, trans-rectal palpation, progesterone assay

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## **INTRODUCTION**

The Muturu is an indigenous breed of cattle in Nigeria that thrives well especially in the Southern part of the country [1]. The breed has the advantage of being rugged and hardy, and highly adaptable to the environment. It is also resistant to some diseases, especially trypanosomosis, which has been acknowledged as one of the major constraints to cattle productivity and militating against adequate supply of animal protein in Nigeria [2]. Going by the rate of reduction in their numbers, the extinction of the Muturu breed of cattle from Nigeria is very imminent, without proper reproductive strategy to increase its population [3].

Pregnancy diagnosis is an important aspect of livestock production. It guarantees animal multiplication. Early pregnancy diagnosis is important for evaluation of the success of a recent insemination, determination of foetal numbers for institution of required adequate nutrition, estimation of day of gestation so that preparation is made for calving and its associated eventualities such as dystocia, and the recognition of abnormalities of pregnancy for appropriate management, all of which are central to enhanced reproductive efficiency of the herd [4].

Conventional methods employed for diagnosis of pregnancy in cattle include non-return to oestrus, abdominal ballottement, development of mammary gland and trans-rectal palpation (TRP). Others are hormonal assay for progesterone ( $P_4$ ), ultrasonography and X-ray imaging [5,6]. The last three methods are more specific and confirmatory of pregnancy than the earlier methods. However, the sophistication of the equipment as well as their size may constitute limitations to their application and use in the field. Among the earlier methods mentioned, TRP is considered to be more reliable than the rest. Trans-rectal palpation has been an age-long method of pregnancy diagnosis that was first described by Zemjanis [7]. In spite of many methods of pregnancy diagnosis today, TRP is still very relevant and useful in the diagnosis of pregnancy in large animals because it is simple, requires less facilities and readily applicable in the field [8]. Trans-rectal palpation is one of the most frequently used techniques by the practicing veterinarians across the globe. The simplicity of the technique in terms of facilities required, and its higher degree of accuracy has made it a method of choice to practicing veterinarians in the field.

Progesterone ( $P_4$ ) is otherwise known as the hormone of pregnancy, because of the crucial roles it plays in the sustenance and maintenance of pregnancy. In most domestic mammals, concentrations below a certain threshold may be detrimental to embryo survival [9]. Progesterone is produced by the corpus luteum and placenta [10] and characteristically causes down-regulation of oxytocin receptors that induce the release of prostaglandin  $F_2\alpha$  for luteolysis [11]. Therefore, increasing serum progesterone levels over an 11-day period have been used as confirmatory of pregnancy in most farm animals [12].

Early pregnancy diagnosis is vital for efficient cattle reproductive management so that open cows after insemination may be served at the next oestrus, while pregnant cows are carefully monitored and catered for to enhance the sustenance of pregnancy. Previous reports have shown  $P_4$  levels at different stages of pregnancy in the cow [13,14]. Several studies have also detected pregnancy with the aid of TRP [15,16]. However, very few studies have evaluated pregnancy using TRP and validating the result with a definitive diagnostic technique such as progesterone concentration in the serum. There is also inadequate information on serum  $P_4$  concentrations in the Muturu cow either during oestrous cycle or pregnancy, despite being one of the most popular breeds of cattle in Nigeria. Therefore, this study aims to validate the use of TRP as a diagnostic tool for pregnancy diagnosis in Muturu cows with serum levels of progesterone.

## **MATERIALS AND METHODS**

### **Experimental animal and management**

Ethical approval for the study was granted by the Research Ethical Committee of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Nine

adult Muturu cows between the ages of 3 to 5 years were purposively selected out of a herd of 19 cows and 2 bulls and used for the study. The entire herd was kept under semi-intensive system of management and were properly ear-tagged for easy identification. The selection of cows into the study was based on pregnancy (those animals who were neither in luteal nor follicular phase of the oestrous cycle) according to TRP findings. The cows had been bred at different periods of time by natural mating with either of the two bulls in the herd. They were then subjected to pregnancy diagnosis using both the TRP and serum P<sub>4</sub> assay. Thereafter, the animals were monitored for 9 months. Calving record was kept and used to estimate the trimester stage of pregnancy for each of the 9 cows in the study group.

### **Trans-rectal palpation**

Trans-rectal palpation of the reproductive tract was carried out as earlier described by Leigh [17]. Briefly, the animal was properly restrained inside an improvised wooden crutch with the horns tied to the pole and a figure-eight rope restraint applied to the hind limbs. An obstetrical glove was worn on the hand to be used for palpation, followed by lubrication of the glove with gel. The fingers were made into a conical shape and the arm gently inserted into the anus after using the other un-gloved hand to raise the tail. The cervix was first located before proceeding to the uterus and uterine horn, and thereafter the ovary. The ovary was held between the middle and ring fingers while the thumb was used to feel its surface to determine presence of follicle(s), corpus haemorrhagicum or corpus luteum. The uterus was also evaluated for tone and picked to check for the presence of foetal membrane or amniotic vesicle. The correlation of the findings on the uterus and ovary as well as surrounding structures such as cotyledon, and foetal structures, were used to arrive at a definitive diagnosis of the pregnancy status of the cow [15]. Sometimes, when the results on different segments of the reproductive tract did not correspond with one another, the cow was regarded as uncertain especially when one of the four major confirmatory signs mentioned earlier could not be felt. All pregnant and uncertain cows as certified by the TRP findings were included in the study. The exercise was repeated with the selected nine cows eleven days later.

### **Hormonal assay for progesterone**

Blood samples were collected through the jugular vein of each selected cow into a plain sample tube, allowed to clot for one hour and then before centrifuged at 1100 g for 10 minutes. Serum samples were retrieved, labeled and stored at -4°C before assay. Enzyme linked immune-sorbent assay (ELISA) was used as previously described [18] with a P<sub>4</sub> commercial kit (Progesterone AccuBind ELISA Test Kit®; Monobind; Lake Forest, CA, USA). The limit of detection of the assay was between 0.3 ng/ml and 60 ng/ml. The serum P<sub>4</sub> assay was repeated for each cow after 11 days interval. Pregnancy diagnosis using this method was based on the sustenance of P<sub>4</sub> over an eleven-day period in a cow.

### **Calving rate**

At the end of the study, nine months from the time of first palpation, the number of calving within the herd was recorded. The overall calving rate of the whole herd was based on the percentage of cows that calved (7) to the total number of cows (19), while the calving rate in experimental animals for the study was calculated as the number of cows that calved in the study (6) over the total number (9) in the study.

### **Statistical analysis**

Descriptive statistics (percentage) was used to determine the efficiency of each technique. Based on calving record at nine months after the commencement of the study, the overall accuracy of the TRP and progesterone assay was calculated using the calving record of the selected animal for the study. The average percentage efficiency of TRP for pregnancy diagnosis was calculated by dividing the sum of the percentage efficiency during the first and the second palpations by two. One cow that was missed out completely from the study counted as part of error of TRP at first palpation, and its progesterone profile was not available. The percentage efficiency of progesterone assay was based on the number of correctly diagnosed cows over the total number of experimental animals in the study.

## RESULTS

### Trans-Rectal Palpation

The results of the TRP indicated that 7 animals were pregnant during the first palpation, while the pregnancy statuses of two cows were not certain, and required a second assessment. During the second palpation, six cows were also indicated pregnant while three were not. Out of the non-pregnant cows, two were in the luteal phase while one was in the follicular phase. One cow was completely missed out during the first palpation used for inclusion of only pregnant cows in the study. This cow calved 7 months later.

It is important to note that the 6 individual cows detected to be pregnant during the first and second palpation were not the same. Out of the two cows tagged uncertain during the first palpation, one was certified to be pregnant at second palpation while the other one was in the luteal phase. Unfortunately, one of the pregnant cows as per the first palpation was discovered to be open during the second palpation and in the luteal phase, since the initial confirmatory signs of pregnancy such as palpation of membrane slip were no longer present in this animal.

### Progesterone Assay

The results of serum P<sub>4</sub> assay for the nine cows are shown in Table 1. Progesterone levels in all the animals ranged from 0.86 to 36.83 ng/mL during the first assay, while a range of 0.98 to 28.75 ng/ml was observed during the second assay. The results of the two assays showed seven cows to be pregnant because of sustained high levels of progesterone during the first and the second assays. Out of the two non-pregnant cows, one cow had a P<sub>4</sub> concentration of 1.58ng/ml (luteal phase) while the concentration in the latter was 0.86 ng/ml (follicular phase) during the first assay. The former was in the follicular phase with 0.98 ng/ml as P<sub>4</sub> concentration while the other was in the luteal phase and had P<sub>4</sub> concentration of 1.51ng/ml during the second P<sub>4</sub> assay.

### Calving rate and Accuracy of TRP and Progesterone assay for pregnancy diagnosis

One cow that was not included because it was diagnosed wrongly during the first palpation calved 7 months after. The overall calving rate in the selected nine animals for this study was 66.7% whereas it was 52.6% for the whole herd within the period of study. The percentage efficiency of using TRP for pregnancy diagnosis was 60% during the first palpation and 100% during the second palpation giving an average of 80.0%, while that of serum progesterone assay was 88.9%.

**Table 1. Pregnancy detection in Muturu cow by the Trans-rectal palpation and serum Progesterone concentration assay.**

S/N	Tag No.	Trans-rectal palpation		Serum progesterone concentration (ng/ml)		Stage of gestation (Trimester)
		First	second	First	Second	
1	262	Positive	Positive	17.6	12.08*	2 <sup>nd</sup> Trimester
2	265	Uncertain	Negative	0.86	1.51	Non pregnant
3	274	Positive	Positive	8.16	13.63*	3 <sup>rd</sup> Trimester
4	275	Positive	Negative	4.42	1.98*	Embryo loss
5	277	Positive	Positive	15.59	11.23*	3 <sup>rd</sup> Trimester
6	283	Positive	Positive	36.83	25.99*	3 <sup>rd</sup> Trimester
7	291	Positive	Negative	1.58	0.98	Non pregnant
8	294	Uncertain	Positive	5.48	10.51*	2 <sup>nd</sup> Trimester
9	300	Positive	Positive	33.55	28.75*	3 <sup>rd</sup> Trimester
10	299**	Missed	ND	ND	ND	1 <sup>st</sup> Trimester

\*Certified pregnant by the hormonal assay results; \*\*Pregnant cow that was erroneously missed out from the study during the first palpation; Gestation age was based on parturition date of each cow and ND=Not determined.

### **Reconciling the TRP finding and the serum P<sub>4</sub> concentration**

One cow that was certified as pregnant by P<sub>4</sub> assay and declared non-pregnant during the second palpation was not pregnant according to the calving record after 9 months. This cow (cow 275) had plasma P<sub>4</sub> concentrations of 4.42 ng/ml and 1.98 ng/ml during the first and second assays respectively. The two cows that were tagged to be of uncertain status during the first palpation actually had 0.86 (follicular phase) and 5.48 (luteal phase) ng/ml P<sub>4</sub> concentrations, although during the second palpation, the former had transcended into luteal phase with P<sub>4</sub> concentration of 1.51 ng/ml. On the contrary, the second animal with 5.48 ng/ml that could not be detected as pregnant during the first palpation was detectable as pregnant at second palpation with plasma P<sub>4</sub> concentration now at 10.51 ng/ml.

### **DISCUSSION**

Prompt and accurate pregnancy diagnosis is essential for a productive livestock breeding. Such will allow management of pregnant animals or prompt breeding of non-pregnant animals at the next period of oestrus. From available literature, this is the first study reporting the validation of TRP for pregnancy diagnosis with the aid of P<sub>4</sub> assay in Muturu cows.

The use of progesterone assay for pregnancy diagnosis is based on the survival of the corpus luteum producing progesterone for more than 11 days in pregnant cows but not in cycling cows. Therefore, the two dates (11 days interval) of blood collection for P<sub>4</sub> assay done in this study agree with this concept such that one of the periods would have fallen within the time of follicular phase when plasma progesterone level would be low or at basal level [19]. The plasma concentration in non-pregnant cows of this study ranged between 0.85 ng/ml and 1.58 ng/ml and similar to the reports in previous studies [10]. However, Arimbawa *et al.* [20] reported a lower P<sub>4</sub> concentration of 0.52 ng/ml in non-pregnant Bali cows. The disparity may be attributed to differences in breed. Plasma P<sub>4</sub> levels in cow serum of less than 1 ng/ml indicates that the animal is in the follicular phase of the oestrus cycle while a value higher than 1 ng/ml indicates that the animal is in the luteal phase [21]. The P<sub>4</sub> in luteal phase is reduced on transition of the cycle from dioestrus to oestrus due to luteolytic action of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) produced by the endometrium [22]. During pregnancy, interferon tau, the agent of maternal recognition of pregnancy in ruminant is generated by the blastocyst to cause abrogation of the luteolytic effect of the PGF<sub>2α</sub> [23] to elongate the lifespan of corpus luteum for continued progesterone synthesis. Sequel to this is sustained level for P<sub>4</sub> beyond eleven days interval.

The uncertain cow (cow 291) during the first palpation was in the luteal phase based on the serum P<sub>4</sub> concentration of 1.58 ng/ml. The uncertainty was actually due to straining of these animals which impaired further manipulation, thus making conclusive diagnosis on their status impossible. At the second palpation, the cow was in the follicular phase with a serum P<sub>4</sub> concentration of 0.98 ng/ml which agrees with earlier reports of low and high P<sub>4</sub> levels during the follicular and luteal phases of cows respectively [24].

The serum P<sub>4</sub> concentration of pregnant cows as revealed by TRP findings in the present study are at par with previous reports of Astiti and Panjaitan [25] in Bali cows where serum concentrations ranged between  $7.43 \pm 5.27$  ng/ml and  $16.7 \pm 2.29$  ng/ml from the first to seventh months of pregnancy, respectively. The results of TRP indicated that 7 cows were pregnant during the first palpation while only six cows were detected pregnant 11 days later, during the second palpation. The pregnant cows were confirmed by the sustained high levels of serum P<sub>4</sub>. Interpretation of the TRP findings and the subsequent clinical diagnosis thereof, requires a synergy between the anatomical structures felt and the physiology of those organs. The structures found on the ovary include follicles (at different stages of development from primordial to antral), corpus haemorrhagicum, corpus luteum and corpus albicans [26]. Follicle is filled with follicular fluid [27] and so felt softer, while the corpus luteum is felt harder to touch. Non-liftable cervix, flaccid uterine body, asymmetry of the left and right horns as well as the presence of fremitus were all suggestive of pregnancy. Note that these could be observed during pathological conditions of the

uterus that may include hydrometra or mucometra. The presence of corpus luteum on the ovary ipsilateral to the bigger horn may only suggest a strong indication of pregnancy but not confirmatory. Four major findings of the TRP confirmatory of pregnancy include presence of amniotic vesicle, membrane slip, cotyledon, and foetal part [15]. The allanto-chorion constitutes the membrane slip and amniotic sac can both be picked during TRP and are responsible for the membrane slip earlier mentioned.

Membrane slip is felt by compressing the pregnant uterine horn and allowing the chorio-allantoic membrane to slip between the fingers in palpated cows [17]. The placenta in ruminant is said to be synepitheliochorial and cotyledonary [28]. The foetal cotyledon fuses with the maternal caruncle to form a placentome, a basic functional unit of the placenta. The caruncles are region of placentation. There are about 150 caruncles in the two uterine horns and could be felt since they are more palpable in pregnant cows than during oestrous cycle [17]. Foetal parts could be felt once organogenesis has occurred. Organogenesis marks the transformation of the embryo into foetus and that starts about 42 days post fertilisation in cattle. Therefore, foetal parts can be felt in TRP at about 75 days of gestation through ballottement of the uterine horn content.

The uncertainty of the pregnancy status of two cows at first palpation was due to straining by the cows and attempt to calm the cow by knocking on the sacral bones proved abortive. Henceforth, further manipulation within the tract was paused in order to forestall injury to the rectal mucosa and associated hemorrhage as recommended by Ajala and Oladipupo [29]. The diagnosis was inconclusive and hence the pregnancy status of the cows was marked uncertain.

In a similar study done in Egypt, plasma progesterone was reportedly reduced at the 4<sup>th</sup> month of gestation and remained fairly constant until the 9<sup>th</sup> month when a linear decrease was observed 3 days prior to calving [30]. The stability of the plasma P<sub>4</sub> concentration could not be ascertained in the present study since the hormonal assays were done only twice at 11 days interval and because the cows were actually at different gestation age having been mated at different times by the bull via natural service. Multiple assays were also constrained by financial limitation.

Importantly, there is dearth of information on plasma progesterone concentration of Muturu breed of cattle in the literature either during oestrous cycle or pregnancy. The P<sub>4</sub> concentration of pregnant cows obtained in this study is similar to the values from other breeds of cattle such as Bali and Norwegian red cows [25,31]. Astiti and Panjaitan [25] reported P<sub>4</sub> concentrations of  $16.7 \pm 2.29$  ng/ml at the 7<sup>th</sup> month of pregnancy. Nevertheless, further studies are required in Muturu cows to establish the findings of Eissa and El-Belely [30], where progesterone levels were reportedly stable from 4 to 9 months of pregnancy.

The gestation age in the study was done retrospectively from the days of parturition and was categorized based on the three stages of trimester. The practice and expertise of using TRP for clinical diagnosis reportedly required higher frequency of palpation on as much as 200 cows [32]. The investigator, in this study, incidentally has about ten years' experience in the teaching and practice of using TRP for pregnancy diagnosis and fertility investigation in cows. However, no attempt was made to use the TRP findings to estimate the gestation stage and expected date of delivery although the TRP had previously been used to estimate gestational length and predict expected calving date. In a previous study involving 108 dairy farms in Italy, TRP was used to correctly predict expected date of calving within a time frame of  $\pm 10$  days [16].

The cow that showed positive pregnancy with TRP during the first palpation was also confirmed to be pregnant with P<sub>4</sub> concentration of 4.42 ng/ml but failed to show pregnancy during the second palpation, and subsequently had reduction in progesterone (1.98 ng/ml) with no parturition 9 months after. This cow possibly had an embryonic loss the cause of which could not be immediately ascertained in this study and definitely not attributable to the TRP. This is consistent with previous report where low progesterone

level is indicative of embryonic loss [9]. Criticism of TRP for diagnosis of pregnancy in some quarters is based on the possibility of causing abortion especially during the early stage of pregnancy, when rupture of the CL by inexperienced examiner is detrimental to pregnancy. In this case, the loss was not considered to be due to the TRP. Previous studies have allayed this fear of pregnancy loss attributable to TRP during early pregnancy [15,33,34]. Also, TRP did not cause foetal abnormalities [35]. In a study with 800 lactating dairy cows, pregnancy diagnosis with either foetal membrane slip or amniotic sac palpation during late embryonic or early fetal period did not increase pregnancy loss, affect calving rates, or produce calves with congenital abnormalities [36]. Possible causes of embryo loss in cattle are related to genetic factors, maternal age/parity, progesterone level, nutrition and energy balance [37].

In conclusion, the results of this study have confirmed the high efficiency of using TRP for diagnosis of pregnancy in cattle relative to progesterone assay (80% vs. 88.9%). Trans rectal palpation is simple, requires minimal equipment, and its use is proposed to be taken advantage of by veterinarians, especially in poor-resource settings like Nigeria, where access to highly sophisticated equipment such as portable ultrasound machine may not be feasible. Moreover, the results of this study further strengthen the efficacy of TRP in diagnosing pregnancy in cattle particularly when a second palpation is done to verify the result of the first palpation.

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