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Comparison of estrus parameters and haemato-biochemical profile in normal cyclic and follicular cystic Ongole cows

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Abstract
Estrus parameters and haemato-biochemical profiles were compared between normal cyclic (Group 1) and follicular cystic Ongole (Group 2) cows. Results revealed that duration of estrus was significantly (p<0.01) longer and estrus cycle length was significantly (p<0.01) shorter in cystic compared to normal cyclic cows. Most cystic cows exhibited an intense type of estrus and displayed typical fern pattern in their cervico-vaginal mucus. A non-significantly (p>0.05) lower spinbarkeit value with pH ranging between 7-8 was recorded in cervico-vaginal mucus of cystic cows. The serum glucose and neutrophil levels were significantly (p<0.05) higher, while serum cholesterol and lymphocyte levels were significantly (p<0.05) lower in cystic compared to normal cyclic cows. Additionally, monocyte, TLC and eosinophil counts were non-significantly (p>0.05) lower in cystic cows. In conclusion, assessing of estrus and haemato-biochemical parameters may be helpful to enhance the health and future fertility of cows affected with follicular cysts.

Keywords: Follicular cysts, Ongole cows, estrus parameters, haemato-biochemical parameters

1. Introduction
Cystic ovarian degeneration impacts dairy cows at an occurrence of 6 to 19 % and is a significant contributor to infertility and economic losses in the dairy sector (Gumen and Wiltbank 2002) [11]. The cysts were defined as fluid-filled or solid structures with a diameter of 2.5 cm or greater persisting on the surface of the ovary for a duration of 10 days or longer. Ovarian cysts may develop in high milk-producing animals because of metabolic adaptations that occur to sustain elevated production levels (Butler, 2003) [4]. Various factors such as dystocia, metabolic irregularities, retention of fetal membranes, twin births, stress, milk fever (Roberts, 1986) [27], lameness and uterine infections are associated with an elevated prevalence of cystic ovaries. Various types of hematological changes could be observed in association with reproductive disorders. The hematological variations were observed because of stress, attributed to elevated glucocorticoid secretion (Tiwari *et al*., 2020) [35]. The presence of stress-induced biochemical alterations was evident, with increased production of free radicals (Butler, 2003) [4]. Consequently, it is crucial to examine the alterations in both hematological and metabolic profiles in normally cycling and follicular cystic conditions, as this offers valuable diagnostic and prognostic insights. With these considerations in mind, the current study was devised to evaluate changes in estrus and haemato-biochemical parameters in cows affected with follicular cysts in comparison to normal cyclic cows.

2. Materials and Methods
The current work was carried out on Ongole cows afflicted with follicular cysts, which were brought to the Large Animal Obstetrical ward, Department of Veterinary Gynaecology and Obstetrics, NTR CVSc, Gannavaram, Andhra Pradesh during the research span from January 2023 to December 2023. The cervico-vaginal mucus from 10 cystic and 10 normal cyclic cows was aspirated by using a blue sheath connected to a syringe. The pH and physical characteristics including the spinbarkeit and fern pattern of these samples were assessed within 2-3 hours of being collected. The pH was determined by using pH Merck paper with indicators ranging from 0 to 14. Alteration in the colour of the paper is compared with the attached standard value.

Spinbarkeit was quantified in centimeters (Panigrahi, 1964) [21] and the fern pattern was examined in estrual mucus smears using both low-power (10X) and high-power (40X) microscopes and it was categorized into typical, atypical, and nil types (Luktuke and Roy, 1967) [16]. Blood samples were collected in EDTA vacutainers to assess the hematological parameters, while blood collected in clot-activating vacutainers was utilized to obtain the necessary serum for assessing biochemical parameters. Samples were obtained from both cows with follicular cysts and normal cyclic cows, serving as controls. Total leukocyte count (TLC) and Differential leukocyte count (DLC) were promptly determined by following standard procedures given by Jain (1993) [12] by using a semi-auto analyzer (Multiskan go, Thermo Scientific Instruments). Biochemical parameters, specifically Glucose and Cholesterol were assessed using commercially available biochemical kits. The statistical analysis of the data was done as per the procedures outlined by Snedecor and Cochran (1994) [32].

3. Results and Discussion

3.1 Estrus Parameters

In the present study, significantly (p<0.05) prolonged duration of estrus was observed in follicular cyst affected compared to normal cyclic cows (Table 1, Figure 1). The present findings agreed with the reports of Shah and Andrabi (2012) [29], Mimoune *et al.* (2017) [19] and Smith (2021) [31] who also recorded significantly prolonged estrus duration of 48-72, >48 and >50 hrs, respectively in follicular cyst affected cows. On the contrary, Talukder *et al.* (2014) [34] and Smith (2021) [31] reported the presence of persistent and enlarged follicular structures with anestrous in follicular cystic cattle. The prolonged estrus duration of cystic cows in the present study could be attributed to a lack of sufficient luteinizing hormone. Meanwhile, adequate amounts of Follicle-Stimulating Hormone (FSH) might endure, which may promote the development and secretion of follicles that do not ovulate (Shah and Andrabi, 2012) [29].

A significantly (p<0.05) decreased length of the estrus cycle was observed in follicular cyst afflicted cows compared to normal cyclic cows (Table 1, Figure 2). The findings in the present study were in conformity with those of Perez-Marin and Espana (2007) [23], Enginler *et al.* (2012) [8], Beggs (2013) [3], and Purohit (2018) [25] who reported significantly lower estrus cycle lengths in cattle with follicular cysts as <18, 10-15, <18-21, and 16-19 days, respectively. The decreased estrus cycle length of follicular cystic cows in the present study could be due to the persistence of unchanged granulosa cells which may lead to increased estrogen production from the dominant follicle of the first or second follicular wave (Purohit, 2018) [25].

In the current study, a significantly more intense type of estrus was observed in follicular cystic cows compared to normal cyclic cows (Table 1, Figure 3). The present findings were in accordance with Gad *et al*. (2022) [9] who recorded the occurrence of an intense type of estrus in follicular cystic cows. An intense type of estrus in follicular cystic cows in the present study could be attributed to elevated estrogen levels from cystic follicles (Chakurkar *et al*., 2008) [5].

In the present study, there was an inappreciable difference in the pH of normal cyclic and follicular cystic cows (Figure 4). These findings were analogous to the observations of Gohel *et al.* (2012) [10] who reported a non-significant increase in repeat breeder cows compared to normal cyclic cows. The variations in the pH of cervical mucus in the present study could be linked to decreased concentrations of sodium chloride, mucoproteins, and water content, all of which are influenced by elevated estrogen levels detected in cows with follicular cysts during the estrus period (Tsiligianni *et al.*, 2001) [36].

A significantly (p<0.05) lower spinbarkeit was noted in follicular cystic than in normal cyclic cows (Figure 5). These findings were in close harmony with the report of Gohel *et al.* (2012) [10] who recorded a significantly lower value of Spinnbarkeit in repeat breeder Gir cows compared to normal cyclic cows. The lower value of Spinnbarkeit in cystic cows in the present study could be under the influence of progesterone at subluteal concentrations which may cause disturbances in strong intermolecular forces within the long molecules and branching of the chains in cervicovaginal mucus (Tsiligianni *et al.*, 2001) [36].

In the present study, a greater percentage of atypical fern pattern in follicular cyst affected and typical fern pattern in normal cyclic cows was observed (Figure 6). The findings in the present study were in close agreement with the reports of Sharma *et al.* (2008) [30] and Modi *et al.* (2011) [20] who observed more percentage of atypical fern pattern in cervicovaginal mucus of repeat breeding than in normal cyclic cattle. The higher percentage of atypical fern patterns in cystic cows may be attributed to subluteal concentrations of plasma progesterone which may cause a reduction in the degree of crystallization in cervicovaginal mucus (Gohel *et al.*, 2012) [10].

Table 1: Estrus parameters in both groups of Ongole cows

Estrus parameters		Mean±SE (hrs) (Range)	
		Group-1 (Normal cyclic cows)	**Group-2 (Follicular cystic cows)**
1. Duration of estrus (hrs)		17.4±0.45 (10-24)	51.9±0.98** (24-72)
2. Estrus cycle length (days)		21.2±0.53 (19-25)	15.9±0.74** (10-24)
3. Intensity of estrus	a) Intense	40% (4/10)	60% (6/10)
	b) Intermediate	50% (5/10)	40% (4/10)
	c) Weak	10% (1/10)	0

Mean with different superscripts (**) in a row differed significantly (p<0.05)

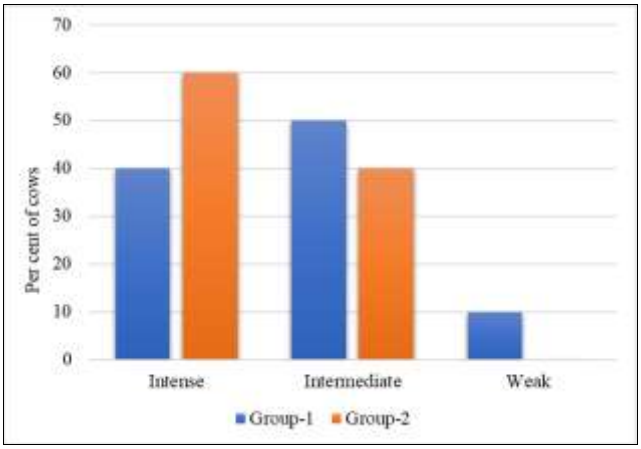

Fig 1: Duration of estrus in both group of Ongole cows

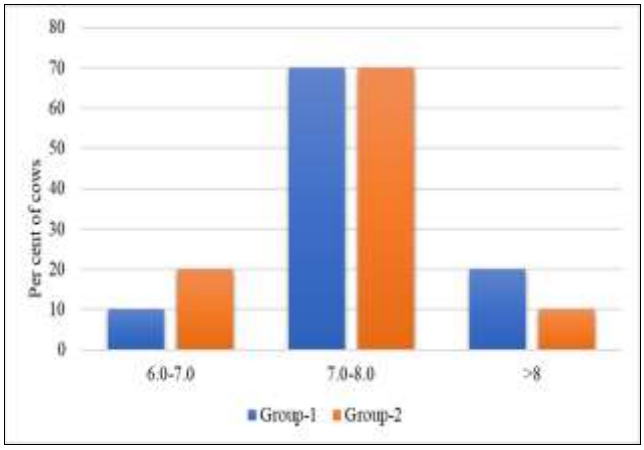

Fig 2: Estrus cycle length in both group of Ongole cows

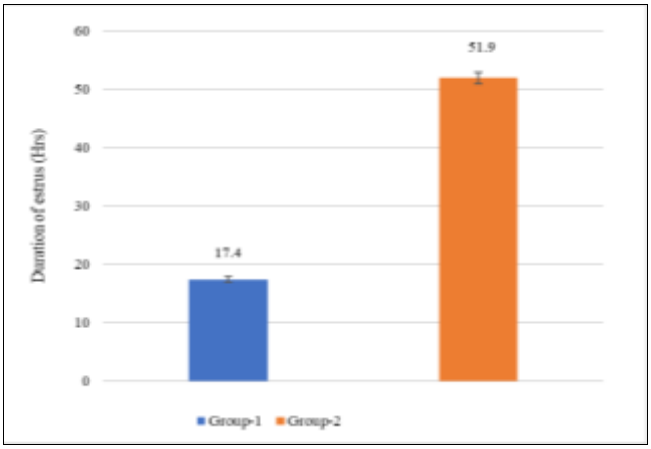

Fig 3: Intensity of estrus in both groups of Ongole cows

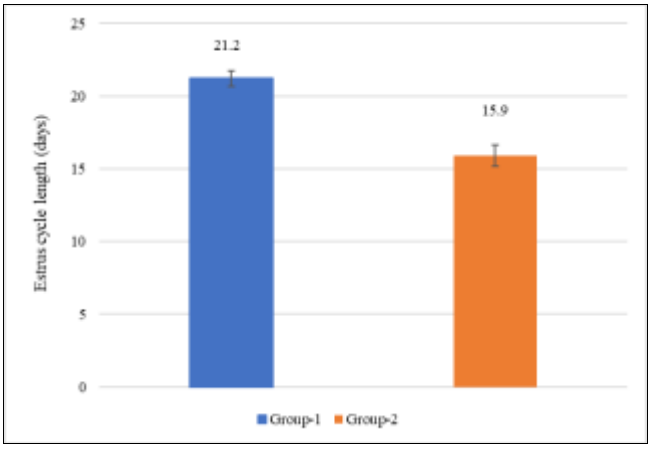

Fig 4: pH of cervico-vaginal mucus in both groups

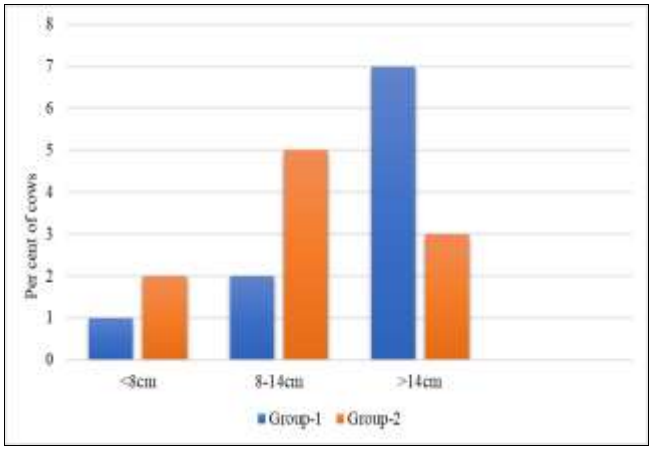

Fig 5: Spinnbarkeit values of cervico- vaginal mucus in both groups

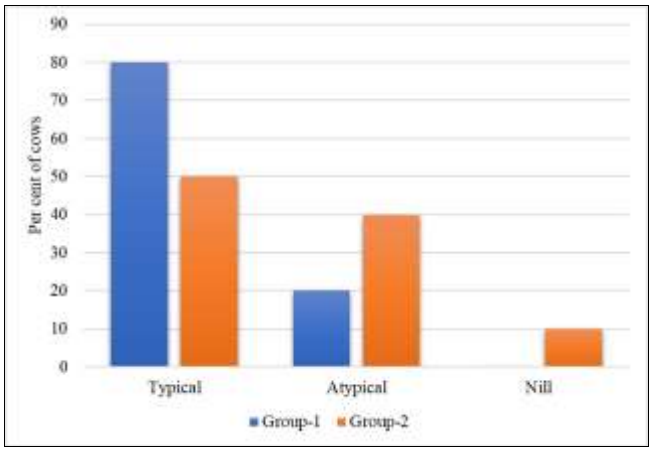

Fig 6: Fern pattern of cervico-vaginal mucus in both groups

3.2 Haematological parameters

The mean TLC count was non-significantly ($p>0.05$) lower in follicular cystic compared to normal cyclic cows in the present study (Table 2 Figure 7). The current findings were in close agreement with the reports of Perumal *et al.* (2013) [24] who also recorded a non-significant decrease in TLC count in repeat breeding cattle compared to normal cyclic cattle. On the contrary, Allam *et al.* (2019) [2] found a significantly higher TLC count in cattle with cystic ovaries than in normal cyclicity. The possible variations in the TLC count in the present study could be due to stress arising from a negative energy balance during calving and in the later stages of lactation which may linked to a reduction in leukocyte function.

The mean neutrophil count was significantly ($p<0.05$) higher in follicular cystic compared to normal cyclic cows in the current study (Table 2 Figure 7). These findings were analogous to the observations of Allam *et al.* (2019) [2] who found a significant increase in neutrophil count in cattle with cystic ovaries than in normal cyclicity. The potential explanation for variations in neutrophil count in the current study could be stress encountered during the postpartum period because of negative energy balance (Allam *et al.* 2019) [2]. This imbalance can result in elevated cortisol/adrenaline levels, triggering the mobilization of marginal neutrophil pool cells through the leukocytosis-inducing factor (Pathan *et al.*, 2011) [22].

The mean lymphocyte count recorded was significantly ($p<0.05$) lower in follicular cystic cows than in normal cyclic cows (Table 2 Figure 7). The findings in the present study were in close agreement with the report of Kekan *et al.* (2005) [13] and Sultana *et al.* (2022) [33] who recorded a significantly lower lymphocyte count in repeat breeding cattle compared to

normal cyclic cattle. On the contrary, Kumar *et al.* (2015) [15] found a non-significant increase in lymphocyte count in postpartum buffaloes with cystic ovaries compared to normal cyclic buffaloes. In the present study, a significant decrease in lymphocyte count in follicular cystic cows could be due to alteration in the environment (postpartum) which can trigger stress, resulting in elevated cortisol concentrations from adrenals. This may be associated with the sequestration of lymphocytes in the lymphoid tissues, ultimately causing lymphopenia (Mimoune *et al*., 2017) [19].

The mean monocyte count recorded was non-significantly (p>0.05) lower in follicular cystic cows when compared to normal cyclic cows (Table 2 Figure 7). The present findings were in accordance with the findings of Allam *et al.* (2019) [2] who reported a non-significant decrease in monocyte count in cows with cystic ovaries compared to normal cyclicity. Similarly, Ali *et al.* (2010) [1] reported a significantly lower monocyte count in Najdi female camels with ovarian cysts compared to normal cyclic camels.

The mean eosinophil count recorded was non-significantly (p>0.05) lower in follicular cystic cows when compared to normal cyclic cows (Table 2 Figure 7). The present findings were similar to the observations of Kekan *et al*. (2005) [13] and Sultana *et al.* (2022) [33] who recorded a non-significant decrease in eosinophil count in repeat breeding cows compared with normal cyclic. On the contrary, Kumar *et al.* (2015) [15] observed a significant increase in eosinophil count in postpartum cystic buffaloes compared to normal cyclic buffaloes. The possible reason for decreased eosinophil count in cystic cows might include stress induced by a state of negative energy balance that initiates a hypothalamo-hypophyseal-adrenocorticotropic response, leading to the release of ACTH. This, in turn, stimulates the adrenal cortex to generate cortisol which results in the reduction of eosinophils released from the bone marrow and promotes eosinophilic diapedesis, ultimately causing a decrease in eosinophilic count (Dukes, 2013) [6].

Table 2: Haematological parameters (Mean ± SE) in follicular cystic and normal cyclic cows

S. No	Haematological parameters	Group 1 (Normal cyclic)	Group 2 (Follicular cystic)
1	Total leucocyte count (10^3/µL)	10.15±0.34	9.42±0.22
2	Differential leucocyte count (DLC)		
A	Neutrophils (%)	43.65±0.95	47.1±0.99*
B	Lymphocytes (%)	42.45±0.93	38.71±1.02*
C	Monocytes (%)	4.24±0.19	4.12±0.17
D	Eosinophils (%)	5.27±0.12	5.04±0.09

Values bearing superscript (*) along the row differ significantly (p<0.05).

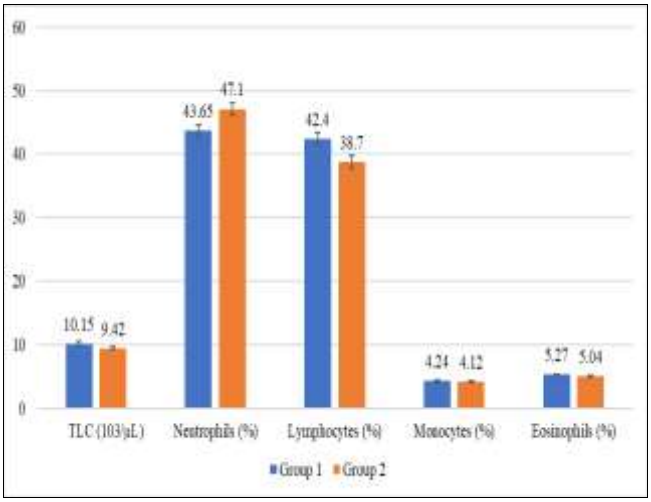

Fig 7: Haematological changes in normal cyclic and follicular cystic Ongole cows

3.3 Biochemical parameters

The mean serum glucose (gm/dL) concentration recorded was significantly (p<0.05) higher in cows with follicular cysts when compared to normal cyclic cows (Table 3 Figure 8). The findings in the present study were in close accordance with the reports of Safonov *et al*. (2021) [28] who recorded a significantly higher glucose concentration in follicular cyst affected cows compared to normal cyclic cows. On the contrary Mimoune *et al*. (2017) [19] and Allam *et al*. (2019) [2] reported a significantly lower glucose concentration in follicular cyst affected cows compared to normal cyclic cows. In the present study, the potential explanation for elevated glucose levels in follicular cystic cows could be an imbalance between the hepatic output and the peripheral absorption of the glucose or disruptions in the endocrine regulatory mechanisms that may impact the ovulatory processes (Safonov *et al*., 2021) [28].

The mean serum cholesterol (gm/dL) concentration recorded was non-significantly (p>0.05) higher in cows with follicular cysts when compared to normal cyclic cows (Table 3 Figure 8). The findings in the present study were in close agreement with the reports of Allam *et al*. (2019) [2] who observed a non-significant increase in serum cholesterol levels (gm/dL) in follicular cyst afflicted compared to normal cyclic cows. On the contrary, Markiewicz *et al*. (1999) [18] and Luthfi *et al*. (2023) [17] recorded significantly lower cholesterol levels in cows with follicular cysts compared to normal cyclic cows. The variations in cholesterol levels in the present study might be due to elevated steroidal hormones like estrogen and progesterone (subluteal) which were derivatives of the cholesterol itself (El-badry *et al*. 2020) [7]. Also, cholesterol serves as a precursor to produce steroidal hormones, which play a crucial role in fertility and fecundity (Kouamo *et al*., 2019) [14].

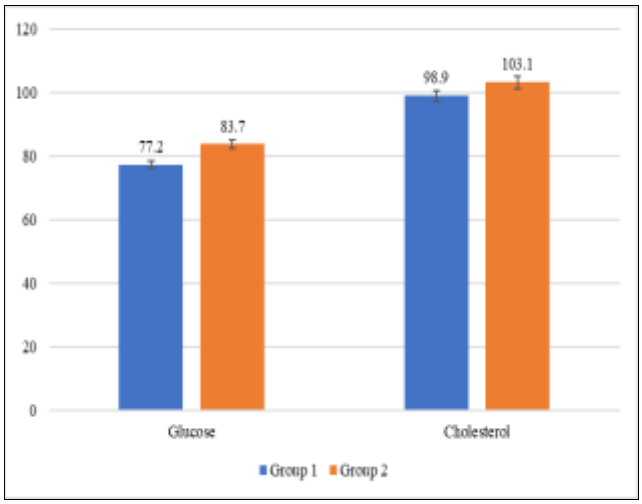

Fig 8: Biochemical changes in normal cyclic and follicular cystic Ongole cows

Table 3: Biochemical parameters (Mean ± SE) in both groups of cows

S. No	Biochemical parameters	Group 1 (normal cyclic)	Group 2 (follicular cystic)
1	Glucose (gm/dL)	77.2±1.20	83.7±1.50*
2	Cholesterol (gm/dL)	98.9±1.62	103.1±2.06

Mean with different superscript (*) in a row differed significantly ($p<0.05$)

4. Conclusion

According to the findings of the present study, the follicular cystic condition was characterized by a combination of symptoms and manifestations of hormonal imbalance and dysfunction of the ovaries. The negative energy balance in postpartum and dysregulation in hypothalamo-hypophyseal-gonadal axis contribute to alterations in estrus parameters such as prolonged estrus period and shortened inter estrus intervals. Also, results in haemato-biochemical alterations which were evident through mild leukocytopenia, neutrophilia, lymphopenia, mild monocytopenia and eosinopenia. Metabolites like glucose levels are elevated and cholesterol levels are slightly inclined. In conclusion, the estrus parameters and haemato-biochemical profile could be used to assess the severity of the condition and future fertility.

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