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Clinical evaluation of platelet rich plasma in surgical repair of contracted tendon in calf

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Abstract
A total of 21 calves presenting with joint issues were screened for the presence of knuckling. Out of these, 16 calves that met the inclusion criteria were randomly divided into two equal groups. Group I calves underwent Z-tenotomy alone, while Group II calves received Z-tenotomy combined with a single Platelet-Rich Plasma (PRP) injection at the tendon site during surgery. Among the 16 selected calves, 14 (87.5%) exhibited bilateral tendon contracture, while 2 (12.5%) had unilateral involvement. Moreover, 9 (56.25%) calves were male, and 7 (43.75%) were female. All calves in Group II achieved full weight-bearing status and were essentially free of lameness by day 14. In contrast, calves in Group I reached full weight-bearing ability after 14 post-operative day, except for case number 8. The PRP injection significantly accelerated healing, enhanced tendon strength, reduced pain and swelling, and lowered the risk of infection. Furthermore, deficiencies in zinc and Vitamin D3 were identified as contributing factors to flexural deformities. This suggests that supplementing the diets of pregnant dams with zinc may help prevent such deformities in calves.

Keywords: Platelet rich plasma, contracted tendon, knuckling

Introduction
Calves represent the future herd, making their health and well-being essential from birth onward. Thus, it is vital to raise them both economically and in a sound manner to promote early maturity. Congenital anomalies may affect one or more systems, presenting as structural and functional abnormalities caused by genetic factors, environmental agents, or a combination of both, with many causes remaining unknown. Congenital malformations are a leading cause of culling, with knuckling playing a significant role (Sato *et al.*, 2020[11] which ultimately has serious economic implications. One of the most prevalent abnormalities of the musculoskeletal system in newborn calves is contracted flexor tendons, a condition also referred to as knuckling (Kamble *et al.*, 2009; Steiner *et al.*, 2014)[5,12] as depicted in Figure 1.

Fig1: Calf with bilateral knuckling on the day of presentation

Necessary surgical intervention promotes quicker recovery from these conditions, ultimately reducing morbidity and enhancing the animal's future performance. Currently, contracted flexor tendon issues are managed surgically through Z tenotomy and immobilization, often accompanied by local infiltration with 2% lignocaine hydrochloride. Local anaesthetics have a significant toxic effect on tenocytes, myocytes. It impairs tenocyte proliferation and extracellular matrix production, inhibits mitosis in tendon cells and suppresses collagen production. This highlights the necessity of establishing an anaesthetic protocol that achieves an adequate surgical plane of anaesthesia and the use of autologous and recombinant products to enhance the healing of soft tissue.

One such product is platelet-rich plasma (PRP) (Foster *et al.*, 2009) [3]. PRP is defined as a concentration of platelets that exceeds the physiological levels found in healthy whole blood (Foster *et al.*, 2009; Wroblewski *et al.*, 2010) [3, 16].

Platelets release a variety of growth factors that influence haemostasis, regulate inflammation, promote angiogenesis (including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF)), and facilitate tissue remodeling. (Arumugam *et al.*, 2021; Kaux *et al.*, 2020; Tarpada *et al.*, 2018) [1, 6, 13].

Considering the importance of the topic and the merits and demerits having with balanced anaesthesia using dissociative anaesthetic and Platelet Rich Plasma (PRP) during the surgical treatment of contracted tendon in newborn calves, the present study was undertaken with aim to evaluate the effect of PRP in the treatment of contracted tendon "Knuckling" during the surgical procedure in newborn calf.

Materials and Methods

A total of 21 calves presented with joint issues were screened for the presence of knuckling. Out of which 16 calves met with the inclusion criteria were included and divided equally into two groups. In all the animals, premedication was done by administering meloxicam (0.1 mg/kg body wt), chlorpheniramine maleate (0.5 mg/kg body wt) and amoxicillin sodium and sulbactam sodium (12.5 mg/kg body wt) administered intramuscularly. The anaesthesia was induced in both groups with Inj. Butorphanol (0.05 mg/kg) intramuscularly and Inj. Midazolam (0.2mg/kg) intravenously. After 10 minutes, Inj. Ketamine (5mg/kg) was used for induction. Calves under Group I calves underwent Z-tenotomy, while Group II received Z-tenotomy combined with a single PRP injection at the tendon site during the surgery. Post-operative immobilization affected limbs by applying plaster of Paris cast. In both groups a small window was made in POP at surgical site. The clinical effectiveness of Platelet-Rich Plasma (PRP) during tendon healing was evaluated by assessing various parameters such as clinical and physiological factors, wound healing, weight bearing, locomotion, and hematological-biochemical analysis.

Physiological parameters like rectal temperature, heart rate and respiratory rate, haematological parameters like Hb, PCV, TEC, TLC were recorded 0^{th} (zero), 3^{rd}, 7^{th} and 14^{th} post-operative days. Biochemical estimations such as Calcium (mg/dL) Magnesium (mg/dL), Vitamin D_3 (ng/ml), Zinc (µg/dl) and Alkaline Phosphatase (IU/L) were done on 0^{th} and 21^{st} postoperative day. Affected limbs were subjected to pre-operative radiography, post-operative tendography and ultrasound examination. Data gathered for this study was statistically analyzed using ICAR WASP 2.0 software's Two-Way Factorial Experimental Design and Two Sample t-test.

Results and Discussion

This study revealed that the contracted tendon deformity constituted 92% of the overall joint affection during the study period with bilateral deformity being most commonly occurred (87.5%) followed by unilateral contracted tendon (12.5%) (Table 1). Nine calves (56.25%) out of 16 were males and seven calves (43.75%) were females (Table 2).It might be because of male calves have more birth weight than females, there may be weight disparity between fetus and mother limiting the fetus mobility within the uterus and eventfully leading to the development of contracted tendon These observations are in accordance with Chakraborty *et al.*, (2023) [2].

Table 1: Unilateral/Bilateral incidence of contracted tendon

Total no of Calves	Calves with bilateral Contracted tendon	Calves with unilateral Contracted tendon
16	11 (87.5%)	5 (12.5%)

Table 2: Sex wise incidence of contracted tendon.

Total no of Calves	Males	Females
16	9(56.25%)	7(43.75%)

The physiological parameters, including temperature and respiration rate, remained within the normal physiological range of the calves from both the groups on the day of presentation and throughout the postoperative period. Heart rate values were manifested a statistically non-significant increase on 3^{rd} and 7^{th} postoperative day. It might be due to the release of corticosteroid in a state of stress, surgical manipulation and slight inflammation at the surgical site. But there was statistically insignificant drop of this value on 14^{th} day, this might be due to the gradual decrease in the inflammatory reaction. These results are in unison with Mander, (1999), Ochube *et al.*, (2014) and Vasanthkumar, H. (2017) [8, 10, 14].

Postoperatively, no calves exhibited knuckling. It was observed that by day 14, all calves in Group II achieved full weight bearing and were essentially not lame, whereas calves in Group I reached full weight bearing after 14 days postoperatively, with the exception of case number 8 (Figure 2 and 3).

Fig 2: Calf exhibiting proper angulation and weight-bearing capability on the third post-operative day

Fig 3: Calf exhibiting proper angulation and weight-bearing capability on 7th post-operative day

Notably, by the 14th postoperative day, all calves showed no swelling in the affected limb, except for case number 8 in Group I. Furthermore, no calves in Group II, except for case number 4, exhibited signs of pain by day 14. In contrast, six calves in Group I showed mild pain, while two calves (case numbers 3 and 6) displayed no pain by the 14th postoperative day (Figure 4).

Fig 4: Calf exhibiting weight bearing capability with normal ambulation on 14th post-operative day

The results of group I are corroborated with the findings of Mander, P. S. (1999) [8] who also stated that the high pain, swelling on day '0' might be due to the inflammatory reaction initiated by the tenotomy followed by tenorrhaphy. This inflammation gradually subsided at the end of the study indicated by significant reduction in pain and swelling and increasing the weight bearing capacity and locomotion.

Postoperatively more rapid reduction in pain scores, swelling, increase in the weight bearing capacity and locomotion was observed in all calves in Group II as compared to those in Group I. This may be attributed to the administration of a single PRP injection at the surgical site during tenorrhaphy, which led to an increase in growth factors, enhanced anti-inflammatory mediators, improved collagen production, and antimicrobial activity. Additionally, it contributed to the strengthening of healing tissues by releasing key structural proteins. These findings are consistent with those reported by Madison (2024) [9], who also noted the efficacy of PRP in the repair of tendon, joint, and ligament injuries in horses.

Regarding hematological parameters, i.e. hemoglobin, packed cell volume, total erythrocyte count and total leucocyte count, a statistically non-significant increasing trend was observed throughout the study period. The current haematological findings align with Kumar *et al.* (2014); Mander, (1999) and Vijay *et al.* (2022) [7, 8, 15]. This non-significant increase may be attributed to erythropoiesis and the effects of the haematinic supplement used as supportive therapy. TLC values showed a statistically significant increase on day 3, which may be attributed to the release of corticosteroids during stress, anesthesia, surgical manipulation, and mild inflammation at the surgical site. A statistically significant decrease in the TLC values was observed thereafter, likely reflecting the gradual reduction in the inflammatory response. Despite these fluctuations, the leucocyte counts remained within normal physiological limits throughout the study period at all intervals in both groups. Additionally, lymphocyte, monocyte, neutrophil, eosinophil, and basophil count showed statistically insignificant changes, though all values were within normal physiological limits. These findings align with those of Vijay *et al.* (2022) [15] who observed no significant changes in lymphocyte counts. Similarly, Mander PS (1999) [8] reported non-significant changes in lymphocyte counts across the four groups, with values fluctuating within normal physiological limits throughout the study period (Table 3).

Table 3: Haematological parameters of calves under both the groups at different intervals

Parameter	Group	Day 0	Day 3	Day 7	Day 14
Hb	Group 1	11.26±0.42	11.27±0.44	11.33±0.44	11.33±0.44
	Group 2	11.75±0.29	11.93±0.27	12.0±0.28	12.17±0.28
PCV	Group 1	35.31±1.2	35.25±1.2	35.27±1.2	35.27±1.25
	Group 2	36.55±0.72	36.35±0.77	36.51±0.79	36.67±0.79
TEC	Group 1	7.28±0.32	7.35±0.33	7.56±0.39	7.86±0.42
	Group 2	6.49±0.16	6.54±0.17	6.58±0.16	7.12±0.30
TLC	Group 1	7.03±0.51	7.71±0.49	7.32±0.44	7.04±0.49
	Group 2	7.06±0.44	7.6±0.42	7.20±0.45	7.09±0.44
Lymphocyte	Group 1	54.15±2.37	55±2.38	54.87±2.32	54.99±2.41
	Group 2	54.25±2.33	55.12±2.31	54.99±2.34	55.11±2.44
Monocytes	Group 1	4.91±0.21	4.44±0.18	4.39±0.25	4.29±0.34
	Group 2	4.8±0.29	4.52±0.25	4.44±0.18	4.33±0.3
Neutrophils	Group 1	23.16±1.07	23.39±1.09	23.72±1.41	23.42±1.30
	Group 2	22.88±1.21	23.16±1.19	23.±1.47	23.45±1.29
Eosinophils	Group 1	11.12±0.60	11.38±0.82	11.52±0.73	11.44±0.69
	Group 2	11.37±0.50	11.58±0.72	11.58±0.70	11.4±0.70
Basophils	Group 1	1.2±0.18	1.32±0.10	1.1±0.02	1.3±0.308
	Group 2	1.23±0.17	1.35±0.12	1.22±0.11	1.32±0.305

Table 4: Biochemical parameters of calves under both the groups at different intervals

Parameter		Day 0	Day 21
Calcium	Group 1	10.04±0.25	10.22±0.25
	Group 2	10.017±0.36	10.29±0.34
Magnesium	Group 1	2.3±0.12	2.45±0.10
	Group 2	2.41±0.15	2.45±0.41
Vitamin D3	Group 1	15.02±2.49	35.09±6.92
	Group 2	18.46±2.8	32.84±3.12
Zinc	Group 1	38.60±5.66	54.70±3.83
	Group 2	37.76±5.1	55.89±3.28
ALP	Group 1	145.60±1.88	142.61±2.23
	Group 2	143.48±1.83	142.32±2.02

Biochemical analysis of serum revealed a non-significant increase in Calcium and Magnesium levels (Chakraborty *et al.*, 2023) [2]. Alkaline phosphatase values showed a non-significant decrease by day 21. However, these values

remained within normal reference limit. Present findings are aligned with findings of Kumar *et al.* (2014) [7]. They also observed a non-significant decrease in alkaline phosphatase levels in calves (Table 4).

On the day of presentation, the mean levels of zinc and vitamin D3 were below the normal physiological range, but by the 21st day, both showed a statistically significant increase due to oral supplementation of supportive therapy containing vitamin D3 and zinc during postoperative period. These findings are in harmony with those of Genccelep *et al.* (2019) [4] who reported lower zinc levels in calves affected by contracted tendons compared to healthy calves. They suggested that zinc deficiency, which has been linked to myotonic dystrophy type II, could contribute to flexural deformity in calves.

Plain radiographic examination of all the affected calves with contracted tendon on the day of presentation revealed that fetlock joint was only involved in all these cases of newborn calves (Figure 5).

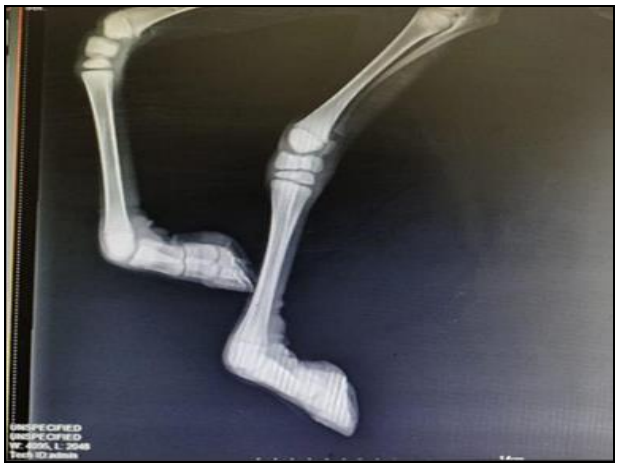

Fig 5: Pre-operative plain radiograph on the day of presentation

Post-operative tendograms on day of POP cast removal revealed restoration of continuity across the tendon evidenced by the clear demarcation among these structures (Figure 6).

Fig 6: Post-operative contrast radiograph on the day of plaster removal

Ultrasonograms on day of POP cast removal revealed longitudinal smooth hypoechoic pattern of operated tendon. Demarcation was well established between SDFT-DDFT (Figure 7).

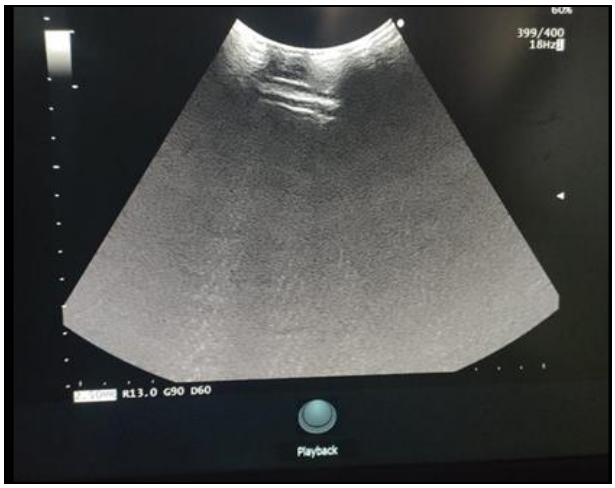

Fig 7: Post-operative ultrasonograms on the day of plaster removal

No intra-operative complications were recorded during the study period. However, resolvable postoperative complication like wound dehiscence was observed in case 8 from group I due to self-mutilation caused by the affected calf.

Functional outcome was evaluated based on clinical parameters such as pain, swelling, weight bearing at standing posture, lameness score, weight bearing in motion. It was found to be excellent in thirteen cases, good in two cases and poor in one case (case 8) from group I.

Conclusion

It can be concluded that administration of a single injection of PRP at the site of the tenotomy promotes faster tendon healing, stronger tendons and reduction in infection rates. It also helps in managing post-surgical inflammation, swelling and alleviates surgery-associated pain, making the recovery process more efficient.

Conflict of interest

Not available

Financial support

Not available

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