MEASUREMENT OF STRESS LEVEL IN BUFFALOES BY THE EFFECT OF REPEATED RECTAL PALPATION IN TEACHING ENVIRONMENT

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ABSTRACT: Livestock contributed 60.1 percent to the agriculture value added and 11.5 % in overall GDP during 2020-21 (Economic Survey 2020-21). Livestock plays a major and vital role in socio economic development of rural region of country. According to economic survey of Pakistan, 42.4 million buffalo population is present in Pakistan. Small holding buffalo farming system is present in rural areas of Pakistan. Rectal palpation is the most useful method for conducting pregnancy diagnosis, artificial inseminations and detection of various reproductive anomalies in large domestic animals like cows and buffaloes. This procedure, however, can prove to be stressful and painful for these animals which raise an animal health and welfare issue. Blood picture in terms of changed levels of cortisol, glucose, cholesterol and blood cells may occur in animals subjected to this procedure. Similarly, behavioral symptoms of elevated heart rate and respiratory rates may also occur. The aim of the current project, therefore, was to study the effects of repeated rectal palpation-related stress response in blood constituents in buffaloes so that the appropriate counter measures could be adapted to save the animals from impending pain and stress. The study was conducted on ten buffaloes divided into two groups each i.e. experimental and control. Buffaloes were subjected to rectal palpation procedure. Blood samples were collected before and after rectal palpation for serum cortisol and complete blood cells count. Blood samples were also collected from buffaloes of control group without rectal palpation. Physical parameters were also noted. No statistically significant difference found in cortisol levels, CBC or physical parameters i.e. TPR that demonstrates that rectal palpation procedure inflicts no harm to buffaloes and can be used for the training of students.

Key words:

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INTRODUCTION

Rectal Palpation is the most convenient and cheap way for artificial insemination, cyesiognosis (pregnancy diagnosis), gynecologic conditions, and transplantation of embryos in large animals including cattle, buffalo, horses and camels. However, it can be painful inflicting stress to t h e animal. Rectal palpation of the uterus is used for the detection of postpartum uterine diseases (LeBlanc *et al*., 2002), during AI, and is the most common method, used for early diagnosis of pregnancy in dairy cattle (Romano *et al*., 2007). Transrectal palpation in large animals for diagnosing gynecological problems is a necessary skill for a veterinary student to learn. The procedure is usually quickly done by a skilled veterinarian, though, can last for five to ten minutes for an apprentice. Palpation of uterus per rectum is a non-traumatic procedure; however, it can result in physiological stress on palpated animals (Cingi *et al*., 2012; Nakao *et al*., 1994; Waiblinger *et al*., 2004). The word stress is much frequently used in everyday language, it is surprisingly difficult to define. Stress is generally a symptom resulting from an exposure of an individual to a hostile environment. It is an individual's physiological or biological response to a stressor such as adverse environment condition. Students of veterinary sciences are trained on experimentalanimals the world over where they perform substantial rectal palpation on such animals. Stress of various kinds on animals may lead to conditions that range from discomfort to death (Dantzer *et al*., 1983). Many researches have explored the quantity of stress by various means in different environments, however, a little work is done to gauge stress level in teaching environment. Due to the potential stress on animals, the repeated rectal examinations of animals by veterinary students is questioned for animal welfare reasons (Berghold *et al*., 2007). Studies have shown that mean heart rate increased during 5 minutes palpation of the reproductive tracts in cattle (Kovács *et al*., 2016; Kovács *et al*., 2014). Besides behavioral changes like heart rate and respiratory rates, changes in different blood constituents like the number of white blood cells (WBCs) and red blood cells (RBCs), the level of glucose and cortisol hormone and values of other parameters have been noted after rectal palpation in cattle. WBCs and Packed cell volume values were significantly increased following the rectal palpation procedure as compared to the initial values however, no difference was observed in hemoglobin concentrations and red blood cell levels. Serum cholesterol concentrations were decreased after rectal palpation while serum glucose and cortisol concentrations were markedly

increased (Cingi *et al*., 2012). An increased cortisol levels in saliva have been demonstrated before and immediately after the rectal palpation procedure in cows. However, this was not statistically significant (Steele. 2013). Behaviorally, more reactive animals exhibited increased plasma and salivary cortisol concentrations and a higher cardiac autonomic response to rectal examination than calmer ones (Kovács *et al*., 2016). An increased release of glucocorticoids in response to various physical and psychological stressors can have negative effects on growth, production, reproduction and immune system (Charmandari *et al*., 2005). The detrimental effect of stressors on buffaloes can pose economic inefficiency that ultimately borne by the producer and consumer (Burdick *et al*., 2011). The Rectal Palpation procedure offers many considerable benefits especially due to easy and minimally invasive, requires no special equipment and the results are immediate. Buffaloes can experience stress when confronted with something uncomfortable or frightening. Since procedures like rectal palpation can lead to discomfort and stress in livestock population, their productivity is reduced. The animal becomes incapable to achieve its genetic potential and this is reflected by poor productive and reproductive performance. Stress free herd is a far most important requirement in the economic efficiency of a dairy farm. Common biological materials for measuring cortisol levels are blood, saliva, milk, urine and feces. Plasma cortisol concentration is a physiological marker of stress. (Sathya *et al*., 2018) studied the stress of calving by measuring the increase in plasma cortisol concentration during the close up period peaked on the day of parturition. The very accurate method to quantify physiological stress response is to measure glucocorticoids level in blood (Harlow *et al*., 1990; Widmaier *et al*., 1994). Trans-rectal palpation in large animals including cattle, buffalo and camels is a day one competence for a veterinary student and is a necessary skill to learn. Undergraduate and graduate students in different veterinary institutions in Pakistan usually perform rectal palpation in large animals to be imparted work in relation to Theriogenology. Though innovative models have been developed to learn rectal palpation (Baillie. 2007) but they do not always give a real feel to technician's hand. They are expensive and may limit the ability to find new pathological conditions that one can find while palpating a real cow. Normally in a veterinary college, veterinary college rectal palpation is taught to fourth and fifth year DVM students. This procedure, however, can prove to be stressful and painful for these animals which raise an animal health and welfare issue. Nevertheless, so far there is no study that could provide quantity of stress contracted by animals by this frequent practice. The present study intends to monitor the stress response of buffaloes by rectal palpation so that the appropriate counter measures could be adapted to save the animals from impending pain and stress.

MATERIALS AND METHODS

In the present study, effect of stress on ten apparently healthy, non-pregnant, nonlactating buffaloes (Bubalus bubalis) subjected to repeated rectal palpations was studied. The buffaloes were housed in the animal sheds of Riphah College of Veterinary Sciences for two months prior to the initiation of study and no experiments carried out a month earlier than the study time. The buffaloes were of 5 to 6 years of age as determined by their incisor teeth emergence pattern. During the study tenure the animals were given same diet according to their nutritional requirements. Drinking water was available to buffaloes all the times and they were housed under the same environmental conditions. The procedure of rectal palpation was performed on five buffaloes using the traditional method for five to ten minutes for five consecutive days whereas five buffaloes were kept as control, in which no rectal palpation of uterus was performed.

Procedure of the rectal palpation was performed on experimental group of buffaloes, the procedure was performed using the traditional method for five to ten minutes for five consecutive days. The buffaloes of control group were not subjected to rectal palpation procedure.

Blood Sampling and Assay Procedures: Blood samples were collected from experimental buffaloes by direct jugular venipuncture in plain (red top) and EDTA treated (lavender top) vacutainers before and after the rectal palpation procedure. Blood Samples were also collected from control group of five buffaloes on which no rectal palpation was performed with an interval of one hour. Samples for CBC were immediately processed at RCVetS PG laboratory using an automated hematological analyzer URIT-2900 Vet Plus. Samples for blood serum cortisol level were centrifuged at 3000 rpm for 30 minutes and separated sera were then collected in eppendorf tubes stored at -18˚C till assay was conducted at AM Pets Care Lab. Blood Serum cortisol level was examined using ELISA kit as per the instructions provided by the manufacturer at AM Pets Care lab.

Physical Parameters: Rectal temperature was measured by using a clinical thermometer inserted in animal's rectum at a depth of approximately six cm's for one minute. Respiration rate and heart rate were recorded manually by using stethoscope and a stopwatch for 30 seconds and then multiplying the values with a count of two to get per minute results of both respiration and heart rates expressed in beats per minute (bpm) before and after performing the rectal palpation procedure in experimental buffaloes. The same observation was

recorded in control group buffaloes one hour later in which no rectal palpation was performed.

All samples for CBC were immediately processed at RCVetS PG Laboratory using URIT-2900 Vet Plus. URIT – 2900 Vet Plus is intended to determine different hematology parameters from 25 µl of whole blood sample. White blood cells, red blood cells and platelets are counted and sorted by the electrical impedance method; this method is based on the measurement of changes in the electrical impedance produced by the particle passing through the aperture. Analyzer sucks 25 µl of blood from the presented vacutainer and instantly generates printed report.

ELISA Procedure for Cortisol Measurement: Cortisol assay was conducted at AM Pets Care Lab located DHA phase II Lahore. Procedure of the cortisol assay is listed below: Prior to assay, allow reagents to stand at room temperature. Gently mixed all reagents before use and placed the desired number of coated strips into the holder. Took 25 µL of cortisol standards, control and patient's sera with pipette. 50 µL of Biotin reagent to all wells was added. 100 µL of cortisol enzyme conjugate to all wells was added. Thoroughly mixed for 10 seconds. Incubated for 60 minutes at room temperature (20 - 25 ºC). Liquid from all wells was removed. Wells were washed three times with 300 µL of 1X wash buffer. Absorbent was bloated on paper towels. 100 µL of TMB substrate to all wells was added. Incubated for 15 minutes at room temperature (20 - 25 ºC). 50 µL of stop solution to all wells was added. Plate was shaked gently to mix the solution. Read absorbance on ELISA reader at 450 nm within 20 minutes after adding the stop solution.

Statistical Analysis: Paired t- test and Split-way ANOVA were employed to analyze the statistical data. (Data presented as estimated marginal mean ± SE, %age) Data were analyzed by using Statistical Software (SPSS; version 20.0, IBM Corp. Armonk, NY) and for all statistical analyses, P-value $\leq$ 0.05 was considered significant.

RESULTS

Serum cortisol level:

Table 1.1: Effect of rectal palpation on serum cortisol level in the experimental buffaloes before and after rectal palpation.

Day	N	Before Mean ± SE (ng/ml)	N	After Mean ± SE (ng/ml)	P-value
1	10	26.06 ± 13.98	10	38.25 ± 19.22	N/S
2	10	30.54 ± 19.36	10	21.237 ± 4.84	N/S
3	10	31.24 ± 20.61	10	28.22 ± 11.93	N/S
4	10	19.61 ± 6.3	10	21.38 ± 7.15	N/S
5	10	25.47 ± 8.54	10	36.99 ± 7.51	0.0005

*(N/S = Non-significant)

Daily measurement of serum cortisol levels before and after rectal palpation procedure were not significantly affected from day first to day fourth of study while at day fifth cortisol level was increased. The graphical representation of this table is given following

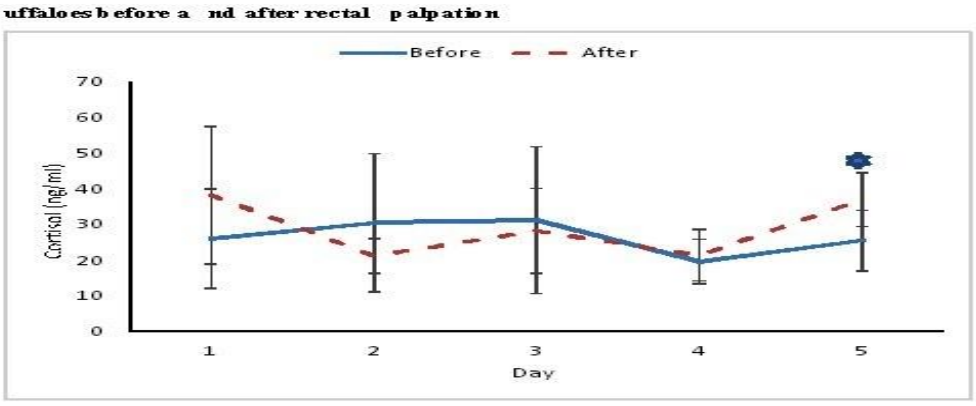


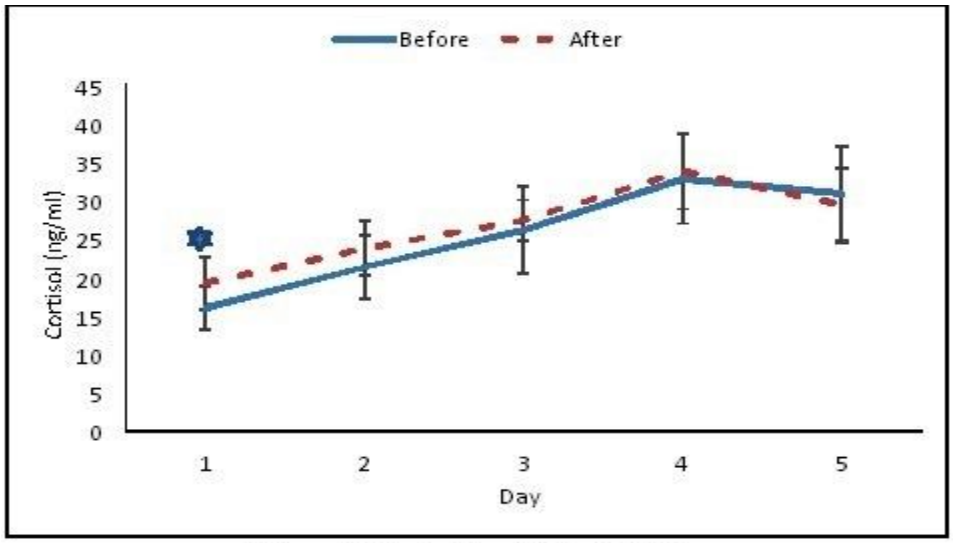
Figure 1.1: Effect of rectal palpation on serum cortisol level in the experimental

Table 1.2: Difference in serum cortisol level in the control buffaloes without subjecting them to rectal palpation.

Day	N	Before Mean ± SE (ng/ml)	N	After Mean ± SE (ng/ml)	P-value
1	10	16.01 ± 2.77	10	19.19 ± 3.44	0.05
2	10	21.31 ± 4.13	10	23.67 ± 3.58	N/S
3	10	26.12 ± 5.7	10	27.37 ± 2.61	N/S
4	10	32.72 ± 5.81	10	33.66 ± 4.84	N/S
5	10	30.75 ± 6.21	10	29.41 ± 4.64	N/S

*(N/S = Non-significant)

Data presented above depicts that difference in cortisol level was evident only on first day of study before and after rectal palpation in control buffaloes while no difference was found in later days of study. The graphical representation of this table is given below



*** Means differ significantly ($P < 0.05$).**

Figure 1.2: Difference in serum cortisol level in the control buffaloes without subjecting them to rectal palpation

Table 1.3: Difference in serum cortisol level compared between experimental group and control group.

Day	N	Experimental Mean ± SE (ng/ml)	N	Control Mean ± SE (ng/ml)	P-value
1	10	32.27 ± 11.93	10	17.6 ± 11.93	N/S
2	10	25.9 ± 8.56	10	22.49 ± 8.56	N/S
3	10	29.73 ± 28.17	10	59.85 ± 28.17	N/S
4	10	20.5 ± 6.03	10	33.19 ± 6.03	N/S
5	10	31.23 ± 6.71	10	30.08 ± 6.71	N/S

*(N/S = Non-significant)

Data presented above depicts that there was no significant difference in cortisol level between experimental and control group during the course of study. The graphical representation of this table is given below

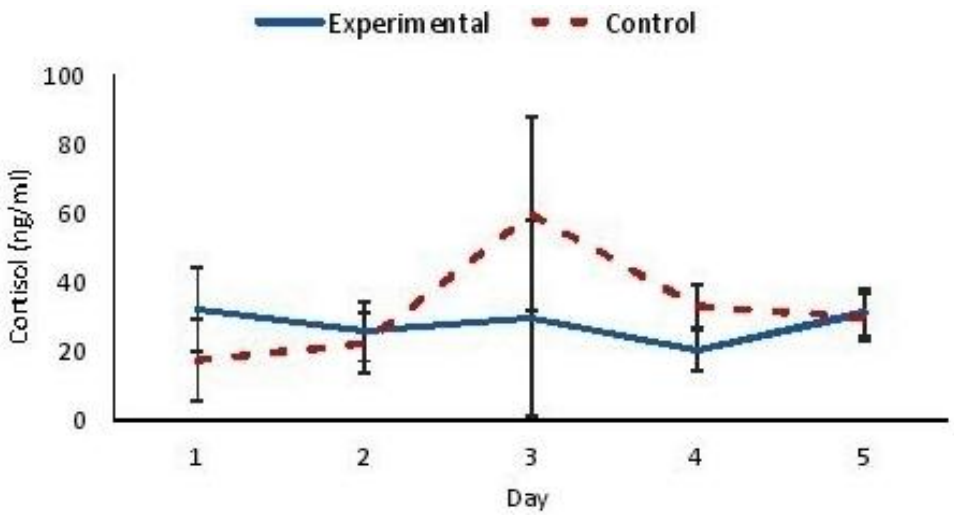


Figure 1.3: Difference in serum cortisol level compared between experimental group and control group

1. White Blood Cell Count:

Table 2.1: Effect of rectal palpation on white blood cell count in the experimental buffaloes before and after rectal palpation.

Day	N	Before Mean ± SE (10^9/l)	N	After Mean ± SE (10^9/l)	P-value
1	10	6.7 ± 0.36	10	6.86 ± 0.56	N/S
2	10	6 ± 0.05	10	6.27 ± 0.16	N/S
3	10	6.48 ± 0.29	10	6.76 ± 0.36	N/S
4	10	6.78 ± 0.15	10	6.76 ± 0.28	N/S
5	10	6.42 ± 0.47	10	6.98 ± 0.12	N/S

*(N/S = Non-significant)

No significant difference was observed in WBC count before and after rectal palpation procedure. The graphical representation of this table is given below

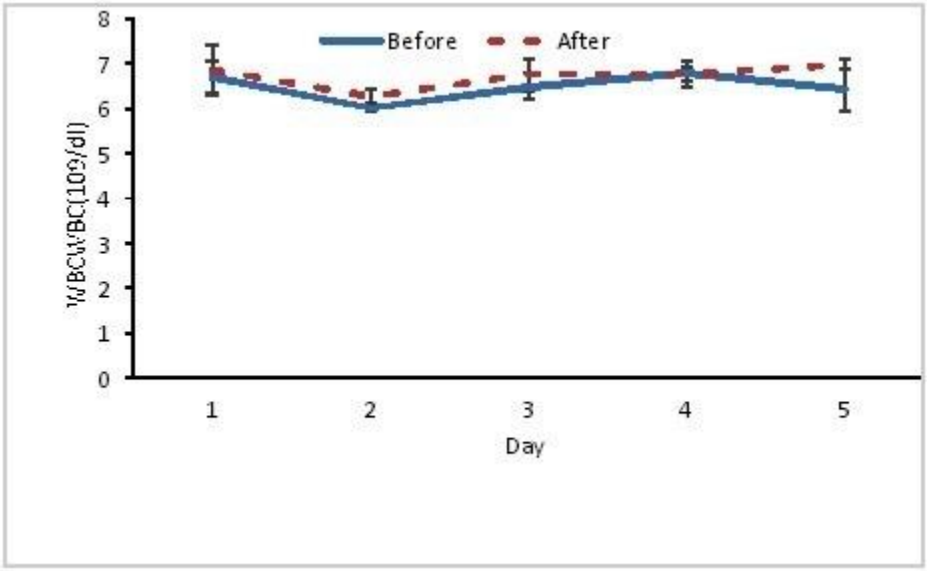


Figure 2.1: Effect of rectal palpation on white blood cell count in the experimental buffaloes before and after rectal palpation

Table 2.2: Difference in white blood cell count in the control buffaloes without subjecting them to rectal palpation.

Day	N	Before Mean ± SE (10^9/l)	N	After Mean ± SE (10^9/l)	P-value
1	10	5.72 ± 0.12	10	5.46 ± 0.11	0.03
2	10	5.24 ± 0.36	10	5.46 ± 0.3	N/S
3	10	5.79 ± 0.68	10	5.61 ± 0.18	N/S
4	10	5.81 ± 0.3	10	5.6 ± 0.16	N/S
5	10	5.92 ± 0.18	10	5.32 ± 0.07	0.007

*(N/S = Non-significant)

Significant rise in WBC count was observed at day 1 and day 5 between before and after rectal palpation in control group. The graphical representation of this table is given below

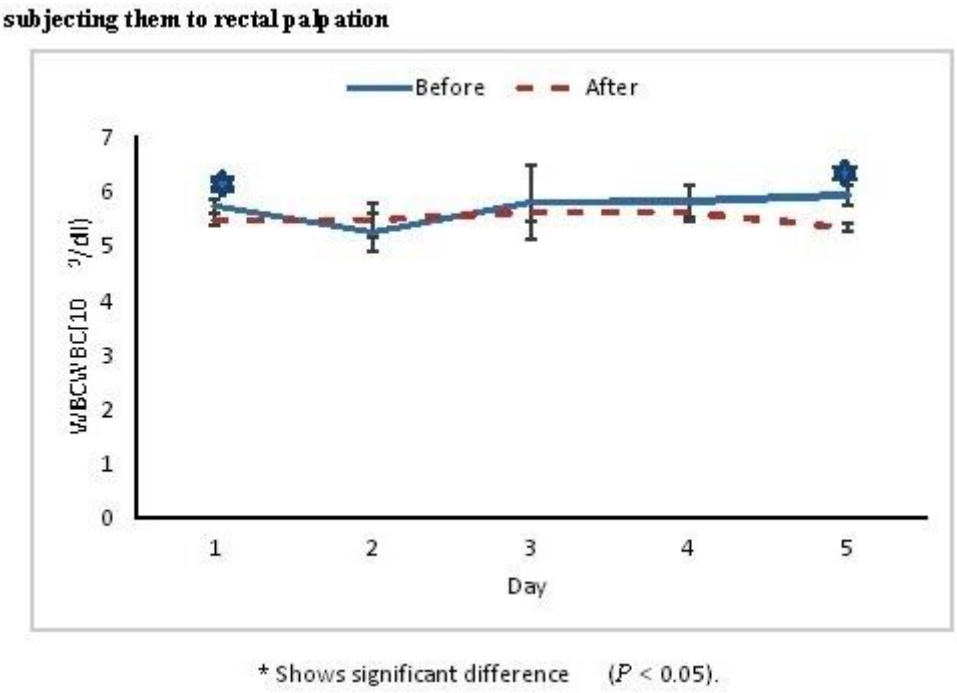


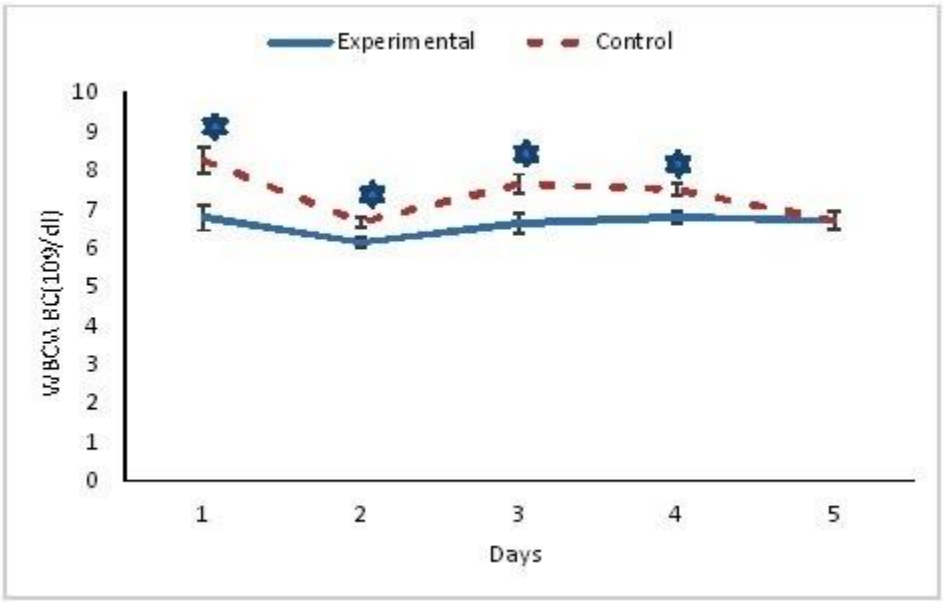
Figure 2.2: Difference in white blood cell count in the control buffaloes without

Table 2.3: Difference in WBC count compared between experimental buffaloes and control buffaloes.

Day	N	Experiment Mean ± SE (10^9/l)	N	Control Mean ± SE (10^9/l)	P-value
1	10	6.78 ± 0.33	10	8.25 ± 0.33	0.01
2	10	6.14 ± 0.14	10	6.66 ± 0.14	0.03
3	10	6.62 ± 0.26	10	7.64 ± 0.26	0.02
4	10	6.77 ± 0.16	10	7.5 ± 0.16	0.01
5	10	6.7 ± 0.22	10	6.7 ± 0.22	N/S

*(N/S = Non-significant)

Significant difference was observed in WBC count between experimental and control group except day 5. The graphical representation of this table is given below



***** Shows significant difference ($P < 0.05$).

Figure 2.3: Difference in WBC count compared between experimental buffaloes and control buffaloes

2. Red blood cell count:

Table 3.1: Effect of rectal palpation on red blood cell count in the experimental buffaloes before and after rectal palpation.

Day	N	Before Mean ± SE (10^{12}/l)	N	After Mean ± SE (10^{12}/l)	P-value
1	10	5.93 ± 0.31	10	6.37 ± 0.49	N/S
2	10	6.5 ± 0.12	10	5.82 ± 0.73	N/S
3	10	6.6 ± 0.2	10	6.09 ± 0.08	0.05
4	10	6.3 ± 0.25	10	6.21 ± 0.25	N/S
5	10	6.18 ± 0.36	10	6.12 ± 0.31	N/S

*(N/S = Non-significant)

Blood sample examined for RBC count in experimental buffaloes reflected significant difference only at day 3 of study. The graphical representation of this table is given below

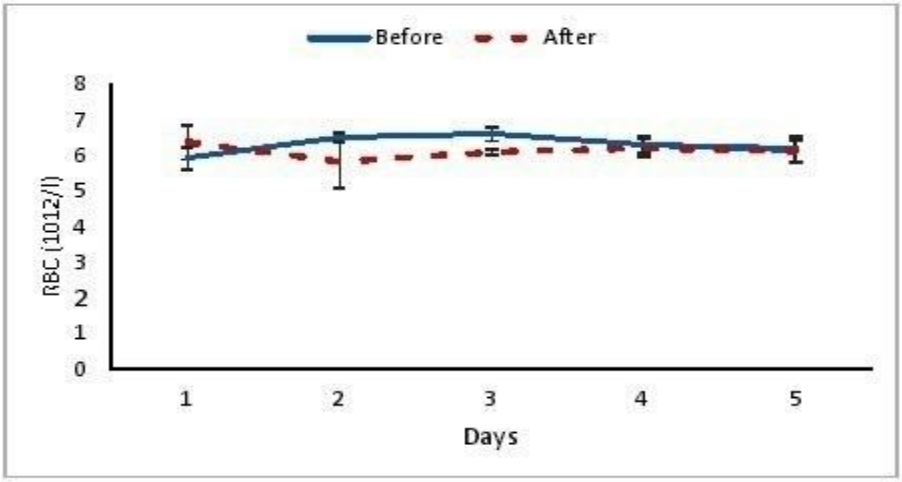


Figure 3.1: Effect of rectal palpation on red blood cell count in the experimental buffaloes before and after rectal palpation

Table 3.2: Difference in red blood cell count in the control buffaloes without subjecting them to rectal palpation procedure.

Day	N	Before Mean ± SE (10^{12}/l)	N	After Mean ± SE (10^{12}/l)	P-value
1	10	7.4 ± 0.42	10	9.1 ± 0.28	0.05
2	10	6.06 ± 0.41	10	7.26 ± 0.08	N/S
3	10	7.44 ± 0.22	10	7.84 ± 0.52	N/S
4	10	7.04 ± 0.21	10	7.96 ± 0.2	0.007
5	10	6.52 ± 0.06	10	6.88 ± 0.32	N/S

*(N/S = Non-significant)

Significant increase was observed in red blood cell count at day 1 and day 4 after rectal palpation procedure in control buffaloes. **The graphical representation of this table is given below**

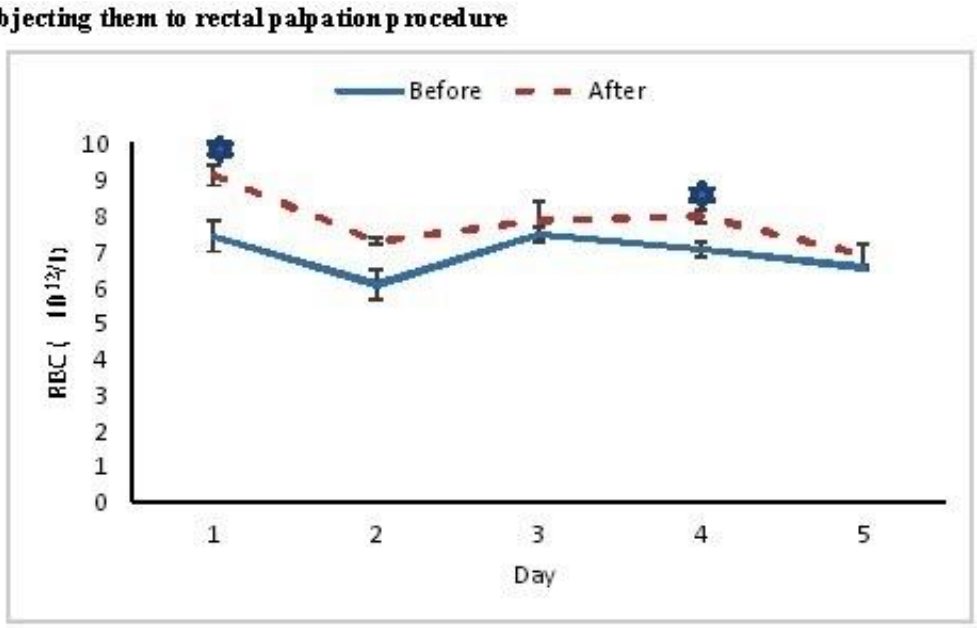


Figure 3.2: Difference in red blood cell count in the control buffaloes without

***** Shows significant difference **($P < 0.05$).**

Table 3.3: Difference in red blood cell count compared between the experimental buffaloes and control buffaloes

Day	N	Experiment Mean ± SE (10^{12}/l)	N	Control Mean ± SE (10^{12}/l)	P-value
1	10	6.16 ± 0.29	10	5.6 ± 0.29	N/S
2	10	6.19 ± 0.21	10	5.36 ± 0.21	0.02
3	10	6.35 ± 0.31	10	5.7 ± 0.31	N/S
4	10	6.25 ± 0.23	10	5.7 ± 0.23	N/S
5	10	6.15 ± 0.25	10	5.62 ± 0.25	N/S

*(N/S = Non-significant)

Red blood cell count showed no significant difference between experiment and control buffaloes except da 2 in which red blood cell count was significantly higher than control buffaloes. The graphical representation of this data is shown below

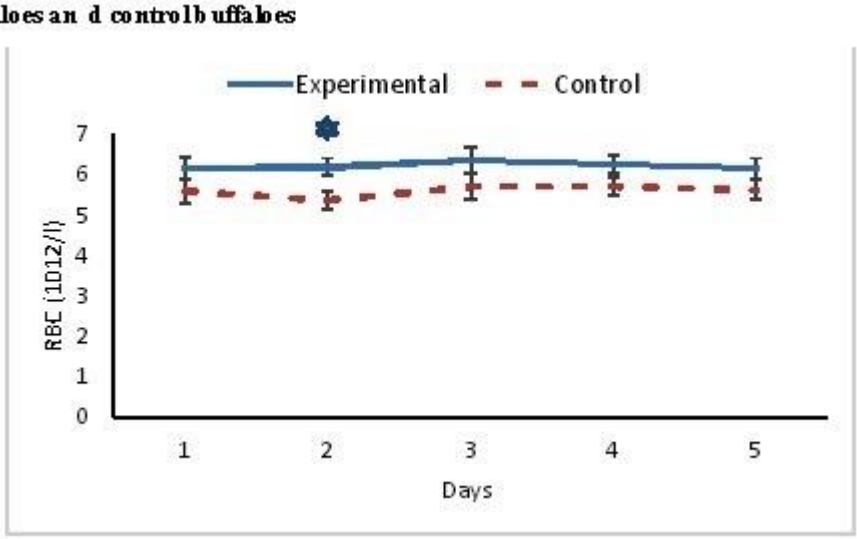


Figure 3.3: Difference in red blood cell count compared between the experimental
***** Shows significant difference **(*P* < 0.05).**

3. Hemoglobin:

Table 4.1: Effect of rectal palpation on Hemoglobin in the experimental buffaloes before and after rectal palpation.

Day	N	Before Mean ± SE (%)	N	After Mean ± SE (%)	P-value
1	10	10.38 ± 0.66	10	11.31 ± 0.77	N/S
2	10	11.96 ± 0.26	10	10.22 ± 0.56	0.03
3	10	12.36 ± 0.65	10	11.52 ± 0.36	N/S
4	10	11.34 ± 0.64	10	11.26 ± 0.56	N/S
5	10	11.16 ± 0.75	10	11.02 ± 0.68	N/S

*(N/S = Non-significant)

Daily measurements of blood hemoglobin levels before and after rectal palpation procedure were not significantly affected except day 2. The graphical representation of this data is given below

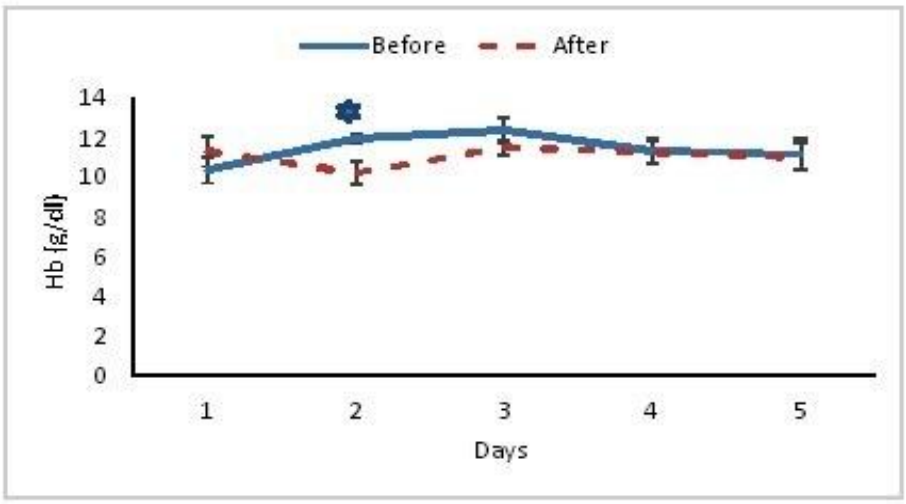


Figure 4.1: Effect of rectal palpation on Hemoglobin in the experimental
***** Shows significant difference **(*P* < 0.05).**

Table 4.2: Difference in Hemoglobin level in the control buffaloes in samples taken after an interval of one hour.

Day	N	Before Mean ± SE (%)	N	After Mean ± SE (%)	P-value
1	10	19.8 ± 5.51	10	18.74 ± 5.33	0.004
2	10	16.72 ± 3.96	10	18.74 ± 5.18	N/S
3	10	20.04 ± 5.58	10	19.54 ± 5.25	N/S
4	10	19.65 ± 5.19	10	19.32 ± 5.3	N/S
5	10	19.97 ± 5.25	10	18.12 ± 4.67	0.05

*(N/S = Non-significant)

Data presented above depicts that there was no significant difference in Hemoglobin level between before and after rectal palpation in control buffaloes except day 1 and day 5. **The graphical representation of this data is shown below**

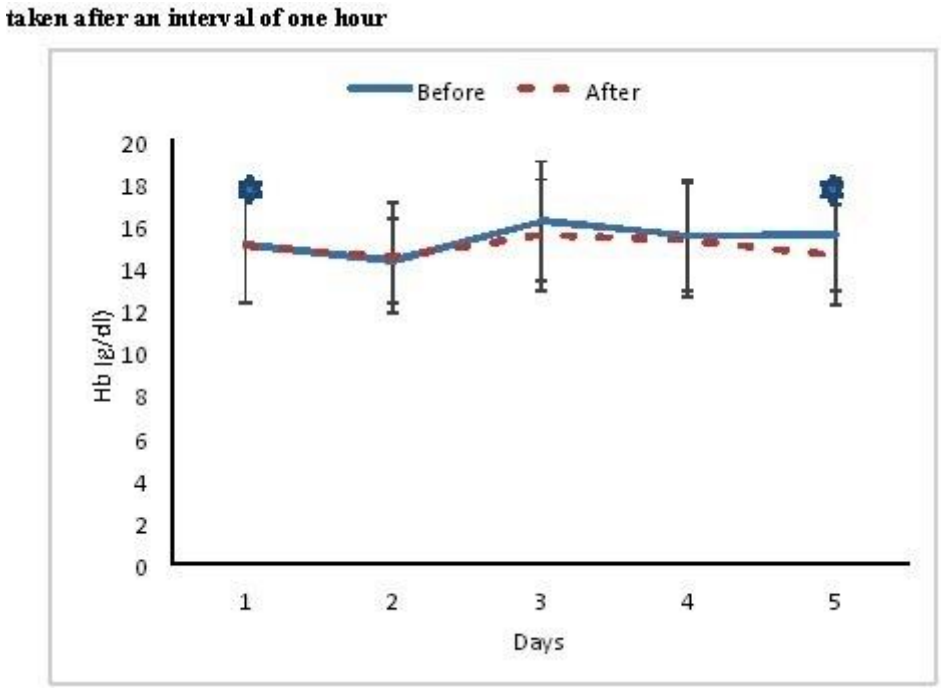


Figure 4.2: Difference in Hemoglobin level in the control buffaloes in samples

***** Shows significant difference **(*P* < 0.05).**

Table 4.3: Difference in Hemoglobin level compared between experimental buffaloes and control buffaloes.

Day	N	Experiment Mean ± SE (g/dl)	N	Control Mean ± SE (g/dl)	P-value
1	10	10.85 ± 3.87	10	19.27 ± 3.87	N/S
2	10	11.09 ± 3.2	10	17.73 ± 3.2	N/S
3	10	11.94 ± 3.83	10	19.79 ± 3.83	N/S
4	10	11.3 ± 3.73	10	19.49 ± 3.73	N/S
5	10	11.09 ± 3.54	10	19.05 ± 3.54	N/S

*(N/S = Non-significant)

No significant difference was observed between experimental and control buffaloes in hemoglobin level. **The graphical representation of this data is given below**

buffaloes and control buffaloes

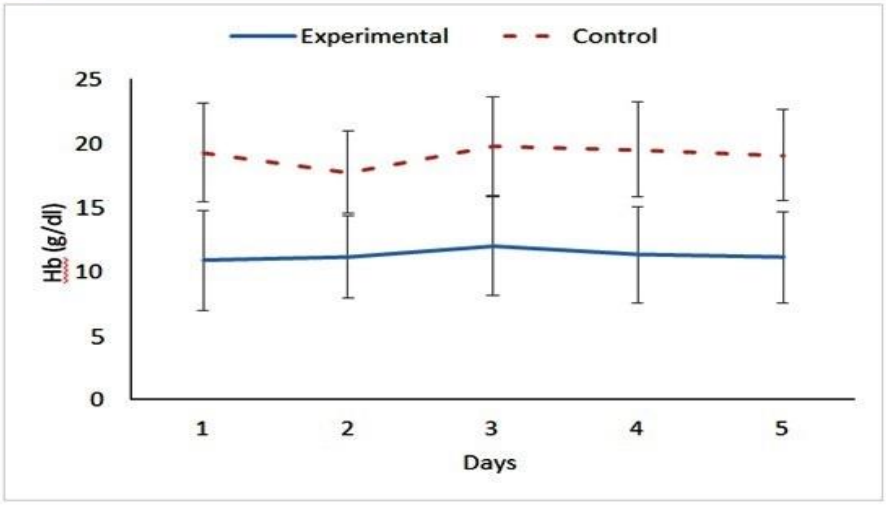


Figure 4.3: Difference in Hemoglobin level compared between experimental

(Data presented as estimated marginal mean ± SE, %age)

4. Hematocrit:

Table 5.1: Effect of rectal palpation on HCT in the experimental buffaloes before and after rectal palpation

Day	N	Before Mean ± SE (%)	N	After Mean ± SE (%)	P-value
1	10	33.1 ± 2.67	10	34.12 ± 2.81	0.05
2	10	35.1 ± 1.45	10	30.26 ± 1.88	N/S
3	10	36.1 ± 1.97	10	33.52 ± 0.84	N/S
4	10	34.12 ± 2.23	10	33.24 ± 1.88	N/S
5	10	33.52 ± 2.44	10	32.82 ± 2.26	N/S

*(N/S = Non-significant)

No significant difference was observed in HCT before and after rectal palpation procedure. The graphical representation of this data is given below

before and after rectal palpation

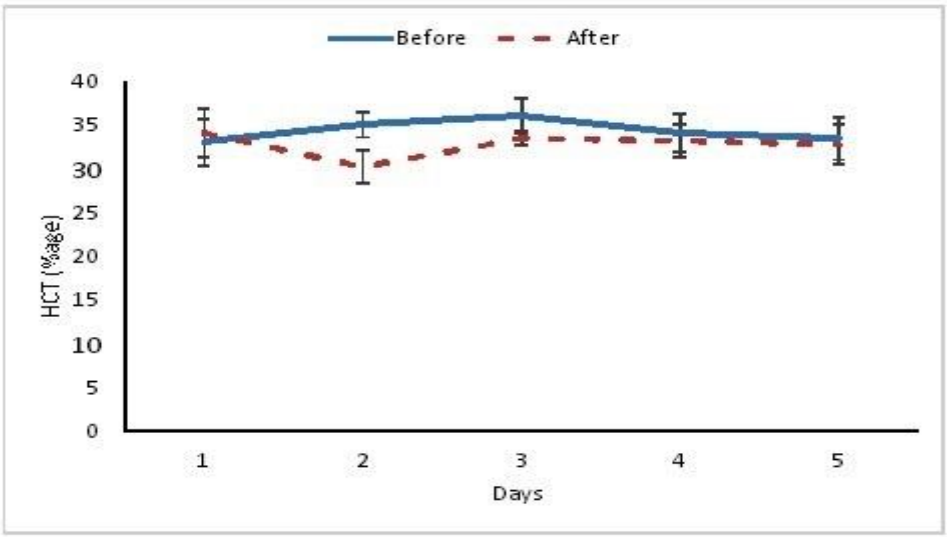


Figure 5.1: Effect of rectal palpation on HCT in the experimental buffaloes

Table 5.2: Difference in HCT in the control buffaloes without rectal palpation.

Day	N	Before Mean ± SE (%)	N	After Mean ± SE (%)	P-value
1	10	32.42 ± 0.65	10	30.98 ± 0.78	0.01
2	10	28.94 ± 1.9	10	30.58 ± 1.34	N/S
3	10	32.52 ± 3.48	10	31.54 ± 0.68	N/S
4	10	32.65 ± 1.33	10	31.61 ± 0.59	N/S
5	10	33.52 ± 0.63	10	30.18 ± 0.33	0.007

*(N/S = Non-significant)

Data presented above depicts that there was no significant difference in HCT level between before and after rectal palpation in control group except day 1 and day 5. The graphical representation of this data is given below

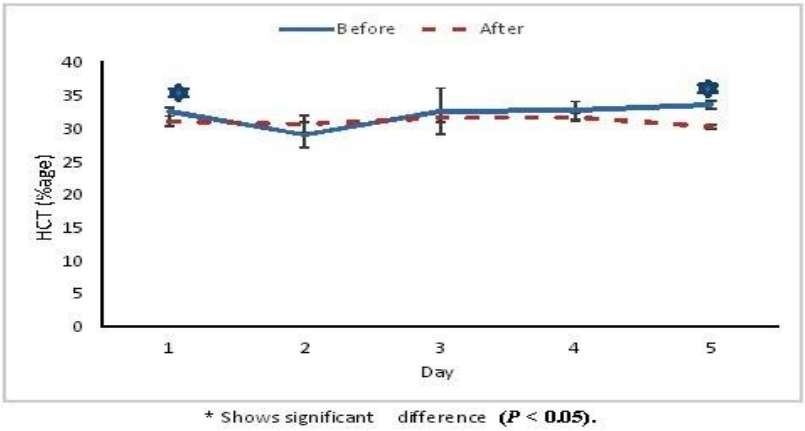


Figure 5 .Difference in HCT in the control buffaloes without rectal palpation

Table 5.3: HCT compared between the experimental buffaloes and control buffaloes.

Day	N	Experiment Mean ± SE (%)	N	Control Mean ± SE (%)	P-value
1	10	33.61 ± 2	10	31.7 ± 2	N/S
2	10	32.68 ± 1.27	10	29.76 ± 1.27	N/S
3	10	34.81 ± 1.74	10	32.03 ± 1.74	N/S
4	10	33.68 ± 1.57	10	32.13 ± 1.57	N/S
5	10	33.17 ± 1.67	10	31.85 ± 1.67	N/S

*(N/S = Non-significant)

No significant difference was observed between experiment and control buffaloes in HCT level. **The graphical representation of this data is given below**

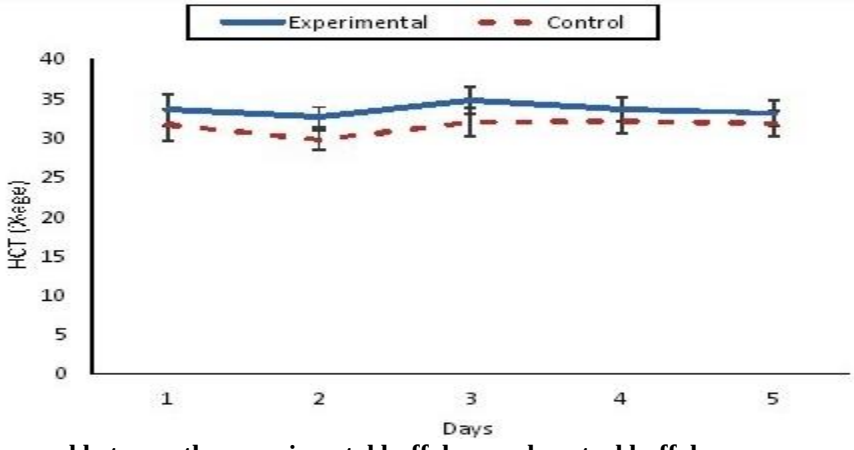


Figure 5.3: HCT compared between the experimental buffaloes and control buffaloes

5. Temperature:

Table 6.1: Effect of rectal palpation body temperature in the experimental buffaloes before and after rectal palpation.

Day	N	Before Mean ± SE (^{o}F)	N	After Mean ± SE (^{o}F)	P-value
1	10	99.92 ± 0.10	10	99.98 ± 0.14	N/S
2	10	99.98 ± 0.16	10	100.18 ± 0.17	N/S
3	10	100.14 ± 0.16	10	100.1 ± 0.20	N/S
4	10	100.24 ± 0.22	10	100.18 ± 0.25	N/S
5	10	100.18 ± 0.16	10	100.42 ± 0.17	0.04

*(N/S = Non-significant)

There was no difference between before rectal palpation and after rectal palpation except at day 5 in experimental group. **The graphical representation of this data is given below**

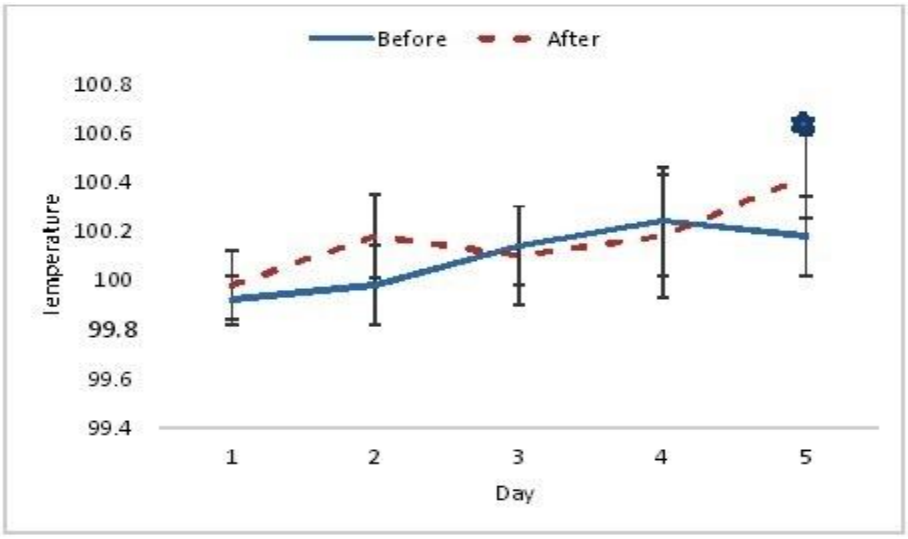


Figure 6.1: Effect of rectal palpation on body temperature in the experiment buffaloes before and after rectal palpation

***** Shows significant difference **($P < 0.05$)**

Table 6.2: Difference in body temperature in the control buffaloes before without rectal palpation procedure.

Day	N	Before Mean ± SE (^{o}F)	N	After Mean ± SE (^{o}F)	P-value
1	10	100.4 ± 0.17	10	100.18 ± 0.11	N/S
2	10	100.14 ± 0.19	10	100.2 ± 0.20	N/S
3	10	100.24 ± 0.13	10	100.14 ± 0.19	N/S
4	10	100.18 ± 0.16	10	100.1 ± 0.15	N/S
5	10	100.14 ± 0.15	10	100.12 ± 0.10	N/S

*(N/S = Non-significant)

There was no difference between before rectal palpation and after rectal palpation in experimental buffaloes. **The graphical representation of this data is given below**

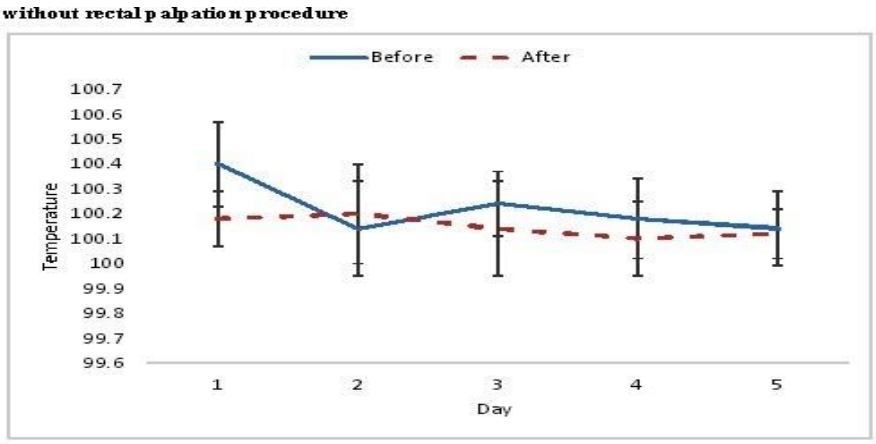


Figure 6.2: Difference in body temperature in the control buffaloes before

Table 6.3: Difference in body temperature compared the experimental buffaloes and control buffaloes

Day	N	Experiment Mean ± SE (^{o}F)	N	Control Mean ± SE (^{o}F)	P-value
1	10	99.95 ± 0.12	10	100.29 ± 0.12	N/S
2	10	100.08 ± 0.18	10	100.17 ± 0.18	N/S
3	10	100.12 ± 0.14	10	100.19 ± 0.14	N/S
4	10	100.21 ± 0.19	10	100.14 ± 0.19	N/S
5	10	100.3 ± 0.14	10	100.13 ± 0.14	N/S

*(N/S = Non-significant)

There was no difference between experimental and control group. The graphical representation of this data is given below

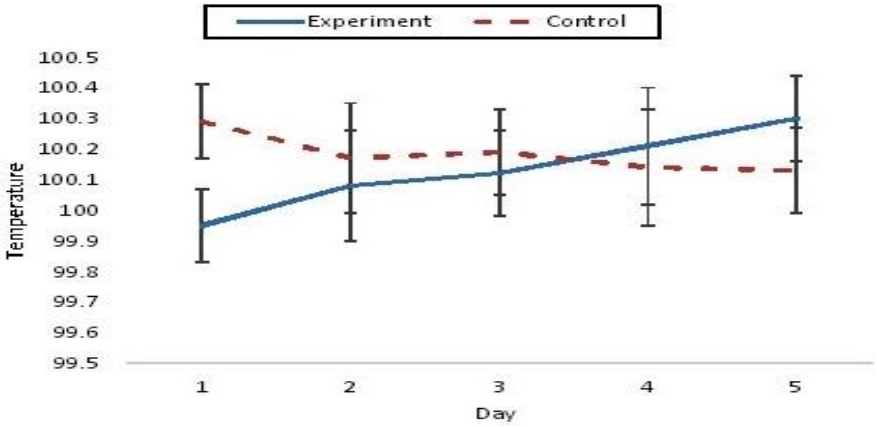


Figure 6.3: Difference in body temperature compared the experimental buffaloes and control buffaloes

6. Pulse:

Table 7.1: Effect of rectal palpation on pulse rate in the experimental buffaloes before and after rectal palpation

Day	N	Before Mean ± SE (/bpm)	N	After Mean ± SE (bpm)	Pvalue
1	10	44.4 ± 0.98	10	46.8 ± 0.8	0.004
2	10	43.8 ± 0.49	10	46 ± 0.63	0.01
3	10	43.6 ± 0.75	10	44.6 ± 0.75	N/S
4	10	37.58 ± 7.71	10	38.32 ± 7.95	N/S
5	10	35.96 ± 7.31	10	37.36 ± 7.65	0.03

*(N/S = Non-significant)

Pulse rate was increased after rectal palpation as compared to before rectal palpation in experimental group at day 1, day 2 and day 5.

The graphical representation of this data is given below

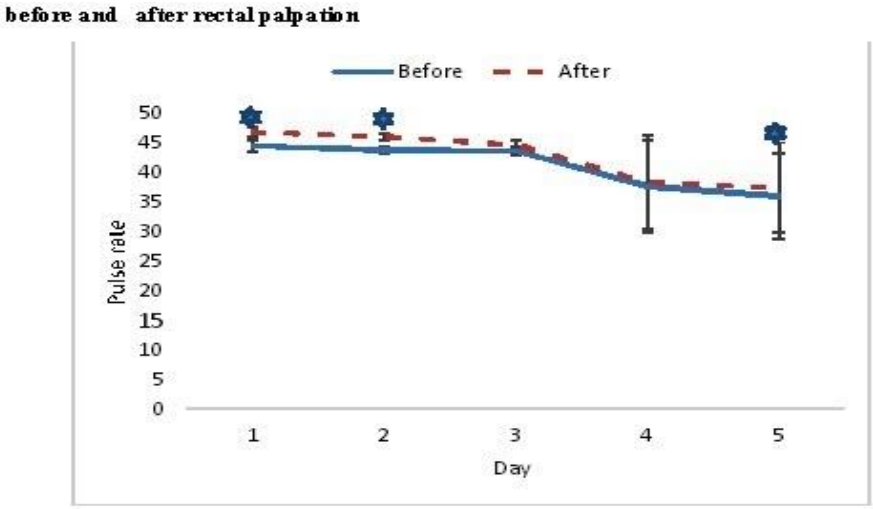


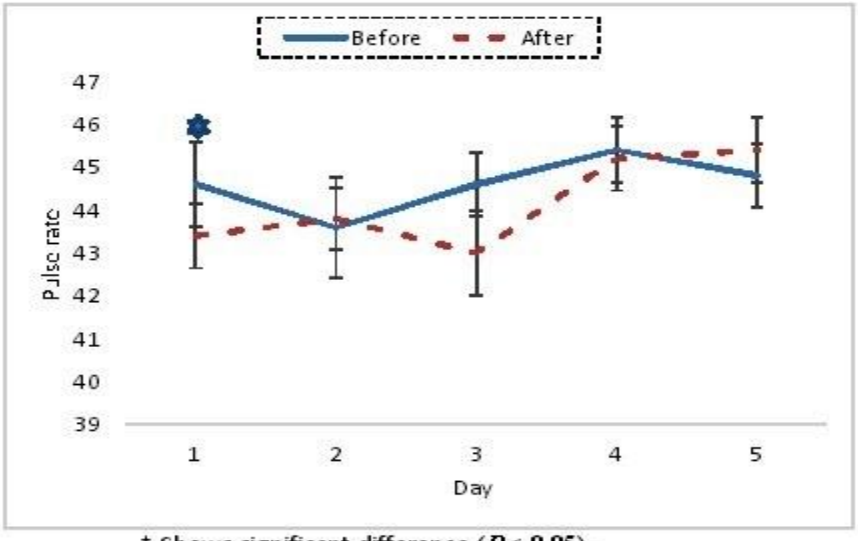
Figure 7.1: Effect of rectal palpation on pulse rate in the experiment buffaloes
***** Shows significant difference **($P < 0.05$)**

Table 7.2: Change in pulse rate in the control buffaloes without rectal palpation procedure.

Day	N	Before Mean ± SE (bpm)	N	After Mean ± SE (bpm)	P-value
1	10	44.6 ± 0.98	10	43.4 ± 0.75	0.03
2	10	43.6 ± 1.17	10	43.8 ± 0.73	N/S
3	10	44.6 ± 0.75	10	43 ± 1	N/S
4	10	45.4 ± 0.75	10	45.2 ± 0.74	N/S
5	10	44.8 ± 0.74	10	45.4 ± 0.75	N/S

*(N/S = Non-significant)

There was no difference between before rectal palpation and after rectal palpation in control buffaloes except day 1. **The graphical representation of the data is given below**



* Shows significant difference ($P < 0.05$).

Figure 7.2: Change in pulse rate in the control buffaloes without rectal palpation procedure

Table 7.3: Change in pulse rate compared between the experimental buffaloes and control buffaloes.

Day	N	Experiment Mean ± SE (bpm)	N	Control Mean ± SE (bpm)	P-value
1	10	45.6 ± 0.86	10	44 ± 0.86	N/S
2	10	44.9 ± 0.51	10	43.7 ± 0.51	N/S
3	10	44.1 ± 0.73	10	43.8 ± 0.73	N/S
4	10	37.95 ± 5.56	10	45.3 ± 5.55	N/S
5	10	36.66 ± 5.3	10	45.1 ± 5.3	N/S

*(N/S = Non-significant)

There was no difference between experimental buffaloes and control buffaloes. **The graphical representation of the data is given below**

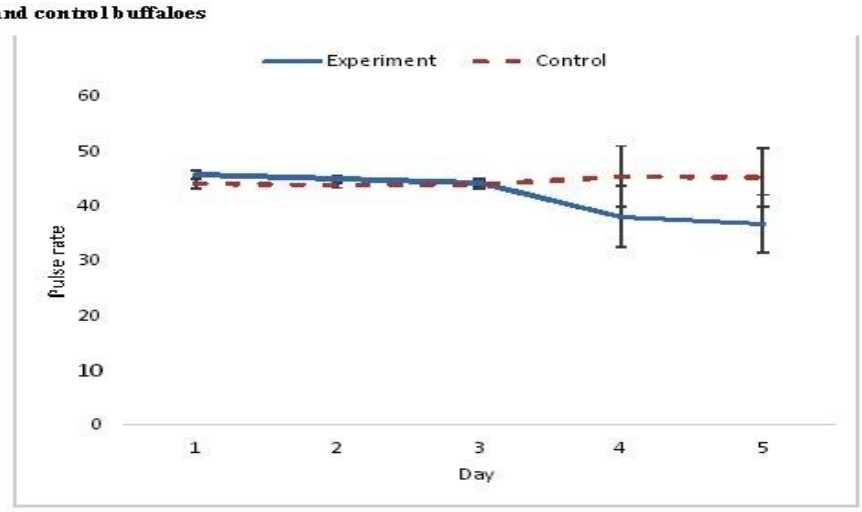


Figure 7.3: Change in pulse rate compared between the experimental buffaloes

7. Respiration:

Table 8.1: Effect of rectal palpation on respiration rate in the experimental buffaloes before and after rectal palpation

Day	N	Before Mean ± SE (/mnt)	N	After Mean ± SE (/mnt)	P-value
1	10	19 ± 4.56	10	19.24 ± 4.79	N/S
2	10	18.8 ± 5.14	10	18.4 ± 4.43	N/S
3	10	20.9 ± 5.68	10	19.84 ± 4.14	N/S
4	10	20.2 ± 5.29	10	20.78 ± 4.34	N/S
5	10	19.62 ± 5.13	10	19.4 ± 4.91	N/S

*(N/S = Non-significant)

There was no difference between before rectal palpation and after rectal palpation in experimental buffaloes. **The graphical representation of the data is given below**

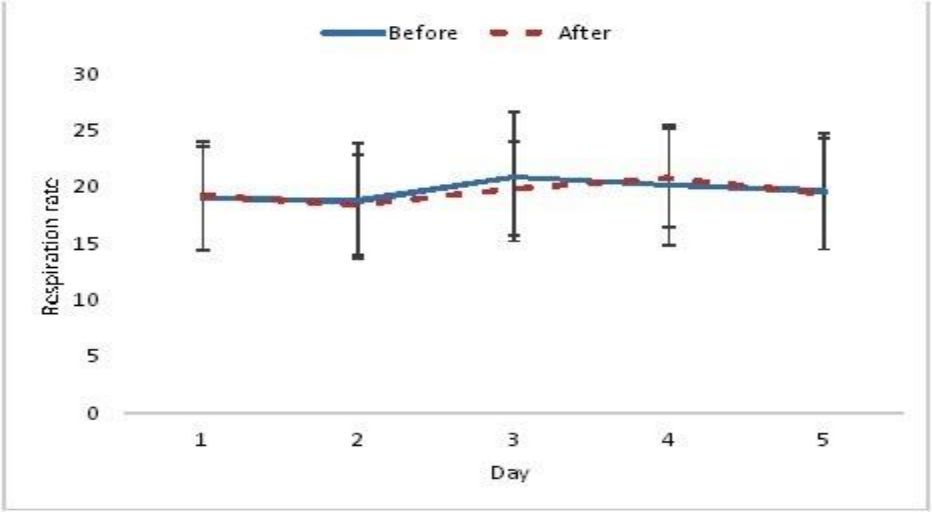


Figure 8.1: Effect of rectal palpation on respiration rate in the experimental buffaloes before and after rectal palpation

Table 8.2: Change in respiration rate in the control buffaloes without rectal palpation procedure.

Day	N	Before Mean ± SE (/mnt)	N	After Mean ± SE (/mnt)	P-value
1	10	14.4 ± 0.75	10	13.8 ± 0.8	N/S
2	10	13.4 ± 0.6	10	13.8 ± 1.02	N/S
3	10	14.4 ± 0.93	10	15.4 ± 0.75	0.03
4	10	13.2 ± 0.49	10	14.2 ± 0.58	N/S
5	10	14.8 ± 0.97	10	14.2 ± 0.66	N/S

*(N/S = Non-significant)

There was no difference between before rectal palpation and after rectal palpation in control buffaloes except day 3. **The graphical representation of the data is given below**

palpation procedure

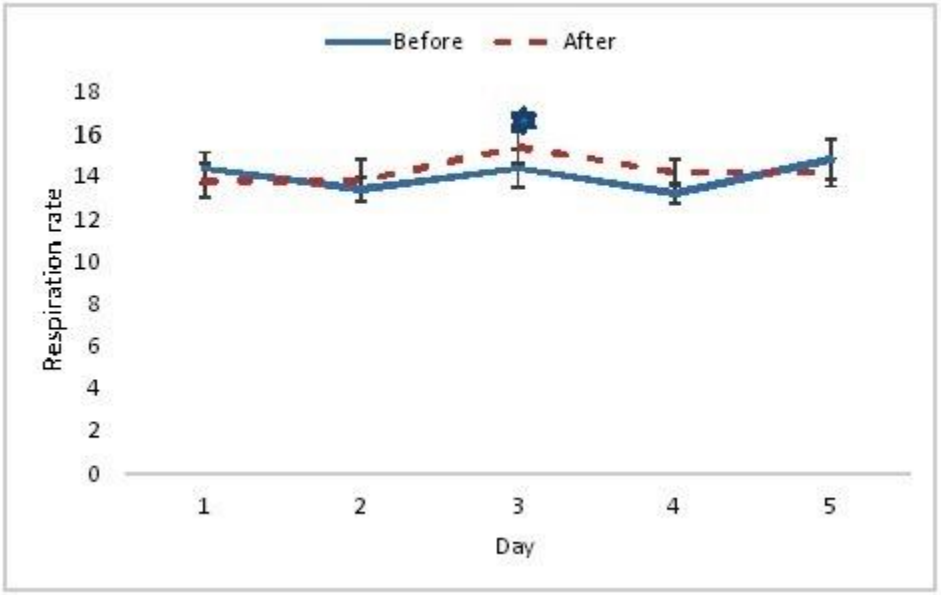


Figure 8.2: Change in respiration rate in the control buffaloes without rectal

***** Shows significant difference **(*P* < 0.05)**

Table 8.3: Respiration rate compared between the experimental buffaloes and control buffaloes.

Day	N	Experiment Mean ± SE (/mnt)	N	Control Mean ± SE (/mnt)	P-value
1	10	19.12 ± 3.34	10	14.1 ± 3.34	N/S
2	10	18.6 ± 3.42	10	13.6 ± 3.42	N/S
3	10	20.37 ± 3.52	10	14.9 ± 3.52	N/S
4	10	20.49 ± 3.41	10	13.7 ± 3.41	N/S
5	10	19.51 ± 3.59	10	14.5 ± 3.59	N/S

*(N/S = Non-significant)

There was no difference between experimental buffaloes and control buffaloes.

The graphical representation of the data is shown below

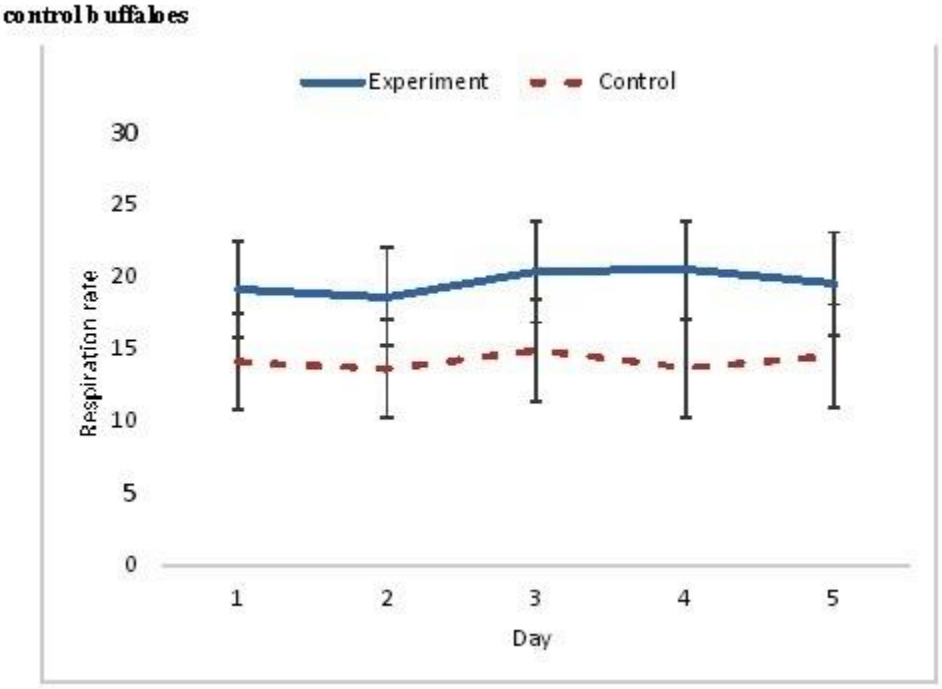


Figure 8.3: Respiration rate compared between the experimental buffaloes and

DISCUSSION

Rectal palpation is the most convenient and cheap method for conducting pregnancy diagnosis, artificial insemination and detection of various reproductive anomalies in large domestic animals like cattle, buffaloes, mare and camel. However, it can be painful and inflicts stress to t h e animal. Prominent change was seen in behavior and physiology of animals in response to stress (Nakao *et al.*, 1994; Waiblinger *et al.*, 2004). Stress is response of animal due to change in environment (Doney *et al.*, 1976). Homeostatic level is maintained by the biological systems adaptation according to environment (Boissy. 1998). Disruption of physiological functions due to stress can pose serious threat to animal's health state and life. Stressful conditions stimulate HPA axis that cause a ten-fold rise in the circulating cortisol concentration (Avci *et al.*, 2008). Disturbance in physiological functions appeared in response to stress resulting in production loss (Moberg. 1987). Cortisol is the conventionally accepted biomarker of stress (Olvera, 2004). Earlier, an increase in plasma cortisol concentration has been reported due to rectal palpation (Nakao *et al.*, 1994). In the present study, an increased serum cortisol concentration is seen only once at the last day of experiment following rectal palpation, which is contrary to the previous findings of Cingi *et al.*, (2012): who found rectal palpation a stressful procedure with markedly high serum cortisol concentration difference after the rectal palpation procedure.

Andrade *et al.*, (2001) studied that restraining is a common procedure for handling cattle and may have detrimental effects on production. Serum cortisol concentration in control group after the rectal palpation procedure is also high only on first day of study which could be due to restraining stress since no change was observed on subsequent days of study. These findings are similar to those of (Boandl *et al.*, 1989) who reported an

increase in cortisol concentration in response to handling. These findings are also similar to those reported by (Szenci *et al.,* 2011) who studied the amount of restrained stress in cattle and concluded that the cortisol concentrations remained elevated for two hours after restraint in crush for two consecutive days; but when the restraint was applied for third consecutive day the cortisol concentration remained fairly constant this supports the postulate that cattle can rapidly adapt to repeated stress. Bovines are subjected to stress during the normal husbandry practices.

Serum cortisol level was higher in experimental group buffaloes as compared to control group buffaloes but it was not statistically significant. In line with our findings, Herskin *et al.,* (2005) reported that plasma cortisol concentrations may peak during initial days of adverse environmental conditions but when this remain for consecutive days cattle rapidly adapt to changing environment.

Several blood parameters can be altered due to stress response. Firstly, sympathetic nervous system is activated in stress response which stimulates adrenal medulla resulting in increased secretion of catecholamines. Contraction of spleen started due to catecholamines act on adrenergic receptors, resulting in an increase in packed cell volume and hemoglobin concentration (Avci *et al.,* 2008; Fidan *et al.,* 2010). In present study, rectal palpation procedure caused significant decrease in hemoglobin level at day-2 in experimental group buffaloes which can be due to rough palpation of genital tract per rectum causing erosions in mucosal layer of rectum resulting in bleeding. In buffaloes of control group, hemoglobin values significantly decreased which can be due to inappropriate handling of animals. While, there was no significant difference between experimental and control group buffaloes in hemoglobin and HCT level. In addition, no difference in blood HCT level was observed after rectal palpation in buffaloes of experimental group. In this study, increase in RBC level was significantly higher in experimental group buffaloes as compared to control group buffaloes in response to stress imposed by rectal palpation at day-2 of study. Along with this, white blood cell count was significantly lower in experimental group buffaloes as compared to control group buffaloes while in experimental group buffaloes no difference was observed following the rectal palpation procedure.

Previous handling and gentle interactions affect behaviour and heart rate of dairy cows during a veterinary procedure (Waiblinger *et al.,* 2004). Body temperature was increased at day 5 after rectal palpation in experimental group buffaloes while there was no significance difference between experimental group buffaloes and control group buffaloes. Cingi *et al.,* (2012) reported a significant increase in pulse and respiration rates following the rectal palpation procedure. Many researchers have studied the changes in heart rate during different handling procedures like restraint and venipuncture (Stephens *et al.,* 1975), transportation (Stermer *et al.,* 1981), handling by humans (Hemsworth *et al.,* 1989) and during milking (Royle *et al.,* 1992). Significant increase in pulse rate was observed after rectal palpation in experimental group buffaloes as compared to before rectal palpation which is concordant with the findings of Cingi *et al.,* (2012). Furthermore, no difference in pulse rate was observed between experimental group and control group buffaloes. In present study no difference was observed in the respiration rate of experimental buffaloes following the rectal palpation procedure, also there was no difference in respiration rate of control group buffaloes.

According to the data of this study, serum cortisol level was increased in response to rectal palpation stress at only few instances. Experimental animals of this study were used to physical stress caused by handling, restraining and rectal palpation during training of veterinary students which may mask the effect of rectal palpations stress during this study. This might be the major reason for subtle change in serum cortisol level due to the effect of rectal palpation.

Further studies with increased number of animals can be beneficial for the comprehensive identification of physiological and biological changes during the rectal palpation procedure in terms of accurate management practices and industry.

Conclusion: During the course of our study, except at few instances, no significant difference was observed in serum cortisol level, related blood and physical parameters in relation to stress. In conclusion, rectal palpation is the most convenient and an atraumatic procedure for artificial insemination, cyesiognosis, diagnosis of various reproductive ailments and transportation of embryos in large animals.

Recommendations: It inflicts no stress to animals and can be used for the training of veterinary students to impart skill in relation to theriogenology. However, more research is needed to elucidate the amount of stress caused by the increased number of palpations by students.

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