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Research Article

Investigation of some fertility parameters: Development of pregnancy predictive models following Synchromate® administration and double fixed-timed artificial insemination in the bunaji cow

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Fertility parameters prior to insemination at 72 and 96 hours were investigated in forty Bunaji cows (≥ 1 parity, weight range: 245 kg to 350 kg), that were treated with two shots of intramuscular 500 µg Synchromate® (11 days apart) in order to develop pregnancy predictive models. Saliva Fern Pattern (SFP), External Cervical Diameter (ECD), Site of Semen Deposition (SSD), Reproductive Tract Score (RTS) and Mucous Discharge (MD) were analyzed using descriptive statistics and Multinomial Logit Regression with significance at P<0.05. Pregnancy was diagnosed *via* ELISA (DG Blue Eyes® at 30 days post insemination). Following double fixed-timed artificial insemination at 72 and 96 hours, good pregnancy outcomes were obtained with SFP (Fair/Fair, Good/Fair and Good/Good), ECD (>15 or 20 mm), SSD (MC/MC, IU/AC or IU/IU), RTS (≥ 2–4) and MD (positive or negative) while low or poor outcomes were obtained with SFP (Fair/None or None/None), ECD (<15 mm), SSD (MC/IU) and RTS of 1. Two pregnancy models were generated with equal (82.35%) predictive accuracies. These findings will reduce losses, increase precisions and improve pregnancy outcomes at cattle breeding stations.

Key words: Bunaji cow, pregnancy models, fertility parameters, synchromate, insemination.

INTRODUCTION

Conception or pregnancy in the livestock industry is subject to various fertility determinants including semen and herd fertility, oestrus detection accuracy as well as the efficiency of the inseminator. The association among these determinants is that of multiplicative alone (ABS 2001), and this portends great danger to pregnancy rates in the event that any of the determinants has a poor score. Artificial Insemination (AI) is a biotechnology tool that has been used for cattle improvement, reducing the risk of disease spread in animal husbandry. Experiences in 3rd world countries (e.g. Nigeria) with AI has however not been consistently satisfactory (Agbugba et al., 2019). This could be partly due, to status of its acceptability among stakeholders as well as availability of the technical know-how in these regions of the world. This opinion for presupposes that the statuses of other pregnancy determinants are good enough, which may not be true in all cases. In order to engender success in terms of high pregnancy rates following artificial insemination, it would be essential to conduct comprehensive investigations on pregnancy determinants as

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often as possible. The development of pregnancy predictive models using various fertility parameters will unequivocally enhance pregnancy outcomes in livestock breeding stations. There have been reports of poor/inconsistent pregnancy rates associated with prostaglandin F2 alpha protocols in cattle (Rekwot et al., 1999), (Leigh et al., 2018). These may be due to several factors including the degree of asynchrony which could have arisen from differences in pre-treatment physiological statuses as well as individual idiosyncrasies. The availability of overwhelming data on the statuses of increasing fertility parameters will not only be helpful in improving pregnancy rates but may be useful in more precise diagnosis of females to be inseminated and thereby reducing associated losses. The present study therefore investigated some fertility parameters along with pregnancy outcomes to develop predictive models for pregnancy in Synchromate®-treated Bunaji cows that were subjected to Double Fixed-Timed Artificial Insemination (DFTAI).

MATERIALS AND METHODS

Animals and management

Forty (40) Bunaji cows with parity ≥ 1 were used for the study. The weight range of the cows was 245 kg–300 kg. The animals were fed with corn silage and crushed elephant grass. They were also allowed to graze in fenced farm lands on daily basis.

Cow restraint

Restraint was achieved by using the cattle chute coupled with head restraint of each cow as was required.

Collection of samples/Investigation of parameters

All samples were collected just prior to insemination at 72 and 96 hours post synchronization.

Saliva fern pattern

Saliva samples were collected using a clean dry gloved finger placed sublingually. The saliva was smeared on a dry dirt free glass slide and air-dried at room temperature. Crystallization was thereafter observed using a light microscope under X100 and X400. The fern pattern was captured using Future Winjoe Software® and recorded as Good, Fair or None (Cortes et al., 2014). According to these authors, Good SFP was when all the fern branches have well defined venation of variable lengths while Fair SFP was when few of the fern branches have irregular venation. The None SFP was when there was no venation in the sample collected.

External cervical diameter

The external cervical diameter was estimated by placing the thumb and the index finger on its lateral borders during rectal examination. Values were recorded in millimeters (Anderson et al., 1991).

Reproductive tract score

The reproductive tract scoring was carried out *via* rectal examination as described by (Anderson et al., 1991). Using the thumb and index finger, the length of the ovary was estimated from pole to pole, the height from the hilus to the free border and the width from side to side. The size of the uterus was measured by placing the widest borders between the thumb and the index finger.

Site of semen deposition

The site where semen was deposited in each animal was recorded i.e. the most anterior possible location, between the Posterior Cervix (PC) and Intra Uterine (IU), including the Middle Cervix (MC) and the Anterior Cervix (AC).

Mucous discharge

Presence of stringy mucous discharge was monitored and recorded as Positive (scanty/sticky or copious) or Negative (no discharge seen).

Procedure for artificial insemination

The recto-vaginal method as described by (Leigh et al., 2018) was used. Briefly, the insemination gun was inserted at an angle of 30˚-45˚ with the tip directed cranio-dorsally into the vulva and vagina. The thumb and the index finger, within the rectum, were used to guide the insemination gun into the external os of the cervix. With combined, gentle, forward advancing of the gun and the manoeuvring of the cervix, penetration through cervical rings into the body of the uterus was attempted. The semen was deposited slowly at the most anterior portion that accessible. Thereafter, the gun was carefully withdrawn and the genitalium was gently massaged.

Pregnancy diagnosis

This was carried out *via* an ELISA principle, using the DG Blue eyes® kit according to manufacturer's instruction. Fresh serum (100 µl) was dispensed into pre-coated wells and incubated for 10 minutes. The content of the wells was discharged by turning the plates upside down without allowing for spillage of content between wells. Freshly prepared washing solution (100 µl) was then dispensed into each well. Washing was repeated 5 times at each instance. Three drops of Detector, Conjugate and Substrate solutions were added separately, incubated for 10 minutes each and washed as before. At the end of the assay, any well having bright blue colouration was regarded as positive for pregnancy while colourless wells were negative. Any cow for which the well was not colourless and yet not distinctly bright blue was queried for pregnancy and marked for a recheck.

Pregnancy rate

This was calculated as the percentage of females pregnant compared to total number that were synchronized and inseminated (Leigh et al., 2019).

Oestrus synchronization

The double Prostaglandin F2 alpha (PGF) protocol was used. Animals were administered intramuscularly with 500 µg of Synchromate® (Schering-Plough Animal Health Corp, Germany) at 11 days apart.

Time of insemination

Each cow was subjected to double fixed-time artificial insemination at 72 and 96 hours after second Synchromate® injection.

Pregnancy rate

This was calculated by dividing the number of cows confirmed pregnant *via* DG Blue Eyes® ELISA kit by the number of cows inseminated.

Data analysis

Data were presented using descriptive statistics (SPSS 23). Pearson Correlation and Multinomial log it regression were computed to develop two models. Following data splitting into training (70%) and testing (30%), forecasting was done to establish the efficiencies of the models.

Ethical consideration

The study was carried out with strict adherence to the approval of the Animal Care and Use of Animals in Research Committee (ACUREC), University of Ibadan, Nigeria under UI-Acurec/19/007.

RESULTS

The results of the study are as shown in Tables 1-7, Equations 1 and 2 as well in Figures 1-3. Table 1 shows the Saliva Fern Pattern (SFP) just prior to insemination in the cows at 72 and 96 hours as well as the pregnancy outcomes. The proportions of cows with Good/Good, Good/Fair, Fair/Fair, Fair/None and None/None SFP at 72/96 hours were 10%, 35%, 25%, 25%, and 5%, respectively. The proportions of pregnant and non-pregnant cows with these SFP were 100% and 0%, 85.7% and 14.3%, 100% and 0%, 20% and 80%, and, 0% and 100%, respectively. Table 2 shows the External Cervical Diameter (ECD) just prior to insemination in the cows at 72 and 96 hours as well as the pregnancy outcomes. The proportions of cows with >20 mm, >15<20 mm and <15 mm ECD at 72 and 96 hours were 55%, 35%, and 10%, respectively. The proportions of pregnant and non-pregnant cows with these ECD were 72.7% and 27.3%, 71.4% and 28.6%, and, 50% and 50%, respectively. Table 3 shows the Site of Semen Deposition (SSD) in the cows at 72 and 96 hours as well as the pregnancy outcomes. The proportions of cows with IU/IU, IU/MC, MC/MC, IU/AC and MC/IU at 72 and 96 hours were 55%, 10%, 25%, 5%, and 5%, respectively. The proportions of pregnant and non-pregnant cows with these SSD were 81.8% and 18.2%, 50% and 50%, 60% and 40%, 100% and 0%, and, 0% and 100%, respectively. Table 4 shows the Reproductive Tract Score (RTS) just prior to insemination in the cows at both 72 and 96 hours. The proportions of cows with 4, 3, 2, and 1 RTS were 20%, 30%, 35%, and 15%, respectively, The proportions of pregnant an non-pregnant cows with these RTS were 75% and 25%, 100% and 0%, 57.1% and 42.9%, and, 33.3% and 66.7%, respectively. Table 5 shows the statuses of Mucous Discharge (MD) just prior to insemination in the cows at both 72 and 96 hours. The proportions of cows with positive and negative MD at 72 and 96 hours were 15% and 85%, respectively. The proportions of pregnant and non-pregnant cows with these statuses of MD were 100% and 0% and, 64.7% and 35.3%, respectively. Tables 6 and 7 show the multinomial logit models estimates for predictions of pregnancy while Tables 8 and 9 show the prediction outcomes and model accuracy (82.35%) at 72 and 96 hours' inseminations, respectively. The resulting equations 1 and 2 show how the parameters relate in the model. Figures 1-3 were the characteristic appearances of Good, Fair and None SFP, respectively.

Table 1. Saliva fern pattern prior to insemination in the cows at 72 and 96 hours and pregnancy outcomes.

Saliva fern pattern at 72- and 96-hour inseminations	Proportion of cows (%)	Proportion of cows that were pregnant (%)	Proportion of cows that were non-pregnant (%)
Good/Good	10	100	0
Good/Fair	35	85.7	14.3
Fair/Fair	25	100	0
Fair/None	25	20	80
None/None	5	0	100
Total number of cows (n)	40	28	12

Table 2. External cervical diameter prior to insemination in the cows at 72 and 96 hours and pregnancy outcomes.

External Cervical Diameter at 72- and 96-hour inseminations (mm)	Proportion of cows (%)	Proportion of cows that were pregnant (%)	Proportion of cows that were non-pregnant (%)
>20	55	72.7	27.3
>15<20	35	71.4	28.6
Total number of cows (n)	40	28	12

Table 3. Site of semen deposition during 72 and 96 hour inseminations in the cows and pregnancy outcomes.

Site of semen deposition at 72- and 96-hour inseminations	Proportion of cows (%)	Proportion of cows that were pregnant (%)	Proportion of cows that were non-pregnant (%)
IU/IU	55	81.8	18.2
IU/MC	10	50	50
MC/MC	25	60	40
IU/AC	5	100	0
MC/IU	5	0	100
Total number of cows (n)	40	28	12

Table 4. Reproductive tract score of cows prior to 72 and 96 hour inseminations and pregnancy outcomes.

Reproductive tract scores at 72- and 96-hour inseminations	Proportion of cows (%)	Proportion of cows that were pregnant (%)	Proportion of cows that were non-pregnant (%)
4	20	75	25
3	30	100	0.00
2	35	57.1	42.9
1	15	33.3	66.7
Total number of cows (n)	40	28	12

Table 5. Status of mucous discharge just prior to 72 and 96 hour inseminations and pregnancy outcomes.

Status of Mucous discharge at 72- and 96-hour inseminations	Proportion of cows (%)	Proportion of cows that were pregnant (%)	Proportion of cows that were non-pregnant (%)
4	20	75	25
3	30	100	0.00
2	35	57.1	42.9
1	15	33.3	66.7
Total number of cows (n)	40	28	12

Table 6. Multinomial logit model estimates ± SEM for prediction of pregnancy in Bunaji cows at 72 hour insemination following Synchromate® treatment.

	Estimate	Std. Error	t value	Pr. (>/t/))
Intercepts	1.153723	0.596448	1.934	0.0583
ECD	-0.006121	0.010447	-0.586	0.5604
SSD	-0.007346	0.153377	-0.048	0.9620
SFP	-0.294904	0.134104	-2.199	0.00322*
RTS	0.101148	0.065500	1.544	0.1284
MD	-0.052990	0.188491	-0.281	0.7797

Note: (*) SSD- Site of semen deposition.

Table 7. Multinomial logit model estimates ± SEM for prediction of pregnancy in Bunaji cows at 96 hour insemination following Synchromate® treatment.

	Estimate	Std. Error	t value	Pr (>/t/)
Intercepts	1.641860	0.471537	3.482	0.000994***
ECD	0.002415	0.010821	0.223	0.824263
SSD	-0.064876	0.092322	-0.703	0.485253
SFP	-0.180194	0.124996	-1.442	0.155191
RTS	-0.220724	0.092959	-2.398	0.019989*
MD	-0.074097	0.173908	-0.426	0.671751

Note: (***) Intercepts, (*) RTS- Reproductive tract score

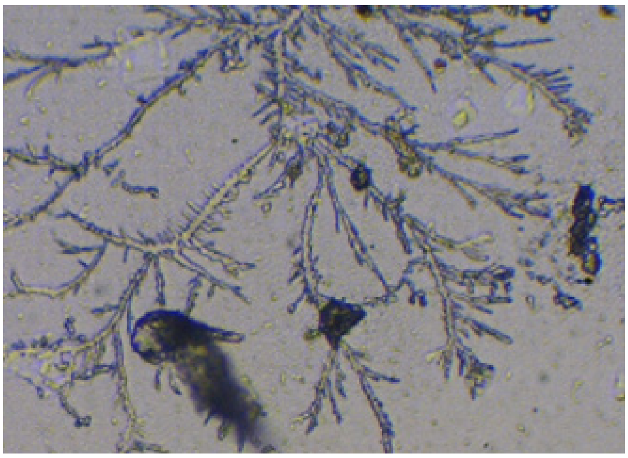

Figure 1. A characteristic Good saliva fern pattern from one of the cows (Magnification ×400).

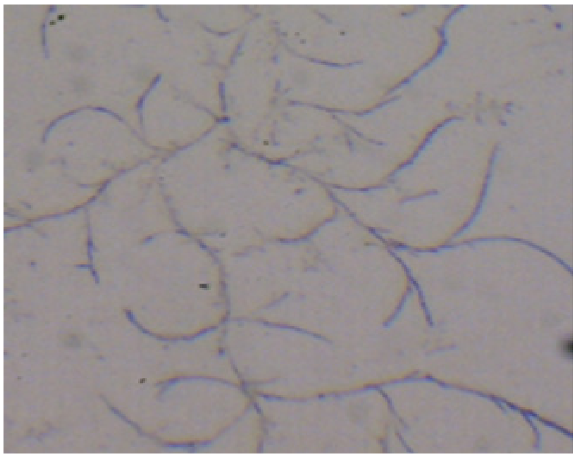

Figure 2. A characteristic Fair saliva fern pattern from one of the cows (Magnification ×400).

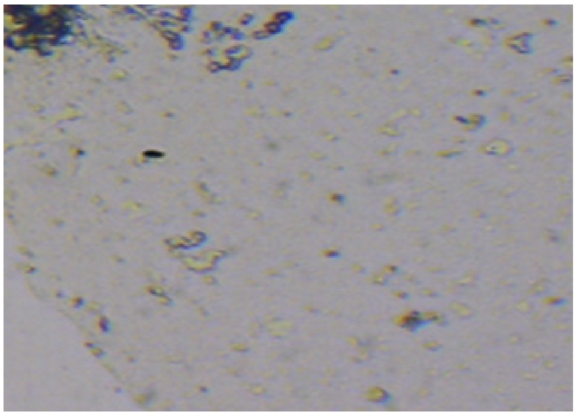

Figure 3. A characteristic none saliva fern pattern from one of the cows (Magnification ×400).

Table 8. Pregnancy Prediction outcome and model accuracy at 72 hour insemination in Synchromate®-treated Bunaji cow.

No of Test	Test data	Predicted class
1	Confirmed	Confirmed
2	Confirmed	Confirmed
3	Confirmed	Confirmed
4	Not	Confirmed
5	Not	Not
6	Confirmed	Confirmed
7	Confirmed	Confirmed
8	Confirmed	Confirmed
9	Confirmed	Confirmed
10	Confirmed	Confirmed
11	Confirmed	Confirmed
12	Not	Confirmed
13	Confirmed	Confirmed
14	Confirmed	Confirmed
15	Not	Confirmed
16	Not	Not
17	Confirmed	Confirmed

Table 9. Pregnancy Prediction outcome and model accuracy at 96 hour insemination in Synchromate®-treated Bunaji cow.

No of Test	Test data	Predicted class
1	Confirmed	Confirmed
2	Confirmed	Confirmed
3	Confirmed	Confirmed
4	Not	Not
5	Not	Not
6	Confirmed	Not
7	Confirmed	Confirmed
8	Confirmed	Confirmed
9	Confirmed	Confirmed
10	Confirmed	Confirmed
11	Confirmed	Confirmed
12	Not	Not
13	Confirmed	Confirmed
14	Confirmed	Confirmed
15	Not	Not
16	Not	Confirmed
17	Confirmed	Not

Equation 1

Equation 1 below shows that for the cows inseminated at 72-hour following Synchromate® treatment, the log odds of external cervical diameter, site of semen deposition, saliva fern pattern, and mucous discharge will decrease by 0.006121, 0.007346, 0.294904 , 0.052990, respectively while reproductive tract score will increase by 0.1011148.

In (p (outcome)=Non-pregnant)

p(outcome)=pregnant=1.153723-0.006121(ECD)-0.007346(SSD)–0.294904(SFP)+0.101148 (RTS)–0.052990 (MD).

Equation 2

Equation 2 below shows that for the cows inseminated at 96-hour following Synchromate® treatment, the log odd of site of semen deposition, saliva fern pattern, reproductive tract score and mucous discharge will decrease by 0.064876, 0.180194, 0.220724, 0.074097, respectively while external cervical diameter will increase by 0.002415.

In (p (outcome)=Non-pregnant)

p(outcome)=pregnant=1.641860+0.002415(CD)-0.064876(SSD)-0.180194(SFP)-0.220724(RTS)-0.074097(MD).

DISCUSSION

Findings with Saliva Fern Pattern (SFP) clearly suggest that a good or fair status at both 72 and 96 hours post Synchromate® treatment or, at least a good SFP at 72 hours, would strongly suggest a high fertile period and also produce good pregnancy rates in timed inseminated cows. Though, the gap in the statuses of SFP that produced good pregnancy rates in this study may be enough to regard SFP as having no significant effect on fertility/pregnancy forecasting, the very high proportions of non-pregnant cows associated with the groups with poor status (i.e. None SFP) may faintly project the importance of SFP as a factor to consider in cattle breeding. Be that as it may, a close observation of SFP of cows with good pregnancy outcomes also suggest that, all things being equal, a good or a fair SFP at 72 or 96 hours, respectively, may strongly suggest a high fertile period and a good pregnancy outcome. Much credence is given to this position by the fact that none or perhaps, a very negligible proportion of the cows in the groups with poor pregnancy outcomes had fair to Good SFP status prior to the inseminations. Though, this trend appears to be novel, it would require more investigations before much scientific claim may be attached to it. According to Holm et al. (2009), crystallization of salts in the saliva which is the origin of the fern pattern reaches a peak at oestrus. It may not be unscientific therefore to associate the good SFP with the oestrus-ovulation or highest fertility window and the Fair or Poor (i.e. None) SFP with other periods (pre or post oestrus-ovulation/highest fertility window) of the oestrous cycle where fertility of the cow is reduced. With the DFTAI used in this study, the twin inseminations occurred within 24 hours, which falls within the 20-40-hour peak fertility period in the cow (Saumande and Humblot, 2005). This apparently further suggests the relevance of SFP as a critical fertility parameter for good pregnancy outcomes. For other cows in the study, it may suffice to opine that a larger proportion of them were inseminated farther away from this fertility window, resulting in the observed poor pregnancy outcomes. The reasons for the deviation of these groups of cows, having been subjected to same synchronization protocol, may be related to the usual asynchrony associated with prostaglandin synchronization programmes in cattle (Gokhan et. al., 2010). Observations with External Cervical Diameter (ECD) apparently suggest that larger ECD favoured higher pregnancy outcomes. Though, this position requires careful interpretation as there are numerous pregnancy determinant factors in the female mammal. It is not unlikely however that the present finding is complimentary to the ease with which the inseminator was able to traverse the tract and deposit semen at the right location. This takes into cognisance the difficulties inseminators encounter with small-sized as well as the various anatomic variations in terms of shapes and curvatures of cervices. While these positions may be true, further observations with present findings especially in cows with the least ECD (<15 mm), also suggest that small ECD may not badly affect pregnancy outcomes as long as all other determinants are optimal. The most effective site for semen deposition during artificial insemination is the uterine body (Lopez-Gatius, 2000). It may not be unexpected therefore to have very good pregnancy outcomes in the groups of cows which had twin intrauterine or at least the first (i.e. 72-hour) as intrauterine insemination. In these groups though, a small proportion of the cows were not pregnant. The highest pregnancy outcome observed with IU/AC group need not be blown out of proportion. In the first instance, the site of deposition of the second semen is inferior compared with those of IU/IU or IU/MC. Therefore, the pregnancy outcome may only be accounted for by the factor of the proportion of cows in the group. The findings also show that at least, a middle cervix insemination is capable of producing pregnancy in the cow. Although, such semen deposition are associated with comparatively lower pregnancy outcomes as reflected in the groups of cows that had IU/MC or MC/MC inseminations. The observation with MC/IU insemination appears not to be consistent with other groups e.g. MC/MC. These two groups had semen deposited MC with the first insemination and the earlier had IU with second insemination but still with zero pregnancy compared to the latter which had MC at the 96-hour. While the presence of other factors that may culminate in the absence of pregnancy establishment may be involved, it appears again that observation with MC/IU may be accounted for by the factor of the proportion of cows in the group. The findings with SSD do not only appear to support the fact that IU insemination is the most effective for pregnancy establishment but also strongly suggest that where DFTAI is used as in the present study, ensuring the first insemination at 72-hour is intrauterine may be most favourable for good pregnancy outcomes. Findings with reproductive tract score (RTS) shows that cows with high scores (3-4) produced better pregnancy outcomes while those with smaller RTS produced correspondingly low outcomes. The observation in this present study is in agreement with earlier reports (Anderson et al. 1999), (Pineda et al., 2003) with claims that there were positive correlation between RTS and conception rate. Present findings therefore support the fact that routine assessment of RTS in heifers and cows, prior to breeding, will improve reproductive efficiency at breeding stations. The observation of profuse mucous discharge is a frequent occurrence during proestrus in the cow (Loeffler et al., 1999), (Mahmoud, 2009). In synchronized cows awaiting insemination, its presence assures the veterinarian that cows are responding well to treatment, giving an indication that breeding time was imminent. This may explain why present observations show optimum pregnancy outcome in the group of cows that had mucous discharge. Though, findings with the other group (negative i.e. no mucous discharge) showed above average pregnancy outcome, it strongly suggests that presence of mucous discharge may not be a critical predictor of pregnancy in cattle breeding. As have been discussed earlier, several other factors that are not related to mucous discharge may be accountable for the cows found without pregnancy.

Lay summary

Higher successful rates following natural mating or artificial insemination are the joy of every farmer, Inseminator. The reproductive parameters of forty Bunaji cows that have been given birth at least once were used for the study. They were synchronization with Synchromate® double injection at 11 days apart to makes all the forty cows to come to heat period at the same time. Their reproductive parameters (Site of semen deposition, Saliva fern pattern, Reproductive tract score, External cervical diameter, and mucous discharge) were measured and observed at 72 and 96 hours post second Synchromate® injection. Pregnancy was diagnosed *via* ELISA (DG Blue Eyes® at 30 days post insemination). The data collected at the point of insemination at 72 and 96 hours were analyzed using descriptive statistics and Multinomial Logit Regression with significance at $P<0.05$. Two pregnancy predictive models was generated one at 72 and second at 96 hours with (82.35%) predictive accuracies.

With these models one can predict the outcome of the natural mating or insemination even at the point of insemination; this will increase the accuracy of the inseminator, and aids the work of assisted reproduction techniques.

Teaser text

Two pregnancy predictive models were generated that will help, both the farmers and the Inseminators to achieve during the process of insemination, and reduce semen wastage thereby reducing the cost of reproduction.

CONCLUSION

Two (2) models: the first at 72 hours and second, at 96 hours are thus developed with 82.35% predictive accuracy using Saliva fern pattern, Reproductive tract score, External cervical diameter, Site of semen deposition and Mucous discharge, as fertility parameters in Synchromate®-treated cows that were subjected to double fixed time artificial insemination. Additionally, intrauterine semen deposition during the 72-hour insemination appears to be more favourable to good pregnancy outcomes.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

1. ABS, (2011). Artificial Insemination Management Manual. 6th ed.1: 1-240.
2. Agbugba LC, Oyewunmi AO, Ogundumade TP, Leigh OO (2020). Investigation of vaginal mucus parameters: Development of models for staging the oestrous cycle of the Bunaji cow. Reprod Dom Anim. 55: 1044– 1053.
3. Anderson KJ, LeFever DG, Brinks JS, Odde KG (1991). The use of reproductive tract scoring in beef heifers. Agri-Practice. 12: 19-26.
4. Cortes ME, Gonzalez F, VIGIL P (2014). Crystallization of Bovine Cervical Mucus at Oestrus: AnUpdate. Rev. Med. Vet. (online). 2014. 28: 103-116.
5. Gokhan D, Sanban MK, Fikrat K, Ergun Y (2010). The comparison of the pregnancy rates obtained after the ovsynch and double dose PGF2α + GnRH applications in Lactating Dairy cows. Journal of Animal and Veterinary Advances, Vol. 9: 809-813.
6. Holm DE,Thompson PN, Irons PC (2009). The value of reproductive tract scoring as a predictor of fertility and production outcomes in beef heifers. Journal of animal science. 87.
7. Leigh OO, Mustapha L, Agbugba LC, Ibiam AE. (2018) Timed artificial insemination: Pregnancy rates in sokoto gudali cattle treated with prostaglandin f2 alpha at a private dairy farm in Nigeria, Animal Production. 20: 1-6.
8. Leigh OO (2018). Handbook of bovine rectal palpation with notes on the mare for veterinary students. stirling-horden publishers ltd. ibadan, Nigeria.

9. Leigh OO, Henry MM, Linda CA. Guide to clinical and laboratory procedures in Theriogenology.

10. Loeffler SH, de Vries MJ, Schukken YH, de Zeeuw AC, Dijkhuizen AA, de Graaf FM, Brand A (1999). Use of AI technician scores for body condition, uterine tone and uterine discharge in a model with disease and milk production parameters to predict pregnancy risk at first AI in Holstein dairy cows. Theriogenology 51: 1267-1284.

11. Lopez-Gatitus F (2000). Site of semen deposition in Cattle: A review. Theriogenolgy. 15;53: 1407-1414 .

12. Mahmoud GB (2013). Physical and chemical properties of ewes cervical mucus during normal estrus and estrus induced by intravaginal sponges. Egyptian J. Anim. Prod. 50: 7-12.

13. Rekwot PI, Oyedipe EO, Mukasa-Mugerwa E, Sekoni VO, Akinpelumi OP, Anyam AA (1999). Fertility in zebu cattle (bos indicus) after prostaglandin administration and artificial insemination. The Veterinary Journal. 158: 53-58.

14. Saumande, J. and Humblot, P. (2005). The variability in the interval between estrus and ovulation in cattle and its determinants. Anim a. Reprod. Sci. 85 (3-4): 171-182.