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# Turnover Flap of External Rectus Sheath as Autologous Graft for Correction of Scrotal Hernia in Rams

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ABSTRACT

The objective of this study was to evaluate the outcomes of external rectus sheath turnover flap (ERF) with and without platelet-rich plasma (PRP) for reconstruction of large scrotal hernia in rams. A total of 13 baladi rams suffered from large sized unilateral voluminous scrotal swellings were studied. All rams were randomly allocated into two groups according to the type of surgical interference; group ERF (n=6) were subjected to surgical correction by external rectus sheath turnover flap while group ERF-PRP (n=7) were subjected to surgical correction by ERF with PRP. The scrotal hernia was successfully corrected, and the graft turnover procedure was easily carried out in all rams. Clinical assessment of pain and heat in ERF-PRP depicted the lowest score at all time points compared to ERF group. Hematological analyses showed significant changes in white blood cells, neutrophil, lymphocytes, eosinophils, basophils count, serum amyloid A, haptoglobin, tumor necrosis factor-α and interlukin-1β in ERF-PRP group (lower) when compared to ERF group at days 3 and 7 postoperative. Ultrasonographic examination of all rams in the ERF and ERF-PRP groups showed that the graft was fixed and correctly positioned. Ultrasound color doppler recorded higher vascularity in ERF-PRP in the form of a high-color signal at the margins of turnover flap at days 7, 21 and 35-days post-operative. Conclusively, autologous grafting with ERF, is a simple performance procedure, with low cost impact and low expected complications. ERF-PRP's showed dominance over the ERF and could be used as a novel autologous composite for reconstruction of large scrotal hernia in ram.

## 1. INTRODUCTION

Surgical treatment of large abdominal wall defects and their complications in ruminant remains an area of debate. Scrotal hernia accounts for approximately 23.5 percent of common abdominal wall defects in small ruminants and has several detrimental impacts (Pathak and Poston, 2006; *Greber et al.*, 2013). Scrotal hernia correction through surgical intervention experienced many complications, including dehiscence, rapture, seroma, mesh extrusion, enterocutaneous fistula, erosion, adhesion, contraction, and recurrence (Langbach, 2015; Cai *et al.*, 2018; Nasralla and Tsang, 2019). Recently, autografting has been widely used in human and veterinary practice to correct various abdominal defects (Lee and Lee, 2014). Autografting showed promising results, including battling infection, high

healing rate, decreased adhesion and contraction (Pratummintra *et al.,* 2013; Jang *et al.,* 2014). The most frequently used loco-regional flaps in autografting involves external oblique muscle, tensor fascia lata, rectus abdominis muscle, rectus femoris muscle, latissimus dorsi muscle, omental flaps and tunical vaginalis (Jang *et al.,* 2014). Although scarce literature addressed the usefulness of autografting in veterinary surgery (Abdelwahed et al. 2012), the anterior rectus sheath turnover flap was used to repair large abdominal defects in human surgery (Kushimoto *et al.,* 2007). Increasing response of tissue and graft incorporation after autografting was achieved through the use of various forms of plasma products as growth factor source. The use of plasma products acts as a scaffold for formation of new fibrovascular tissues and consequently improved mechanical strength, and reduced hernia recurrence incidence (Dubay *et al.,* 2004; Bielecki et al., 2007). One of the widely used plasma products in autografting is platelet-rich plasma (PRP), which considered a rich source of several growth factors including: platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), growth factor insulin (IGF) and hepatocyte growth factor (HGF) (Dean et al., 2015). These factors can control cellular processes such as chemotaxis, angiogenesis, cell proliferation, mitogenesis, differentiation and metabolism within injured tissue with maximum resistance and limited adhesion of tissue (Georg et al., 2010; Geburek et al., 2016; Zuffova et al., 2013). Treatments using PRP have been thoroughly studied in human medicine and experimental animal models to promote bone, tendon and ligament healing processes as well as intra-articular osteoarthritis care ( Dhillon *et al.,* 2012). Similarly, PRP was widely used in veterinary medicine to treat joint inflammation, tendon injuries and lower limb wounds ( Greci et al., 2009; Brossi *et al.,* 2015). Therefore, the purpose of this study was to clinically evaluate the outcomes of of external rectus sheath turnover flap as an autologous graft alone and as a novel autologous composite with autologous PRP for reconstruction of large scrotal hernia in ram.

## 2. Materials and methods

### 2.1. Ethics Statement and Animals

The study procedures were performed in accordance with recommendations of the guidelines for care and use of animals at the College of Veterinary Medicine, Benha University. The research protocol was approved by the Ethical Committee for Institutional Animal Use and Care of the College of Veterinary Medicine, Benha University (BUFVTM 15-09-2018). A total of thirteen baladi rams, aged 18 to 36 months and weighting between 21 to 62 kg were selected and included in this study. All rams were admitted to the veterinary hospitals at college of Veterinary Medicine, Benha University during the period from December 2018 till May 2020. The rams were suffering from large unilateral voluminous scrotal swelling (Figure 1A). All rams were clinically examined to exclude other diseases and to evaluate reducibility and hernial ring dimensions. Hernial ring sizes varied between 7 and 12 cm in width and 10-14 cm in length. Rams were divided randomly into two groups, group-A (n=6) underwent surgical correction with external rectus sheath turnover flap (ERF) of the same side and group-B (n=7) underwent surgical correction by ERF with PRP (ERF-PRP).

### 2.2. Pre-operative preparation and anesthesia

Before surgical intervention, all rams were fasted for 12 hours and water was withheld for 6 hours until surgery. The rams were premedicated using intramuscular injection of Xylazine hydrochloride (Xylaject ®, ADWIA, EL Sharkeya governorate, Egypt) as a pre-anesthetic sedative with a dose rate of 0.2 mg / Kg body weight. Rams were then restrained in dorsal recumbency and the surgical field was prepared aseptically and draped with sterile towels. Finally, 2% of Lignocaine hydrochloride (Depocain ®, Arabco, Cairo, Egypt) was infiltrated locally along the skin incision.

### 2.3. PRP preparation, quantification, and activation

PRP was prepared and activated according to (Efeoglu *et al.,* 2004**).** Briefly, baseline platelet counts were collected from all blood samples prior to processing and after PRP preparation. Platelet count was performed using hematology analyzer (Esco Micro Pte. Ltd., Changi, Singapore). The mean number of peripheral blood platelets count was $146.16\pm15.7\times10^6$ platelets/ml and the mean number of PRP platelets count was $431\pm32.1\times10^6$ platelets/ml. The mean number of platelets in PRP was 3.5 ± 0.4-fold greater than that of whole blood.

### 2.4. Operative techniques

Autologous grafting was accomplished in the ERF group through a prescrotal skin incision (10-15 cm) carried out over the hernial ring and extended along paramedin line (Figure 1B). The status of hernial sac and hernial ring was examined after skin and subcutaneous dissection of the tissue to confirm

the presence or absence of adhesions of hernial contents, testicles, and hernial sac. The spermatic cord was ligated by double transfixation ligatures and transected in-between for castration. Skin and subcutaneous layers were dissected anteriorly from the underlying external rectus sheath. U-shaped sheath flap was incised medially, anteriorly and posteriorly, and dissected from undelaying with flap dimensions varying depending on the dimensions of the defect (the flap was incised 2 cm larger than the ring) (Figure 1C). The flap was gently grasped posteriorly to repair the defect by on-lay technique using interrupted horizontal mattress suture pattern using XLG, No. 2 (Vicryl, Ethicon Ltd. U.K) (Figure 1D). In the ERF-PRP group, autologous grafting was accomplished using same procedures in the ERF group. Before suture fixation, the flap was soaked in activated PRP and the remainder of PRP added to the surface of flap and suture line after suture fixation and prior to closure (Figure 1E). Finally, chromic cat gut, No. 0 (Ethicon Ltd. U.K) was used to close the subcutaneous tissue including the base of scrotum, and then the skin was closed by interrupted horizontal mattress using silk No. 2 (Ethicon Ltd. U.K).

**2.5. Post-operative management**

Post-operatively, all rams were kept in warm, clean well-bedded area. Postoperative treatment lasted for 5 consecutive days, including daily wound dressing using 10% povidon iodine (Betadine ®, Nile Co., Cairo Egypt); and antibiotic spray of Bavitracin twice daily (Neomycin, Bacitracin Aerosol powder Spray 150 ml, ACDIMA International, Giza, Egypt). Broad spectrum antibiotic, 4ml/100 kg Pentomycin (Procaine benzylpenicillin and 200mg / ml dihydrostreptomycin, Univet Vet Pharmaceutical company, Newtownards, UK) and anti-inflammatory 2mg / kg b.wt Flunidine (Flunixin meglumine ®, Arabco, Cairo, Egypt) were also injected intramuscularly. A prophylactic dose of anti-tetanus serum 1500 IU was additionally injected subcutaneously. On the 12$^{th}$ post-surgical day, the skin stitches were removed. The feeding regimen began in low amounts of soft food, with a third in the first week, and gradually increased until regular feeding was resumed.

**2.6. Timeline, postoperative follow up and technique evaluation:**

The hernioplasty technique was evaluated by clinical inspection, hematological examination, and ultrasonography over a period of 5 weeks postoperative (as shown in **Table 1**).

**2.6.1. Clinical evaluation*:*** All rams were evaluated clinically to determine any complications, pain, and heat at surgical site using a modified grading scale system (as shown in **Table 1**).

**2.6.2. Haematological examination*:*** Two blood samples were collected by jugular vein puncture for both hematological and biochemical analyzes. The first blood sample was taken with anticoagulant (EDTA) for determination of total erythrocytes count (RBCs), hemoglobin (HB), packed cell volume (PCV), MCV, MCH, MCHC, WBCs, neutrophil, lymphocytes, monocytes, eosinophils, and basophils using hematology analyzer (Model XF9080, Perlong Medical Machine Co. Ltd, Nanjing, Chin). The second blood sample was centrifuged at 3,000 rpm for 10 minutes, and clear, non-hemolyzed serum samples were stored at −20°C until further testing. Acute phase proteins (SAA and Hp) and inflammatory cytokines (TNF-α and IL-1β) were assessed using commercially available ELISA Kits (CUSABIO Biotech Co. Ltd, Wuhan, China). Hematological examination was performed before (day 0) and 1, 3, 7, 14, 21, 28 and 35$^{th}$ day postoperative.

**2.6.3. Ultrasonography evaluation:** All rams were restrained in dorsal recumbent position and the site of operation was shaved and aseptically prepared with applying of a copious amount of coupling gel. Ultrasonography assessment was conducted using a transcutaneous approach using a portable ultrasound machine (Chison ECO3 Expert, Medical EXPO, Shanghai, China) with an adjusted 8.5 MHz linear transducer (Lacasta *et al.*, 2009). Ultrasonographically, quantitative measurement of subcutaneous edema and visceral adhesion was accomplished and graded according to a modified numerical scale system (Table 1). Colored flow Doppler ultrasonography was used for subjective analysis of the grafting site's vascularity using 2-4 MHz Convex transducer attached to the ultrasonography machine (SonoScape E2 color Doppler, Shenzhen, China).

**2.7. Statistical analysis**

Statistical analysis was performed using JMP® Pro 13 (SAS Institute Inc. Cary, NC, USA). Normality of the data distribution was evaluated by the Shapiro-Wilk test. Comparisons between groups at different time points were performed using a mixed-model ANOVA with the animal as a random effect and time-point as a repeated effect. Significance was set at ($p<0.05$) and all values were presented as the mean ± standard error (SE).

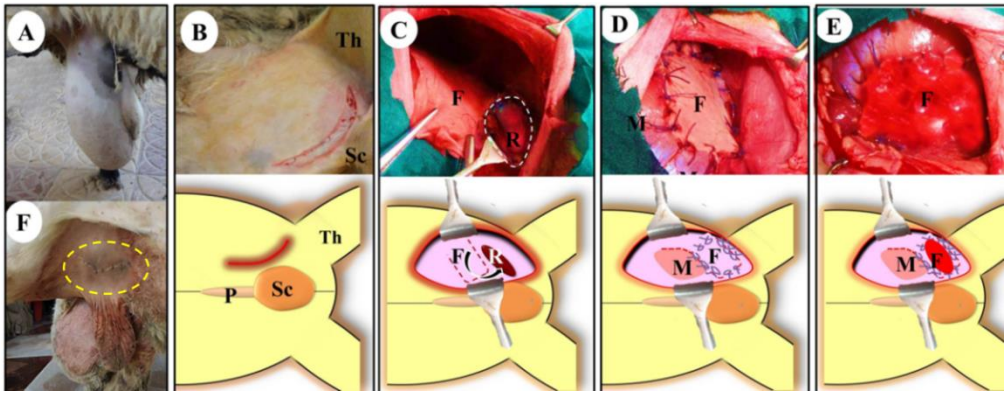


**Figure 1.** Illustrated admitted cases and step sequence of surgical correction of scrotal hernia by autologous graft using external rectus flap with and without PRP. A (admitted cases suffering from unilateral scrotal hernia); B (skin incision); C (Flap dissection); D (closure of the hernial ring with the flap); E (addition of activated PRP at the surface and periphery of turned over flap) and F (wound (yellow circle)at 10th day postoperative). P (penis), Sc (scrotal swelling), Th (thigh medial side), F (flap), R (inguinal ring) and M (rectus abdominis muscle).

**Table 1.** Index score for clinical and ultrasonographic evaluation in rams.

| Parameter | | Evaluation method | Timeline | Score | Description |
|---|---|---|---|---|---|
| Clinical evaluation | Pain | Catching a full skin and subcutaneous fold at the operation site between thumb & index for 30 sec. | Weekly | 0 | No pain (no response to stimuli) |
| | | | | 1 | Mild response (the animal try to move a way) |
| | | | | 2 | Moderate response (kicking) |
| | | | | 3 | Severe response (loud sound) |
| | Heat | Quantitative measuring using a local tape thermometer | Weekly | 0 | No (36-37) |
| | | | | 1 | Mild (37.1-38) |
| | | | | 2 | Moderate (38.1-39) |
| | | | | 3 | Severe (≥ 39.1) |
| | Complications | Clinical evaluation | Daily | 0 | No complications (local inflammatory edema) |
| | | | | 1 | Marked persistent postoperative edema |
| | | | | 2 | Skin dehiscence and sepsis formation |
| | | | | 3 | Graft dehiscence and reherniation |
| Ultrasonography evaluation | Subcutaneous edema | Measuring the depth from probe surface till the graft line. | Weekly | 0 | No (≤ 0.5 cm) |
| | | | | 1 | Mild (0.6 - 1 cm) |
| | | | | 2 | Moderate (1.1 - 1.5 cm) |
| | | | | 3 | Severe (≥ 1.6 cm) |
| | Subcutaneous & visceral adhesion | By image brightness analysis using dedicated software Image J *(Image J, 114 NACL Co. Ltd., and Tokyo, Japan)* | At end of the study | 0 | No (≤ 15 gray brightness value) |
| | | | | 1 | Mild (16-25 gray brightness value) |
| | | | | 2 | Moderate (25-50 gray brightness value) |
| | | | | 3 | Severe (≥ 51 gray brightness value) |

## 3. RESULTS

### 3.1. Clinical evaluation

All surgical procedures were easily performed and well tolerated by all rams from both groups. The scrotal hernia was successfully reduced, and graft turnover procedure was easily carried out in all rams. Clinical examination revealed significant difference in pain, heat and complication scores between both groups (Table 2). Pain and heat assessment in the ERF group showed mild to moderate score and with score 0.33± 0.02 at day 35. While the ERF-PRPgroup depicted the significantly ($p<0.05$) lower score of heat and painat all time points and with score 0 at day 28 (Table 2). No post-operative complications were observed in all rams in ERF-PRP group and the abdominal wall regained its normal integrity without any noticed abnormalities While in the ERF group, assessment of postoperative complication showed marked persistent postoperative edema with a subsequent stitch dehiscence, sepsis formation and closure failure in one ram.

### 3.2. Hematological examination

At day 0 before surgical intervention, no significant differences ($P>0.05$) were noted in any blood hematological and biochemical parameters among the ERF and ERF-PRP groups (Table 3). Assessment of hematological parameter showed that RBCs count, Hb content and PCV%, MCV, MCH, MCHC were non

significantly ($P$>0.05) decreased at day 1 post-operative compared to day 0 (Table 3). Surgical application of ERF and ERF-PRP did not have a significant effect on RBCs count, Hb content and PCV%, MCV, MCH, MCHC count (Table 3).

WBCs, neutrophil, lymphocytes, monocytes, eosinophils, and basophils count were significantly increased ($P$≤0.05) at day 1 post-operative when compared to day 0 (Table 3). The ERF-PRP group revealed significant low WBCs, neutrophil, lymphocytes, eosinophils, and basophils count when compared to the ERF group at days 3, and 7 (Table 3).Assessment of APP and proinflammatory cytokines in both groups were significantly increased at day 1 post-operative compared to day 0. The ERF-PRP group revealed significant low SAA, Hp, TNF-α and IL-1β levels when compared to the ERF group at days 3, 7, 14, 21, and 28 (Table 3).

**3.3. Ultrasonographic examination and adhesion score**

Ultrasonographic examination of all rams in the ERF and ERF-PRP groups showed that the graft was secured and correctly positioned. Postoperative ultrasonographic examination revealed that the rectus flap had a characteristic appearance of fine muscle fibers as an extension of thicker muscle of rectus abdominis accompanied by a slight attenuation of ultrasonic beam (Figure 2). Subcutaneous edema assessment in the ERF group showed mild to moderate edema and with score 1.00±0.00 at day 35. While the ERF-PRP group showed mild degree of edema and with score 0 at day 21. Adhesion evaluation at day 35 showed mild to no adhesion in the ERF-PRP group and mild to moderate adhesion in the ERF group (Table 2). The ERF-PRP group depicted the significantly (p<0.05) the lower score of subcutaneous edema and adhesion. Ultrasound Color Doppler recorded higher vascularity in the form of a higher color signal at the margins of turned over flap in the ERF-PRP group at days 7, 21 and 35-days post-operative (Figure 3).

**Table 2.** Clinical and ultrasonographic scoring of the operated rams (Mean±SE).

| Scored parameter | | Group | 7d | 21d | 28d | 35d |
|---|---|---|---|---|---|---|
| **Clinical scoring** | Pain | ERF-PRP | 1.29±0.49$^{1a}$ | 0.86±0.38$^{1a}$ | 0.00±0.00$^{2a}$ | 0.00±0.00$^{2a}$ |
| | | ERF | 2.00±0.00$^{1a}$ | 1.67±0.52$^{1b}$ | 0.67±0.52$^{2b}$ | 0.33 ±0.02$^{2b}$ |
| | heat | ERF-PRP | 0.86±0.38$^{1a}$ | 0.43±0.53$^{1a}$ | 0.00±0.00$^{2a}$ | 0.00±0.00$^{2a}$ |
| | | ERF | 1.67±0.52$^{1b}$ | 1.16±0.75$^{1b}$ | 0.67±0.52$^{2b}$ | 0.33 ±0.02$^{2b}$ |
| | Complications | ERF-PRP | 0.00±0.00$^{1a}$ | 0.00±0.00$^{1a}$ | 0.00±0.00$^{1a}$ | 0.00±0.00$^{1a}$ |
| | | ERF | 1.16±0.75$^{1b}$ | 0.67±0.02$^{1b}$ | 0.67±0.02$^{1b}$ | 0.67±0.02$^{1b}$ |
| **Ultrasonographic scoring** | Subcutaneous edema | ERF-PRP | 0.43±0.03$^{1a}$ | 0.00±0.00$^{2a}$ | 0.00±0.00$^{2a}$ | 0.00±0.00$^{2a}$ |
| | | ERF | 1.67±0.52$^{1b}$ | 1.5±0.55$^{1b}$ | 1.33±0.52$^{1b}$ | 1.00±0.00$^{1\,b}$ |
| | Visceral adhesion | ERF-PRP | | | | 0.57±0.53$^{a}$ |
| | | ERF | | | | 1.67±0.52$^{b}$ |

**Variable with different superscript numbers in the same row are significantly different at P≤0.05.**

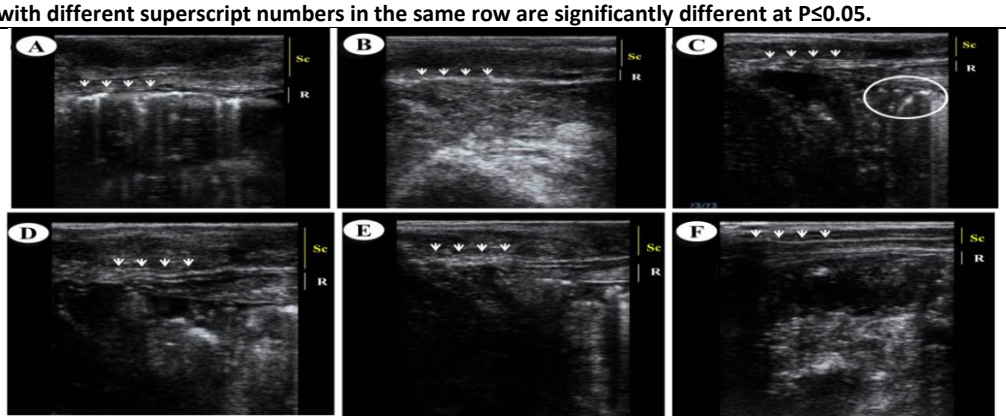


**Figure 2.** Ultrasonographic examination of ERF at 7$^{th}$ (A), 21$^{th}$ (B), 35$^{th}$ (C) days postoperative. Ultrasonographic examination of ERF-PRP at 7$^{th}$ (D), 21$^{th}$ (E), 35$^{th}$ (F) days postoperative. Arrows (flap), R (rectus abdominis muscle) and Sc (subcutaneous tissue edema), white circle (points of adhesion).

**Table 3.** Hematological examination of operated rams (Mean±SE)..

| Days | Group | 0 | 1 | 3 | 7 | 14 | 21 | 28 | 35 |
|---|---|---|---|---|---|---|---|---|---|
| **RBCs (×10³/µ)** | ERF | 5.95±0.49$^{1a}$ | 5.82±0.74$^{1a}$ | 5.08±0.61$^{1a}$ | 4.90±0.62$^{1a}$ | 5.02±0. 70$^{1a}$ | 6.01±0.50$^{1a}$ | 5.98±0.54$^{1a}$ | **6.00±0.57$^{1a}$** |
| | ERF-PRP | 6.06±0.52$^{1a}$ | 6.01±0.41$^{1a}$ | 5.50±0.49$^{1a}$ | 5.05±0.52$^{1a}$ | 5.91±0.31$^{1a}$ | 5.99±0.81$^{1a}$ | 6.00±0.21$^{1a}$ | **6.03±0.39$^{1a}$** |
| **Hb (g/dl)** | ERF | 12.49±0.64 $^{1a}$ | 12.63±0.71 $^{1a}$ | 10.43±0.55 $^{1a}$ | 9.01±0.43$^{1a}$ | 10.91±0.23$^{1a}$ | 11.99±0.31$^{1a}$ | 12.33±0.12 $^{1a}$ | **12.41±0.14$^{1a}$** |
| | ERF-PRP | 12.51±0.67 $^{1a}$ | 12.49±0.88 $^{1a}$ | 10.45±0.71 $^{1a}$ | 8.99±0.42$^{1a}$ | 10.29±0.47$^{1a}$ | 11.89±0.23$^{1a}$ | 12.40±0.21 $^{1a}$ | **12.49±0.57$^{1a}$** |
| **PCV %** | ERF | 28.78±0.86 $^{1a}$ | 28.53±0.81 $^{1a}$ | 24.21±0.63 $^{1a}$ | 20.48±0.45 $^{1a}$ | 22.87±0.61$^{1a}$ | 25.99±0.54$^{1a}$ | 27.79±0.31 $^{1a}$ | **28.63±0.42$^{1a}$** |
| | ERF-PRP | 29.12±0.97 $^{1a}$ | 29.10±0.73 $^{1a}$ | 25.12±0.65 $^{1a}$ | 20.86±0.56 $^{1a}$ | 23.11±0.43$^{1a}$ | 26.01±0.32$^{1a}$ | 28.10±0.22 $^{1a}$ | **29.09±0.60$^{1a}$** |
| **MCV (µm³)** | ERF | 45.35±2.09 $^{1a}$ | 45.22±1.74 $^{1a}$ | 45.08±2.61 $^{1a}$ | 49.65±1.02 $^{1a}$ | 49.12±0.79$^{1a}$ | 44.91±1.50$^{1a}$ | 45.20±2.54 $^{1a}$ | **45.28±1.57$^{1a}$** |
| | ERF-PRP | 46.04±2.52 $^{1a}$ | 46.01±1.41 $^{1a}$ | 45.90±1.29 $^{1a}$ | 50.75±1.52 $^{1a}$ | 50.51±1.91$^{1a}$ | 45.99±1.81$^{1a}$ | 46.00±1.21 $^{1a}$ | **46.03±1.39$^{1a}$** |
| **MCH (pg)** | ERF | 15.49±1.14 $^{1a}$ | 14.83±0.91 $^{1a}$ | 15.43±1.55 $^{1a}$ | 15.01±0.93 $^{1a}$ | 13.91±1.23$^{1a}$ | 14.99±1.31$^{1a}$ | 15.33±1.02 $^{1a}$ | **15.41±1.14$^{1a}$** |
| | ERF-PRP | 14.91±1.13 $^{1a}$ | 14.49±0.88 $^{1a}$ | 14.45±0.71 $^{1a}$ | 13.99±0.92 $^{1a}$ | 13.29±0.87$^{1a}$ | 14.89±1.03$^{1a}$ | 14.40±1.01 $^{1a}$ | **14.49±1.07$^{1a}$** |
| **MCHC (g/dl)** | ERF | 33.78±1.06 $^{1a}$ | 33.53±1.11 $^{1a}$ | 33.41±1.63 $^{1a}$ | 30.18±1.54 $^{1a}$ | 29.78±0.97$^{1a}$ | 32.99±0.84$^{1a}$ | 33.62±1.31 $^{1a}$ | **33.73±1.24$^{1a}$** |
| | ERF-PRP | 33.22±0.97 $^{1a}$ | 32.90±1.03 $^{1a}$ | 33.12±1.65 $^{1a}$ | 29.86±1.16 $^{1a}$ | 29.11±1.23$^{1a}$ | 32.89±1.10$^{1a}$ | 33.10±0.92 $^{1a}$ | **33.09±1.04$^{1a}$** |
| **WBCs (×10³/µ)** | ERF | 8.30±1.09$^{1a}$ | 11.82±1.24 $^{2a}$ | 11.98±1.51 $^{2a}$ | 9.75±1.12$^{2a}$ | 8.96±0. 79$^{1a}$ | 8.06±1.01$^{1a}$ | 8.20±1.21$^{1a}$ | **8.35±1.19$^{1a}$** |
| | ERF-PRP | 8.54±1.52$^{1a}$ | 12.21±1.1$^{2a}$ | 11.09±1.09 $^{2b}$ | 8.35±1.52$^{1b}$ | 8.01±1.10$^{1a}$ | 7.86±1.50$^{1a}$ | 8.20±1.54$^{1a}$ | **8.42±1. 75$^{1a}$** |
| **Neutrophils (×10³/µ)** | ERF | 4.59±0.14$^{1a}$ | 8.71±0.38 $^{2a}$ | 9.58±0.71$^{2a}$ | 7.56±0.29$^{1a}$ | 4.27±0.23$^{1a}$ | 4.33±0.31$^{1a}$ | 4.45±0.02$^{1a}$ | **4.61±0.14$^{1a}$** |
| | ERF-PRP | 4.62±0.13$^{1a}$ | 8.79±0.91$^{1a}$ | 7.97±0.55$^{2b}$ | 5.92±0.53$^{2b}$ | 4.05±0.78$^{1a}$ | 4.18±.031$^{1a}$ | 4.51±0.11$^{1a}$ | **4.58±0.17$^{1a}$** |
| **Lymphocytes (×10³/µ)** | ERF | 2.78±0.66$^{1a}$ | 3.60±1.03 $^{2a}$ | 3.41±0.65$^{2a}$ | 3.71±0.76$^{2a}$ | 2.96±0.23$^{1a}$ | 2.99±1.10$^{1a}$ | 2.99±0.92$^{1a}$ | **3.01±1.04$^{1a}$** |
| | ERF-PRP | 2.55±0.97$^{1a}$ | 2.88±0.19$^{2a}$ | 2.40±0.63$^{1b}$ | 2.00±0. 54$^{1b}$ | 2.73±0.57$^{1a}$ | 2.75±0.84$^{1a}$ | 2.85±0.31$^{1a}$ | **2.76±0. 24$^{1a}$** |
| **Monocytes (×10³/µ)** | ERF | 0.31±0.07$^{1a}$ | 0.36±0.07$^{2a}$ | 0.35±0.05$^{2a}$ | 0.36±0.06$^{2a}$ | 0.33±0.02$^{1a}$ | 0.34±0.03$^{1a}$ | 0.33±0.05$^{1a}$ | **0.34±0.06$^{1a}$** |
| | ERF-PRP | 0.28±0.02$^{1a}$ | 0.35±0.01$^{2a}$ | 0.35±01$^{2a}$ | 0.34±0.02$^{2a}$ | 0.35±0.03$^{2a}$ | 0.35±0.01$^{2a}$ | 0.35±0.0$^{2a}$ | **0.34±0.10$^{2a}$** |
| **Eosinophils (×10³/µ)** | ERF | 0.60±0.09$^{1a}$ | 0.64±0.08$^{2a}$ | 0.65±0.01$^{2a}$ | 0.64±0.10$^{2a}$ | 0.64±0.01$^{2a}$ | 0.62±0.10$^{2a}$ | 0.62±0.04$^{2a}$ | **0.62±0.03$^{2a}$** |
| | ERF-PRP | 0.62±0.07$^{1a}$ | 0.61±0.01$^{1a}$ | 0.59±0.07$^{2b}$ | 0.60±0.10$^{1b}$ | 0.61±0.01$^{1a}$ | 0.61±0.02$^{1a}$ | 0.60±0.01$^{1a}$ | **0.60±0.01$^{1a}$** |
| **Basophils (×10³/µ)** | ERF | 0.02±0.00$^{1a}$ | 0.01±0.00$^{2a}$ | 0.01±0.00$^{2a}$ | 0.01±0.00$^{2a}$ | 0.01±0.00$^{2a}$ | 0.01±0.00$^{2a}$ | 0.00±0.00$^{2a}$ | **0.01±0.00$^{2a}$** |
| | ERF-PRP | 0.02±0.00$^{1a}$ | 0.01±0.00$^{2a}$ | 0.00±0.00$^{2b}$ | 0.00±0.00$^{2b}$ | 0.01±0.0$^{2a}$ | 0.00±0.00$^{2a}$ | 0.01±0.00$^{2a}$ | **0.00±0.00$^{2a}$** |
| **Haptoglobin (µg/ml)** | ERF | 0.064±0.01 $^{1a}$ | 1.85±1.30$^{2a}$ | 1.67±1.21$^{2a}$ | 1.53±1.12$^{2a}$ | 1.03±1.01$^{2a}$ | 0.72±0.98$^{2a}$ | 1.52±0.69$^{2a}$ | **0.13±0.57$^{2a}$** |
| | ERF-PRP | 0.062±0.02 $^{1a}$ | 1.72±1.31$^{2b}$ | 1.51±1.28$^{2b}$ | 1.15±1.02$^{2b}$ | 0.83±0.1$^{2b}$ | 0.61±0.21$^{2a}$ | 0.36±0.19$^{2b}$ | **0.086±0.19$^{1a}$** |
| **Serum amyloid A (µg/ml)** | ERF | 3.36±0.39$^{1a}$ | 35.42±1.67 $^{2a}$ | 28.89±1.50 $^{2a}$ | 22.67±1.23 $^{2a}$ | 18.38±0.99$^{2a}$ | 13.26±0.53$^{2a}$ | 9.00±0.61$^{2a}$ | **5.34±0.46$^{2a}$** |
| | ERF-PRP | 3.87±0.51$^{1a}$ | 31.43±1.43 $^{2b}$ | 24.98±2.12 $^{2b}$ | 19.90±1.32 $^{2b}$ | 14.01±0.89$^{2b}$ | 9.13±0.72$^{2b}$ | 5.83±0.39$^{2b}$ | **4.01±0.35$^{1a}$** |
| **IL-1β (µg/ml)** | ERF | 16.26±3.67 $^{1a}$ | 40.01±3.11 $^{2a}$ | 36.50±4.49 $^{2a}$ | 32.90±3.62 $^{1a}$ | 27.72±2.97$^{1a}$ | 24.19±2.90$^{1a}$ | 21.36±2.64 $^{1a}$ | **17.08±2.57$^{1a}$** |
| | ERF-PRP | 15.96±2.8$^{1a}$ | 36.63±2.84 $^{2b}$ | 33.28±4.61 $^{2b}$ | 28.15±3.02 $^{1b}$ | 23.01±2.21$^{1b}$ | 20.36±1.89$^{1b}$ | 18.01±1.21 $^{1b}$ | **16.00±0.99$^{1a}$** |
| **TNFα (µg/ml)** | ERF | 7.58±1.48$^{1a}$ | 30.25±3.67 $^{2a}$ | 28.13±2.50 $^{2a}$ | 24.70±1.32 $^{2a}$ | 19.31±1.99$^{2a}$ | 14.81±1.53$^{2a}$ | 11.00±1.03 $^{2a}$ | **7.34±0.86$^{1a}$** |
| | ERF-PRP | 7.42±1.51$^{1a}$ | 27.58±3.64 $^{2b}$ | 25.42±2.92 $^{2b}$ | 20.07±1.29 $^{2b}$ | 15.05±1.89$^{2b}$ | 11.23±0.78$^{2b}$ | 8.13±0.59$^{1b}$ | **6.01±0.61$^{1a}$** |

Variables with different superscript letters at the same column are significantly different at P≤0.05
Variable with different superscript numbers in the same row are significantly different at P≤0.05.

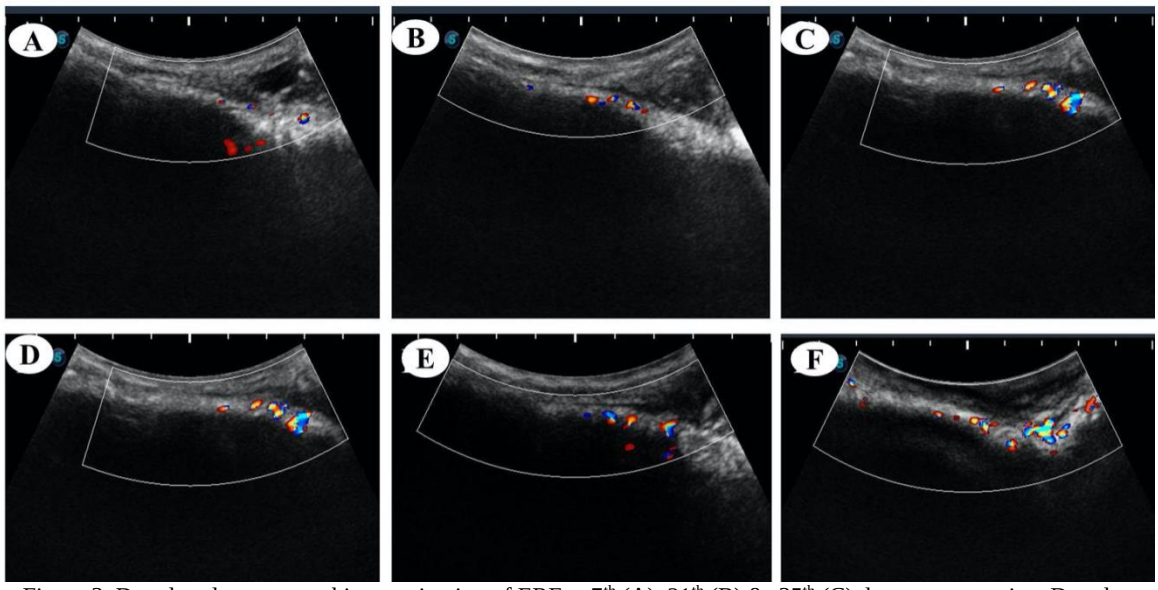


Figure 3. Doppler ultrasonographic examination of ERF at 7th (A), 21th (B) & 35th (C) days postoperative. Doppler ultrasonographic examination of ERF-PRP at 7th (D), 21th (E) & 35th (F) days postoperative.

## 3. DISCUSSION

Although no literature on the use of the external rectus sheath flap for the reconstruction of large abdominal wall defects, several techniques have been suggested in human surgery (Myeroff and Archdeacon, 2011; Kushimoto *et al.*, 2007**).** After grafting, tissue responses have been enhanced using PRP as a source of growth factor and chemokine (Sampson *et al.*, 2008; Malanga and Goldin, 2014**).** Therefore, the present study was planned to determine the effectiveness of external rectus sheath turnover flap as an autologous graft alone and as a novel autologous composite with autologous PRP for reconstruction of large scrotal hernia in ram.

Our results showed that the use of an autologous composite of external rectus sheath turnover flap with PRP to correct abdominal wall defects is correlated with improved clinical outcomes, increased closure strength, and subsequent decreased occurrence of reherniation and other related complications. Similarly, the use of ERF for the reconstruction of large abdominal wall defect was previously explored (Samir *et al.*, 2015). Additionally, no abdominal wall hernia requiring secondary repair without any prosthetic material strengthening the flap was reported (Takahashi *et al.*, 2007).

Evaluation of pain and local heat scores revealed that the rams of the ERF-PRP group experienced a significant lower pain and heat scores when compared to ERF group ($P$<0.05). This might be due to the inflammatory response-managing impact of PRP, that was identified by the significant ($p$<0.05) lower values of serum acute phase proteins (Hp and SAA) and inflammatory cytokines (IL-1β and TNFα) in ERF-PRP group. Several research articles have similarly studied the PRP's anti-inflammatory properties (Tothova *et al.*, 2014**).** PRP can suppress the release of cytokine, promote the release of RANTES, a major monocyte chemoattractant, from its alpha-granules, limit inflammation, encourage tissue regeneration and depress the inflammatory cells specially lymphocytes (El-Sharkawy et al., 2007; Agarwal *et al.*, 2016**).** Additionally, the corresponding functions of PRP correlates with substantial postoperative reductions in circulating lymphocyte in this study. Although one ram in the ERF group revealed marked persistent postoperative edema with a subsequent skin stitch dehiscence, and sepsis formation, the ERF-PRP group exhibited complete recovery without any complications. Recent studies have shown that platelets play a direct role in the detection, sequesteration, and neutralization of invasive pathogens and have indirect role in recruitment of leukocytes to inflammatory sites and killing of microorganisms by activating different signals pathways ( Choukroun *et al.*, 2006; Jenne and Kubes, 2015**).** This theory is confirmed in our study by a substantial postoperative high WBCS during the first three days in the ERF-PRP group.

Postoperative clinical follow up revealed no evidence of hernial recurrence in the ERF-PRP group and reherniation in one ram from the ERF group. The beneficial regenerative healing effects of PRP are due to the release of several growth factors component from the degranulated activated platelets including; PDGF, TGF, IGF, FGF, HGF and VEGF (Dean *et al.*, 2015)**.** Similarly, previous studies showed that the tensile strength of the graft-host scaffold is associated with single-dose PRP therapy involving the development of highly organized collagen and deposition of muscle fibers with predominant angiogenesis (Xu *et al.*, 2008; Van Eps *et al.*, 2016)**.**

In our study, ultrasonographic examination was used for diagnosis and follow-up the healing process following surgical correction of large scrotal hernia in rams. In line with other study, ultrasonographic assessment of graft-visceral and graft-subcutaneous adhesion was achieved, depending on the echogenic signal of the adhesion fibrous tissue (Smereczyński *et al.*, 2013). Ultrasonographic scoring of adhesion manifested advantage of PRP in hernia correction.The result is in accordance with that recorded in a rodent ventral hernia model (Van Eps *et al.*, 2016). This result is important because a decrease in the formation of adhesion would minimize complications such as chronic intestinal pain obstruction and entero-cutaneous fistulae, which would require more surgical intervention (Miller *et al.*, 2000).

In this study, the angiogenesis effect of PRP was confirmed by color doppler ultrasound, which reported higher vascularity in the form of a high-color signal at $7^{th}$, $21^{th}$, $35^{th}$ days post-operative on the margins of the turned over flap in the ERF-PRP group. Nevertheless, the exact role of PRP angiogenesis effect remains unclear. Likewise, (Van Eps *et al.*, 2016) recorded that the PRPs treated patients showed very solid, large, interconnecting networks of more mature neovessels from overlapping tissue, but clearly penetrating deeper into the implanted mesh. This enhanced angiogenesis tended to be linearly related to increased deposition / ingrowth of tissue within the mesh relative to controls. On the other hand, the color doppler ultrasound showed early vascular signals in the ERF group, which attributed to the flap's postoperatively remained clarified blood supply at the base of its U type. It was proposed that the blood supply to the anterior rectal sheath derive mainly from the perforation of intramuscular branches of deep upper and lower epigastric arteries and dispersed within the flap in the form of a broad capillary network (Kushimoto *et al.*, 2007)**.**

In conclusion; autologous grafting with external rectus sheath flap, is a simple performance procedure, with low cost impact and low expected complications. The findings of the current study also showed ERF-PRP's dominance over the ERF which could be used as a novel autologous composite for reconstruction of large scrotal hernia in ram.

**Conflict of interest**

The authors declare that they have no conflicts of interest to disclose.

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