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Comparative evaluation of some surgical approaches to recovery and transfer of caprine embryos in the red Sokoto goat (RSG)

M.A. Umaru^{a,*}, J.B. Adeyanju^b, H.S. Garba^c, E.O. Oyedipe^d, S. Buhari^b, A. Bello^e, A. Jibril^a, U. Adamu^a

^aDepartment of Theriogenology and Animal Production, ^bDepartment of Veterinary Surgery, ^cDepartment of Veterinary Medicine, ^dFormerly of the National Animal Production Research Institute, (NAPRI), Zaria, ^eDepartment of Veterinary Anatomy, Usmanu Danfodiyo University, Sokoto, Nigeria.

*Corresponding author: Department of Theriogenology and Animal Production, Usmanu Danfodiyo University, Sokoto, Nigeria.

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ABSTRACT

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In this study a series of surgical demonstrations were carried out to compare between three approaches to the recovery and transfer of caprine embryos in the Red sokoto Doe (RSD). Attempts were made to surgically demonstrate ease or difficulty to access, manipulate flush, recover, or transfer embryos from or into the genital tract. It was found that the Ventral Midline Approach (VMA) and the Para lumbar flank Approach (PFA) do not give ready access to the genitalia nor do they provide ease for manipulation for recovery and transfer as does a modification of the ventral midline approach (Modified Mid Ventral Approach; MMVA). Measurements were also taken to compare distances from skin incisions to locations on the genitalia in the three approaches. Proximity suggests ease of access. The distance of the uterine horns and the uterine tubes from the skin incisions were found to be $13.5\text{cm} \pm 3.66$, 12.5 ± 1.86 cm and 11.5 ± 3.45 cm in the Para lumbar approach, ventral midline approach and the Modified ventral Midline Approach respectively. Accessibility and ease of manipulation appears to be restricted in the paralumbar approach, it is easier with the ventral midline approach, however, accessibility and ease of manipulation was observed with the modification of the Ventral Midline Approach. The findings were discussed in the paper.

1. Introduction

Embryo recovery and transfer in small ruminants is essentially performed surgically, unlike in larger animals like the cow and the mare in which non surgical procedures are now routinely done. (Betteridge 1980, Agrawal and Battacharya, 1982) Although non surgical procedures have been reported in sheep and goats, their application is not yet widespread and is limited by the size of these animals, because they lack the convenience of a recto-vaginal approach to the manipulation of the genitalia. Embryo transfer techniques are well reported in the sheep, which serve as a low cost model for the cow. These procedures are also being applied in the goat for research to explore its potential as a dairy animal and a source of skin and cashmere wool (Mapletoft, 1987). In this report, surgical experimentations demonstrated the difficulty to access, manipulate, flush and recover embryos for transfer using the conventional Ventral Midline Approach. Accessibility problems were encountered, from the foregoing comparative measurements were taken to estimate proximity and accessibility to the genitalia in the Red Sokoto Doe (RSD) between the two commonly used techniques and a modification of one of them; these are the Para lumbar Approach (PLA), the Ventral Midline Approach (VMA), the Modified Ventral Midline Approach (MVMA). Embryo transfer in goats is rarely performed in this part of the world and is presently performed elsewhere on a commercial basis in several species of animals (Mapletoft, 1987; Betteridge, 1980; Oguri, and Tsutsumi, 1974). This study revealed that in the RSD, a lot of energy and time can be saved using the modified mid ventral approach, which is a modification of the ventral midline approach.

2. Materials and methods

Thirty six (36) Does aged 2- 3 years were used in a series of surgical experiments, to compare accessibility and convenience for the conduct of embryo recovery and transplantation. The Does were conditioned for three weeks before the trials. The does were fasted of food 24 hours before each surgery, and were prepared for surgery by shaving and scrubbing the ventral area or the Para lumbar area depending on the surgical approach to be performed. In those for modified mid ventral approach the area from the umbilicus posteriorly to the mammary gland and beyond to the perineal area. The area of the last lumbar and the first coccygeal vertebra on the dorsum of the donors was shaved, scrubbed and disinfected for the ventral midline approach. The Does were put on dorsal recumbency. A midline incision was made on the *linea alba* 10 cm long 5 cm away from the umbilicus towards the mammary gland. The animals were weighed, vital parameters (temperature, pulse, respiration) were all normal. Anaesthesia was achieved through sedation with Xylazine (chanazine® at a dose of 0.025mg/kg body weight Epidural anaesthesia was instituted using Xylocaine 5mls on the epidural space. complete sterile surgical packs, surgical gloves for surgeon and assistant surgeon, surgical catguts, 0.0,1.0, 2, 2.0, suture needles, Nylon, gowns, lignocaine, xylazine, penicillin, streptomycin, disinfectant (pant®): liquid disinfectant! antiseptic containing chlorhexidine Gluconate B.P. 0.3% w/v and cetrimide B.P. 3.0% w/v) The Fascia was incised; the peritoneum was opened to allow the search for the uterus. The uterus and ovaries were identified exteriorised and properly grasped.

2.1. Comparative accessibility study of surgical embryo recovery, and genital manipulation in the RSD

Comparative Surgical trials were carried out to compare, the three different types of surgical approaches, a comparison of accessibility to surgical recovery and transplantation trials, in the Sokoto Red Does, the approaches being (a) the Ventral Midline Approach. (b) The Para lumbar flank approach and (c) the Modified Ventral Midline Approach; a modification of the Ventral Midline Approach used in the cow and the ewe. It was observed in the VMA that the ovaries can be visualized, and assessed. The corpus luteum on the ovaries too can be assessed to predict ovulations. which from our findings can best be used for the purposes of observations on the ovaries such as assessment of corpus luteum, super ovulation response and possible embryo transplantation. In these series of surgical trials we seek to determine the suitability or otherwise of three different surgical approaches above. Difficulties were encountered with both procedures. This prompted for the modification of the second approach and we came up with the MVMA to surgical embryo collection. In this approach, same procedures were employed as in

the ventral mid line approach, except that in the MVMA, the incision which starts from the umbilicus to just before the udder will be extended in this procedure. The extension will be from just before the udder through the udder and cutting through the *median raphae* of the mammary gland to just after it up to the perineal region (about 3cm behind the udder. the laparotomy incision was kept open by use of tissue retractor. The uterus was located dorsal to the bladder and exteriorised and properly grasped.

3. Results

3.1. Access to ovaries

In the Ventral Midline Approach (VMA) both Ovaries can be visualized. The corpus luteum and ovulations on the ovaries can be seen. The distance measured from the skin incision to the ovaries was 6.0 ± 1.74 cm, while in the Para lumbar flank Approach (PFA) both ovaries can be visualized though one side i. e. depending on where you are standing if you are standing on the left side of the opened animal the right uterine horn and ovaries may not be easily visualized. A distance of 5.0 ± 0.25 cm However, with the Modified Ventral Midline Approach (MVMA) both ovaries can easily be visualized and examined. There was observed a greater accessibility. Corpus luteum and Ovulations can be easily observed. A distance of 4.4 ± 2.2 cm was recorded (Table 1).

Table 1

Comparison of access to various parts of the genitalia in red sokoto doe among the three surgical approaches.

Activity	Ventral midline approach (VMA)	Para lumbar flank approach (PFA)	Modified mid ventral approach (MVMA)
Access to ovaries	Both ovaries can be visualised. Corpus luteum and ovulations can be seen. Distance of ovaries to the skin incision. 6.0 ± 1.74 cm	Both ovaries can be visualised, though one side has a greater access than the other. 5.0 ± 0.25 cm	Both ovaries can be easily visualised and examined, greater accessibility CL and ovulations can be easily observed. 4.4 ± 2.2 cm
Access to uterine horns and tubes	Uterine horns and fallopian tube can be observed, but manipulation difficult. 12.5 ± 2.1 cm	Uterine horns fallopian tube can be visualised, but manipulation difficult.	Uterine horns and fallopian tube can be observed manipulation with greater ease 11.5 ± 0.45 cm
Access to uterine body	Manipulation of uterine body a bit difficult. 14.5 ± 1.6 cm	Manipulation of uterine body difficult. 15.5 ± 1.4 cm	Manipulation of uterine body a bit easier. 13.5 ± 2.5 cm
Bilateral Access to the genitalia	Easy	Difficult, almost impossible	Easier

*Approximate distance from organ/structure to the mid point of the skin incision with the organ in situ.

3.2. Access to uterine horns and tubes

Uterine horns and Fallopian tube can be observed, but manipulation may be difficult in the VMA, a measurement of 12.5 ± 2.1 cm was recorded. In the PFA; uterine horns and Fallopian tube can be visualized, but manipulation difficult. 12.5 ± 1.86 cm, While for the MVMA uterine horns and Fallopian tube can be observed manipulation of the structures is with great ease 11.5 ± 3.45 cm.

3.3. Access to uterine body

In the VMA, manipulation of the uterine body a bit difficult 14.5 ± 1.66 cm, in the PFA; manipulation of the uterine body difficult 15.5 ± 1.4 cm while for MVMA the manipulation of uterine body is a bit easier the distance is shorter 13.5 ± 2.5 cm.

3.4. Bilateral access to the genitalia

Ease in the VNA, Difficulty almost impossible in the PFA. However, the procedure appears to be very easy in the MVMA, (Table 2) Comparative ease of surgical embryo recovery and manipulation in the Red Sokoto Doe using the various approaches. Recovery and manipulation of the genitalia is found to be difficult in both VMA and PFA but easy in the MVMA. The ease of flushing for embryos in the VMA and PFA was found to be difficult and easy in the MVMA for the ease of embryo recovery the procedure was easy but access difficult. In the PFA the procedure is easy but access to the near horn difficult. The procedure was easy Approach highly technical but more convenient considerable room for man receiver.

The distance of the uterine horns and tubes from the skin incision was found to be 13.5 ± 3.66 cm, 12.5 ± 1.86 cm and 11.5 ± 3.45 cm in the PLA, VMA and the MVMA respectively. While access to the genitalia using Para lumbar approach was found to be almost impossible, with the ventral midline approach it was less difficult and very easy using the modified ventral midline approach.

Table 2

Comparative ease of surgical embryo recovery and manipulation in the red sokoto does using the various approaches.

Activity	Ventral Midline Approach(VMA)	Para lumbar Flank Approach(PFA)	Modified Mid Ventral Approach(MVMA)
Ease of cannulation	Difficult	Difficult	Easy
Ease of flushing	Difficult	Difficult	Easy
Ease of embryo recovery	Procedure easy but access difficult	Procedure easy but access to near horn difficult	Procedure easy. Approach highly technical and tedious.

4. Discussion

The ventral midline approach is essentially the commonest method used to access the uterus and ovaries for embryo collection (Smith, 1982; Agrawal and Battacharya, 1982., Armstrong and Evans, 1983). However in this study, some accessibility problems arise which prompted an examination of possible ways to ease surgical accessibility of embryo flushing, collection and transfer in the RSD. The common Procedure used for embryo collection in the Ewe and Doe are surgical. Usually the reproductive tract is opened by a mid ventral incision. In this study after encountering in cannulating and flushing of the reproductive tract of RSDs it became imperative to investigate an easier access route to embryo recovery and transplantation. Table 2, Presents a comparison of accessibility to surgical recovery and transplantation trials, in the Sokoto Red Does, the approaches being (a) the ventral midline approach. (b) The Para lumbar flank approach and (c) the modified Ventral Midline approach; a modification of the ventral midline approach used in the cow and the ewe. It could be seen in VMA that the ovaries can be visualized, and assessed. The corpus luteum on the ovaries too can be assessed to predict ovulations. The distance from the skin incision to the ovaries is 6.0 ± 1.74 cm. While in the Para lumbar flank approach, both ovaries can be visualized through one side i.e. if the approach is either left or right. The side of approach will give better view of the adjoining ovaries and a limited view of the ovary on the other side. The distance of the ovary to the skin incision is 5.0 ± 0.25 cm. It could be seen that this is slightly shorter than the dimension in the ventral midline approach. However, in the modified ventral midline approach (MVMA) the ovaries can be accessed with greater ease and visualizing the ovaries, is found to be very easy. One can visualize the ovaries very closely. The distance from the skin incision to the ovaries is 4.4 ± 2.2 cm. As such it could be seen that the modified ventral midline approach will give better surgical access and greater proximity than the other procedures.

Access to the uterine horns and uterine tubes is much more difficult because they are a little bit more obscure than the ovaries. Similarly the ventral midline approach and the Para lumbar flank approach provide lesser access and ease of manipulation compared to MVMA. In MVMA the uterine horns could be handled and the insertion of a Foley catheter at the base of the uterine horn for flushing is easier. Likewise the uterine tubes which are very tortuous and slippery structures can better be handled and cannulated. Where as, from our experimental trials, we observed that that using procedure it is much difficult and tedious, transplantation of recovered embryos

is easier done using PFA in the RSD. However, tedious operations requiring manipulation of the genitalia including the uterine horns require the use of the extended ventral midline approach.

From the table 1 and 2 it could be seen that access to the uterine body, the ease of cannulation, ease of flushing and ease of embryo recovery are all conveniently achieved going by the MMVA. The distances from opening incision to the various are nearer in the MMVA than the PFA. For the purposes of flushing and recovery the use of the VMA and MVMA approaches are essential, whereas the MVMA gives easier access to the genitalia when compared to the VMA.

There is virtually very few works on embryo collection in Nigeria, only very little have been reported. This is very likely to be among the pioneer attempts the convenience and effectiveness of the surgical approach to embryo recovery knowing the right effective and easier surgical procedure to undertake easily saves the investigator or the researcher a lot of time. This is necessary, due to the obscure and remote position of the genitalia and, the slippery nature of the uterine tube and the cumbersome nature of some the surgical approach, especially looking at the often very little room for manoeuvre approaches such as MVA and PFA.

Goats and sheep eggs embryos are usually obtained from the reproductive organs using the surgical procedure of flushing, first described by Hunter et al., 1955. By this method collection is carried out under general anaesthesia with ovaries, oviducts and uterus exposed by a mid-ventral abdominal incision (Moore 1982). The mid ventral or flank laparotomy incisions permit flushing of the oviducts and uterine horns for the recovery of fertilized eggs. Ovulations and corpora lutea can be easily observed very few reports really dwelled in detail on the type of surgical approach for observing for ovulations or corpora lutea (Hunter, 1955).

From our findings a consideration should be to which procedure will be faster, less tedious and less time consuming depending on what operation is to be done. Here, if the objective is just to visualize the ovaries or to check for ovulations the easier approach is that of ventral midline approach. Uterine tube, especially the fimbriae are very important structures that need to be visualized, and manipulated in order to cannulated them and infuse transfer medium to bring about the flushing of the uterine tube and the uterine horns for recovering the embryos using procedure I uterine horns and fallopian tube can be observed, but manipulation of these structures can be a bit difficult with this approach. In the Para lumbar flank approach manipulating the uterine horns and the fallopian tube can be difficult. However, visualization and manipulation of fallopian tube and uterine body is easier using the extended ventral midline approach. this Procedure will enable an operator to cannulated the fallopian tube using the paediatric catheter also a good unobstructed access to the uterine horn can be achieved to allow for the nick incision that will allow insertion of a Foley catheter, to facilitate drainage of the flushing medium into the receptacle or collecting dish in a retrograde direction.

Access to the uterine body is essential in the technique of embryo recovery because the fixation of the Foley catheter is only possible when the operator grasps the uterus by the corpus. From there a nick incision can be made just a centimetre above the area of the bifurcation. Be it on the right or the left uterine horn depending on which uterine horn is to be flushed. The accessibility to the uterus as a whole is very important due to the tortuous and slippery nature of the genitalia within the pelvic cavity the handling of the tract for transfer is very important, hence the need for a procedure that will give adequate for room for manoeuvre Surgical trials in the present study have made a comparison of ease of access in these approaches (Table: 2) from literature embryo recovery in the ewe, is essentially via the ventral midline approach, it is the approach of choice and the most widely used technique for the ewe (Armstrong and Evans,1983). However, surgical trials in this study have indicated the need for a modification to facilitate accessibility to the reproductive tract for manipulations. Difficulty in accessibility to the genitalia was experienced in these trials. This restricted access limit space for proper grasp of the uterus for catheterization and flushing. Therefore, a modification of the ventral midline approach is necessary extending the ventral midline incision which starts from the umbilicus posteriorly to just before the udder to in this modification an extension through the udder by separating the inter udder fascia. This gives a wider, freer and easier access to the whole reproductive tract beneath.

Accessibility to genitalia for Embryo Recovery: Recovery of embryo and the transfer is to date essentially performed surgically in the small ruminants (Betteridge, 1980). Until the mid 1970s, all successful embryo recoveries in the species were carried out by means of surgery under general anaesthesia, using essentially the method developed by Hunter *et al.*, (1955) .Earlier studies in Embryo Transfer started with surgical recoveries but as time goes on non-surgical recoveries were adopted in large animals. Such non surgical procedures though having numerous advantages have not yet been developed in the Goats. According to Betteridge (1980), the principal advantages of non-surgical method are that the elaborate facilities, costs, risks and post-operative

adhesions associated with surgery are avoided and on the farm collections therefore become possible, lactating cows that are bad surgical subjects can be used and, most importantly, collections can be repeated many times from the same donor. However, until such surgical procedures are developed for the goats, embryo recovery, collection and transfer in these animals will continue to be by surgical procedures. This study revealed that in the RSD, a lot of energy and time can be saved as regards using the Para lumbar flank approach. Table 2 gives a comparison of advantage or accessibility and limitation of each of the procedure.

Access to Ovaries: In the cranial ventral midline approach both ovaries can be visualised, the corpus luteum and ovulations can be seen and evaluated. In the PFA, the near ovary can be better visualised than the far ovary. So there was unilateral access limitation. However in the caudal ventral midline approach, both ovaries can be easily visualized and examined. The corpus luteum, ovulation and manipulation of the ovaries could be done with ease.

Access to Ovaries: In the cranial ventral midline approach both ovaries can be visualised, the corpus luteum and ovulations can be seen and evaluated. In the PFA, the near ovary can be better visualised than the far ovary. So there was unilateral access limitation. However in the caudal ventral midline approach, both ovaries can be easily visualized and examined. The corpus luteum, ovulation and manipulation of the ovaries could be done with ease.

Access to Uterine horns and fallopian tubes: With the midventral approach uterine tubes and fallopian duct can be observed, but manipulation of these can be a bit difficult. For the PFA, the near uterine horn and the fallopian tube can be visualized and manipulation could be done. The far uterine horn and fallopian tube could be visualised with difficulty and manipulation was even more difficult. However, visualization and manipulation of the fallopian tube and the uterine body was easier using the caudal VMA.

Access to Uterine body: Manipulation of the uterine body could be a bit difficult via the cranial ventral midline approach. Similarly manipulation of the uterine body was difficult using the PFA. With the MVMA, manipulation of uterine body was easier; insertion of Foley catheter for flushing was much easier.

Ease of Cannulation and flushing for embryo recovery: In order to flush one would need to cannulate the uterine body with a paediatric catheter and the uterine horns with a Foley catheter; doing this was difficult using ventral midline approach very difficult with Para lumbar flank approach and easier with the caudal ventral midline approach.

Bilateral access to genitalia: There was a bilateral two-way access to the genitalia u Para lumbar flank approach difficult and almost impossible in VMA, while access to genitalia via the MVMA is much easier.

The study also revealed that in the RSD, the routine VMA did not offer adequate accessibility to genitalia to allow for manipulation, cannulation, and flushing for embryos with ease. Due to the problem of accessibility, and remote location of the genitalia in the RSD, alternative surgical approaches that would give easier access to the genital tract were investigated; the study suggested the adoption of the three different approaches depending on procedure to be performed. The PFA do not give convenient access to the genitalia to permit embryo recovery but this approach could possibly offer easier access to genitalia for just the transfer of embryos as the transfer does not require extensive manipulation of reproductive tract. Surgical trials in this project demonstrate that an extension of the incision length in the VMA, as an incision which starts from the umbilicus to the udder) through the fascia separating the two mammary lands provide good access to the genitalia which could easily be seen lying beneath.

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