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ORIGINAL ARTICLE

Comparative Evaluation of Standing and Lateral Recumbency Restraint Positions for Rumenotomy Based on Transforming Growth Factor- β Responses in Kano-Brown Goats

Abubakar Mshelia Saidu^{1*}, Samuel Tanko Fadason², Gabriel E. Ochube², Sani Adamu³

¹ Department of Veterinary Surgery and Radiology, University of Maiduguri, Maiduguri, Nigeria.

² Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

³ Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

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Abstract

Objective- Comparative evaluation of standing and lateral recumbent restraint positions for rumenotomy based on transforming growth factor- β (TGF- β) concentrations of Kano-Brown goats (KBGs).

Design- Experimental study

Animals- Twenty-four KBGs Eighteen KBGs of both sexes diagnosed of rumen foreign body impaction (RFBI), were allocated to groups A, B and D. Six other KBGs free of RFBI were assigned to group C as control.

Procedures- Groups A and B were restrained in lateral recumbency while group D in a fabricated mobile small ruminant surgical chute (MSRSC). Serum samples stored at -20 °C until ELISA, were obtained pre-rumenotomy (Pre) and post-rumenotomy, at 0, 5, 24, 48 and 72 hours, and subsequently at weeks 1, 2, and 3. Group C had no surgery while A, B and D had rumen skin clamp fixation, stay suture rumenotomy and mobile small ruminant surgical chute rumenotomy, respectively.

Results- The post-rumenotomy mean concentrations of TGF- β for groups A, B and D at 0 hour (81.97 ± 24.12 , 71.26 ± 10.28 and 58.51 ± 6.44 ng/L, respectively) were higher than the mean pre-rumenotomy values (38.34 ± 3.66 , 41.31 ± 4.90 and 44.91 ± 4.10 ng/L, respectively) but were not significantly different ($p > 0.05$). As the mean TGF- β concentration in the males of the different experimental groups did not differ significantly ($p > 0.05$), the females of group B had significantly higher ($p < 0.05$) mean concentrations than those of group D and C females at 48 hours post-rumenotomy.

Conclusions and Clinical Relevance- Lateral recumbency restraint position rumenotomy was associated with more severe post-surgical stress than standing restraint based on role switching of TGF- β in this study. This suggests comparative advantage of standing recumbency restraint rumenotomy over the conventional lateral recumbency restraints position in goats.

* Correspondence to: Abubakar Mshelia Saidu; Department of Veterinary Surgery and Radiology, University of Maiduguri, Borno State, Nigeria. E-mail: abubakarsm51@gmail.com

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1. Introduction

In a livestock farm, appropriately selected surgical location and restraint facilities, ameliorates the surgical outcome.¹ Rumenotomy is a surgical entry into the rumen usually through the left flank where the organ dominates. The procedure is a clean-contaminated surgery but effective and safest procedure for retrieving ingested foreign bodies as well as to resolve other conditions of the paunch and its related structures “forestomach”. There are several techniques of rumenotomy including those involving placement of stay sutures or suturing the rumen to the skin prior to rumenotomy. Other methods employ fixation devices to stabilise the rumen such as in Weingarh's ring and Gabel rumen board rumenotomy techniques.²⁻⁴

Rumenotomy studies reported complications that were directly linked to rumenotomy techniques,^{5,6} while others associated most postoperative complication morbidity and mortality to confounding factors at presenting complaint or causes unrelated to the rumen surgical operation.³ There are paucity of information regarding comparative advantage of these techniques in standing and recumbent restraint positions. The many extant techniques of rumenotomy were mostly designed by large ruminant practitioners, thus, are better suited for operations that restrains cattle in standing position using crush rather than the conventional lateral recumbency restraints for sheep and goats.^{5,6} Surgical trauma stimulates a sequence of neuroendocrine, metabolic, immunologic, and inflammatory reaction known as stress response that also comprise immunologic mechanisms.^{7,8} Acute surgical pain can produce significant stress in animals.⁹⁻¹²

Physiological reaction to restore homeostasis following injury is an alteration in the immune response that involve the release of cytokines; inflammatory mediators. Cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the immune responses such that physiologic role in inflammation and pathologic role in systemic inflammatory states are

increasingly recognized. Anti-inflammatory cytokines are a series of immune-regulatory molecules that control the pro-inflammatory cytokine response.¹³ All cytokines offer "half angel – half devil" aspect of inflammatory response and none can be simply labelled either "pro" or "anti".¹⁴ The net effect of any cytokine is dependent on the timing of cytokine release, the local milieu in which it acts, the presence of competing or synergistic elements, cytokine receptor density, and tissue responsiveness to each cytokine.¹⁵ Perturbations of this regulatory network of cytokines by genetic, environmental, or microbial elements may have highly deleterious consequences.¹⁶

Transforming growth factor (TGF- β) is a significant moderator of cell proliferation, differentiation, and formulator of the extracellular matrix.¹⁷ It is produced as an indolent precursor and requires activation before wielding its influence.¹⁸ There are three isoforms of TGF- β (designated TGF- β 1, 2, and 3) expressed in mammalian species. Like many cytokines, TGF- β has both pro- and anti-inflammatory effects. It plays the role of a biological switch, antagonizing or modifying the actions of other cytokines or growth factors. The presence of other cytokines may modulate the cellular response to TGF- β , and the effect may differ depending on the activation state of the cell.¹⁹ TGF- β is capable of converting an active site of inflammation into one dominated by resolution and repair.¹⁷ TGF- β often exhibits disparate effects with immune-enhancing activity in local tissues and immune-suppressive activity in the systemic circulation. TGF- β 1 suppresses the proliferation and differentiation of T and B cells and limits interleukin-2 (IL-2), interferon-gamma (IFN- γ), and tumor necrosis factor (TNF) production. TGF- β 1 acts as a monocyte / macrophage deactivator in a manner similar to IL-10. However, TGF- β is less potent an inhibitor than IL-10 and has little or no effect on IL-1 production.¹⁷ Transforming growth factor mediates various roles of macrophages including the initiation of granulation tissue formation and angiogenesis.²⁰ While anti-inflammatory cytokines are a prerequisite to control the

cascade of pro-inflammatory mediators, their excessive production is associated with a severe immune depression as observed in patients following trauma or major surgeries.²¹ The amount of a given cytokine clearly influences its properties and the best example is given with TGF- β : In addition to its role in controlling inflammation, TGF- β restrains cell proliferation and controls turnover of the extracellular matrix.²²⁻²⁴

There is paucity of information on body responses to surgical stress in the ranking of lateral recumbency and standing restraint positions for rumenotomy, based on the profiles of serum inflammatory / stress biomarker such as of cytokines as an index for the selection of restraint position carrying out rumenotomy in the goat or small ruminants.

The pre- and post-operative comparative evaluation of extant techniques of rumenotomy on the basis of alterations in the inflammatory biomarker responses (TGF- β) could be a guide in the choice of most humane technique of rumenotomy in small ruminants. Findings from this study would provide surgeons with information on the comparative advantages, or otherwise, based on TGF- β profiles, offered by mobile small ruminant chute (standing position) over the conventional lateral recumbency techniques for rumenotomy as guide in choice of restraint positions.

2. Materials and Methods

Ethical clearance was obtained from the Ahmadu Bello University Committee on Animal Use and Care, with approval number ABUCAUC/2018/054.

The study was carried out in the large animal surgery theatre of the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The laboratory phase ELISA was conducted at the National Animal Production Research Institute Laboratory, Ahmadu Bello University, Zaria, Nigeria.

Research Animals

Eighteen Kano-Brown goats were diagnosed on palpation of having rumen foreign body impaction (RFBI) and six other goats diagnosed to be free of the rumen foreign bodies, all of equal number of both sexes, aged between 1 to 2 years and with a mean body weight of 15 ± 0.52 kg were explored in the study. On arrival, the animals were dewormed with ivermectin (Bremamectin, Brema pharma GMBH, Warburg, Germany) at a dose rate of 200 μ g/kg, SC. A prophylactic dose of 20 mg/kg, IM of oxytetracycline (Kepro Oxytet 20%, Kepro, Deventer, The Netherlands) was administered to each goat against bacterial infections. The animals were adequately fed groundnut husk, mixtures of beans, sorghum shafts and maize offal, daily. Clean drinking water was provided *ad libitum*, except where specified. The animals were allowed to acclimatize for two weeks prior to commencement of the study.

Pre-surgical evaluation was carried out on goats in groups A, B, C and D through physical and haematological examinations; the goats were also screened for blood and faecal parasites to ensure the animals were in stable condition for rumenotomy.

Surgical Procedure (Rumenotomy)

The goats were fasted for 12 and 6 hours for feed and water respectively, as preoperative dietary measures for the surgery. All goats in groups A, B and D were first sedated with xylazine hydrochloride (Inovet, Arendok, Belgium) at 0.025 mg/kg, administered subcutaneously. The animals in groups A and B were placed on right lateral recumbency and the left paralumbar fossa of each goat was shaved using a razor blade. The paralumbar fossa of each goat in group D was similarly shaved while in standing position, restrained in the fabricated mobile small ruminant surgical chute. The area was aseptically prepared by scrubbing with 0.2 % Chlorhexidine gluconate (Savlon, Vervaading deur, Johnson and Johnson Ltd, London, UK) and smeared with

povidone iodine (Sawke-10%, Jawa International Limited, Lagos, Nigeria) prior to local anaesthesia and surgery.

The goats in groups A, B and D were anaesthetized regionally with 2 % lidocaine HCl at 4 mg/kg (Syncom Formulations, NCL Pharm Chem Ind. Ltd., India) in an inverted L-block fashion on the shaved left flank.

A polythene rumen shroud was used to drape the goats. A seven centimetre through and through incision was made on the skin via the left mid-flank into the abdominal cavity of all experimental goats in groups A, B and D. Following the skin incision, the muscles were manually separated along the direction of their fibres on each muscle layer that was lifted with a rat tooth forceps. After laparotomy and exteriorization of the rumen, the following techniques of rumenotomy were carried out as prescribed for each group. To perform rumen skin clamp fixation technique (RSCF) in group A, (Figure 1), the rumen was gently pulled out of the incision and its dorsal sac was located and firmly anchored to the skin ventrally and dorsally with two of six towel clamps for the technique. A seven-centimetre (7cm) incision was made at the less vascularised region of the rumen dorsal sac. The edges of the rumen were fixed caudally and cranially to the skin incision with towel clamps just as described by Dehghani and Ghahrdani.⁶ The rumen edges covered the skin edges by 2–3 cm and the remaining clamps were placed between the initially placed clamps. The rumen was explored and foreign materials mostly polythene were removed and weighed given mean weight. A solution of 0.9% normal saline (Juhel, Fabrique par Juhel Nig. Ltd, Anambra, Nigeria) was poured under pressure to rinse the rumen ingesta. Caudal and cranial clamps were removed to aid rumen closure by first leaving the dorsal and ventral clamps in place.

For the stay suture rumenotomy technique (SSR) in group B, the rumen was gently pulled out of the incision, and anchored to the skin with 4 nylon suture knots in a simple interrupted suture pattern. The knots were placed dorsally, ventrally, cranially, and caudally as described by Dehghani and Ghahrdani.⁶ The rumen was then incised and its edges

grasped with artery forceps and the ruminal cavity were then explored. Ruminal and abdominal closure were also as described for RSCF.

To perform the small ruminant surgical chute (MSRSC) rumenotomy in group D, the rumen was exteriorized by gentle pulling and held in place by the non-dominant hand of the surgeon or by an assistant. Rumen shroud was adjusted for a fit while the rumen was still held in place by the surgeon's non-dominant hand. The dorsal rumen sac was identified and a stab incision was made over a less vascular portion with a scalpel, held in the surgeon's dominant hand. The incision was then extended to 7 cm with a sharp-blunt scissors and all foreign bodies were evacuated.

After evacuation of the rumen ingesta in all the restraint positions and techniques of rumenotomy, the rumen was rinsed with 0.9% normal saline. Goats in the MSRSC restraint had their rumen gently pulled away from the abdominal cavity during rinsing which prevented sipping of ruminal fluid into the abdominal cavity (Figure 2). A combination of continuous Lambert was employed followed by a single layer of Cushing suture pattern to invert the rumen edges with a number 2 polyglycolic acid sutures (Atramat, Internactional Farmaceutica, Planta, Mexico). Chromic catgut (Anhui Kangning Industrial group Co, Anhui, China) was used in apposing the muscles in a 3 layers simple continuous suture pattern. The skin was closed using a number 2 nylon suture (Anhui Kangning Industrial group Co, Anhui, China) in a Ford



Figure 1. Lateral recumbency restraint position for rumen skin clamp fixation and stay suture rumenotomy.

interlocking suture pattern. Goats in the control group (group C), were not subjected to any form of surgery but were similarly sampled for blood to obtain serum as described in the treated groups.



Figure 2. Kano-Brown goat undergoing standing restraint position rumenotomy in a fabricated mobile small ruminant chute

TGF- β Analysis

On the day each rumenotomy procedure for the groups A, B and D was performed and just before the surgery, blood sample (5 ml) was collected via the jugular vein to establish pre-rumenotomy status and at 0, 5, 24, 48 and 72 hours, and subsequently at weeks 1, 2, and 3 post-rumenotomy. The blood samples were dispensed into a plain vacutainer tubes and allowed to clot for two hours at room temperature before centrifugation for 20 minutes at 1000 \times g. The harvested serum samples from all the experimental animals were emptied into micro-vials and stored at -20 °C until needed for ELISA. Avoiding repeated freeze/thaw cycles was necessary and was minimized when assaying for serum TGF- β . Analysis of optical densities (OD) of data with duplicate readings were averaged for the standards, and zero standard OD were also subtracted. Standard curve was created by plotting the mean absorbance for each standard on the x-axis against the

concentration for each standard on the y-axis and displaying the trend line for best fit curve through the points on the graph with a computer software (CurveExpert Professional, Hyams Development).

Statistical Analysis

GraphPad Prism (version 5.03 for Windows, San Diego California, USA), was used to determine Mean \pm SE of the variables by column statistics. Two way repeated measures ANOVA with Bonferroni post-test was used to compare between the four groups. Analyses were considered as significant at $p < 0.05$.

3. Results

The post-rumenotomy mean concentrations of TGF- β for groups A, B and D at 0 hour (81.97 ± 24.12 , 71.26 ± 10.28 and 58.51 ± 6.44 ng/L, respectively) were higher than the mean pre-rumenotomy values (38.34 ± 3.66 , 41.31 ± 4.90 and 44.91 ± 4.10 ng/L, respectively), even though the differences were not significant ($p > 0.05$). The mean TGF- β concentration in groups C remained more or less constant (Table 1). The mean concentrations of TGF- β in the females of group B (115.39 ± 36.56 ng/L), was significantly higher ($p < 0.05$) than those of group D (42.57 ± 2.57 ng/L) and C (39.12 ± 3.33 ng/L) at 48 hours post-rumenotomy (Table 2). The mean TGF- β concentration in the males of the different experimental groups did not differ significantly ($p > 0.05$), throughout the experimental period (Table 3).

4. Discussion

Generally, cytokine response in inflammation involves an interplay between pro- and anti-inflammatory cytokines especially following periodic dampening in concentrations of pro-inflammatory cytokines,²⁵ perhaps due to effects of regional anaesthesia, may influence early TGF- β surge.^{26,27} The mean concentration of TGF- β in group A (RSCF) at

Table 1. Mean \pm SE serum concentrations (ng/L) of transforming growth factor- β (TGF- β) pre- and post-rumenotomy in the different experimental groups of the Kano-Brown Goats.

Group	Pre and post-rumenotomy sampling periods								
	Pre	0 hr	5 hrs	24 hrs	48 hrs	72 hrs	Week 1	Week 2	Week 3
A	38.34 \pm 3.66	81.97 \pm 24.12	48.47 \pm 12.30	58.94 \pm 16.39	53.68 \pm 7.06	69.77 \pm 9.97	82.97 \pm 12.50	62.43 \pm 11.11	54.26 \pm 14.00
	B	41.31 \pm 4.90	71.26 \pm 10.28	64.41 \pm 14.45	58.45 \pm 10.21	79.55 \pm 23.66	67.00 \pm 12.41	66.90 \pm 8.46	62.59 \pm 10.12
C		45.11 \pm 2.38	47.26 \pm 4.11	42.95 \pm 2.10	36.71 \pm 1.73	40.39 \pm 1.59	38.91 \pm 2.23	44.99 \pm 4.36	43.22 \pm 3.81
	D	44.91 \pm 4.10	58.51 \pm 6.44	66.87 \pm 5.74	64.52 \pm 7.05	59.62 \pm 7.89	57.93 \pm 9.01	63.09 \pm 11.86	66.29 \pm 8.63

The values within columns are not significantly different ($p > 0.05$). Group A: Rumen skin clamp fixation (RSCF) technique. Group B: Stay suture rumenotomy (SSR). Group C: Control. Group D: Standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC).

Table 2. Mean \pm SE serum concentrations (ng/L) of transforming growth factor- β (TGF- β) pre- and post-rumenotomy in the different experimental groups of female Kano-Brown Goats.

Sampling Time	Group A	Group B	Group D	Control
Pre	35.23 \pm 3.51	37.17 \pm 3.38	41.96 \pm 4.74	43.12 \pm 4.04
0 Hrs	90.12 \pm 52.00	83.24 \pm 13.77	57.97 \pm 10.92	45.11 \pm 6.33
5 Hrs	57.76 \pm 25.00	89.47 \pm 20.31	61.97 \pm 11.85	41.12 \pm 4.27
24 Hrs	74.15 \pm 31.54	80.66 \pm 3.67	51.85 \pm 2.32	36.69 \pm 3.08
48 Hrs	60.11 \pm 10.89	115.39 \pm 36.56 ^a	42.57 \pm 2.57 ^{b,c}	39.12 \pm 3.33 ^b
72 Hrs	77.89 \pm 18.10	91.34 \pm 9.38	53.42 \pm 9.90	35.50 \pm 2.45
Week 1	77.25 \pm 15.94	49.15 \pm 5.94	60.08 \pm 13.91	47.70 \pm 6.02
Week 2	76.31 \pm 16.68	59.84 \pm 9.27	70.08 \pm 4.86	48.81 \pm 3.15
Week 3	42.56 \pm 4.43	47.59 \pm 3.13	44.58 \pm 5.65	52.38 \pm 3.87

Values with different superscripts within a row are significantly different ($p < 0.05$). Group A: Rumen skin clamp fixation (RSCF) technique. Group B: Stay suture rumenotomy (SSR). Group D: Standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC).

Table 3. Mean \pm SE serum concentrations (ng/L) of transforming growth factor- β (TGF- β) pre- and post-rumenotomy in the different experimental groups of male Kano-Brown Goats.

Sampling Time	Group A	Group B	Group D	Control
Pre	41.44 \pm 6.70	45.45 \pm 9.56	47.86 \pm 7.26	47.10 \pm 2.83
0 Hrs	73.81 \pm 11.75	59.28 \pm 13.97	59.05 \pm 9.36	49.41 \pm 6.30
5 Hrs	39.19 \pm 6.75	39.34 \pm 1.64	71.77 \pm 0.58	44.78 \pm 0.71
24 Hrs	43.74 \pm 10.84	36.23 \pm 3.75	77.19 \pm 9.08	36.73 \pm 2.33
48 Hrs	47.25 \pm 9.44	43.71 \pm 13.31	76.68 \pm 3.68	41.65 \pm 0.00
72 Hrs	61.65 \pm 10.15	42.67 \pm 9.46	62.45 \pm 16.97	42.32 \pm 2.71
Week 1	88.68 \pm 22.23	84.64 \pm 2.75	66.10 \pm 22.37	42.28 \pm 7.16
Week 2	48.54 \pm 12.10	65.33 \pm 20.46	62.50 \pm 18.28	37.63 \pm 5.59
Week 3	65.96 \pm 28.69	57.98 \pm 9.53	49.02 \pm 2.37	44.17 \pm 5.36

All values within rows were not significantly different ($p > 0.05$). Group A: Rumen skin clamp fixation (RSCF) technique. Group B: Stay suture rumenotomy (SSR). Group D: Standing position rumenotomy using Mobile Small Ruminant Surgical Chute (MSRSC).

week 1 was almost twice the concentrations in group B (SSR) KBGs, which were suggestive of more severe surgical stress associated with the recumbency restraint positions. The explanation for the significantly higher mean serum TGF- β concentration in group B (SSR) females than group A (RSCF) females at 48 hours post-

rumenotomy could be that female KBGs showed higher TGF- β response than the males,^{28,29} as observed in this study. The significantly higher mean concentration of TGF- β in groups A and B than in group D also suggests that post-surgical stress associated with the recumbency restraint position of rumenotomy was more severe when

compared to the standing restraint position of group D (MSRSC). The TGF- β expressions in this study peaked earlier than could be expected of an anti-inflammatory cytokine in group B female KBGs at 48 hours. This finding agrees with the work of Cavaillon,¹⁴ who reported that a given cytokine may behave as a pro- as well as an anti-inflammatory cytokine, depending on the inflammatory signal. This switching role of cytokines is usually influenced by cytokine amount, nature of the target cell, nature of the activating signal, nature of produced cytokines, the timing, the sequence of cytokine action and even the experimental model are parameters which greatly influence cytokine properties.^{14,26} The release of TGF- β at an early stage of healing process was perhaps to prompt recruitment of inflammatory cells into the injury site, which are later involved in a negative feedback via release of superoxide from macrophages. During this interim stage, granulation tissues are gradually formed and TGF- β prompts the expression of key components of extra cellular matrix (ECM) proteins, such as fibronectin, collagen types I and III, and VEGF. Further, TGF- β improves the angiogenic properties of endothelial progenitor cells to facilitate blood supply to the injured site and stimulates contraction of fibroblasts to enable wound closure. Keratinocyte migration is also promoted by TGF- β .^{30,31} Conclusively, the lateral recumbency restraint position of rumenotomy was more severe when compared to the standing restraint position of group D (MSRSC). The phenomenon that regional anaesthesia (inverted-L block) dampened pro-inflammatory cytokine surge such that a given cytokine behaves as a pro- as well as an anti-inflammatory cytokine depending on the inflammatory signal, termed role switching of cytokines could be linked to the TGF- β expressions in this study that peaked earlier than could be expected of an anti-inflammatory cytokine in group B female KBGs at 48 hours. This TGF- β response suggests comparative advantage of the standing recumbency restraint rumenotomy over the conventional

lateral recumbency restraints position for rumenotomy in the goat.

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Conflict of Interests

The authors state that there was no conflict of interest in respect of this publication.

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