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Morphological and endocrine profile in ewes suffering from uterine infection with special reference to cyclicity

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Abstract

This study was undertaken to study the alterations in morphological and progesterone in the blood serum, follicular fluid and corpus luteum of ewes with or without uterine infection. The uterine infection was diagnosed on the basis of white side test, pH of uterine washings and endometrial cytology. The reproductive stage was determined on the basis of the functional structures present on the ovaries. Both infectious and normal ewes were further sub divided into Follicular infectious (FI), Luteal infectious (LI), Follicular normal (FN), Luteal normal (LN) and Acyclic normal (AC). Serum was collected from the clotted whole blood and preserved at -20°C till further processing. The mean pH of the uterine secretions obtained from ewes suffering from uterine infection was significantly higher ($P<0.001$) than normal ewes. The ovarian weight in the Follicular and Luteal group was higher than the Acyclic ewes. The total number of follicles and number of large follicles was significantly higher in follicular group than the other groups. The serum progesterone concentration (ng/ml) was significantly higher ($P<0.05$) in Luteal group of ewes (LN and LI) as compared to Follicular and Acyclic group of ewes. The mean follicular progesterone concentration was significantly higher ($P<0.001$) in luteal group of ewes (LI and LN) and Follicular infectious groups as compared to Follicular normal and Acyclic group of ewes.

Keywords: Progesterone, uterine infections, morphology, ewes, cyclicity

Introduction

The mammalian uterus is usually a sterile environment, but it is readily contaminated with bacteria during coitus, parturition and more commonly in postpartum period [1, 2]. The term 'uterine infection' implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium and/or release of bacterial toxins that lead to establishment of uterine diseases [3]. Pathogenic bacteria pass through the cervix and contaminate the uterus resulting in endometritis [4]. It causes infertility by disrupting normal uterine environment as well as ovarian function, which has been reflected by the alterations in ovarian cycle [3, 4]. The endometrium and its fluids play a major role in the reproductive process including sperm transport in the oviduct, regulation of the corpus luteum (CL) function, initiation of implantation, pregnancy and parturition [5]. Endometrium, the innermost layer of uterus, forms the first line of defense against infections and it is the first to be affected during ascending uterine infection. Uterine infection has been reported to damage the endometrial health and function leading to infertility conditions in domestic animals [6]. The term uterine infection implies contamination of uterus with pathogenic organisms i.e. adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium and/or release of bacterial toxins that lead to the establishment of uterine disease [7]. But uterine infection (endometritis, metritis and pyometra) is a rather general term and the criteria used to diagnose and classify uterine infections seem to vary among investigators [3]. The classification is mainly based on the degree of severity and clinical signs or by depth of inflammation histologically. Uterine infections have a profound local effect at ovarian level in ewe [8].

Uterine infection, an important cause of infertility, has been reported to be 5 to 10% in sheep [9] and 9.3% in goats [10]. Despite some published reports, the "true" incidence of uterine infection is not known for any livestock species including sheep because the detection and diagnosis are often inaccurate and reporting is not mandatory [11]. Uterine infections cause infertility by not only disrupting endometrial health but also affect an ovarian cycle. The normal mammalian uterus is usually sterile, but can get easily contaminated with microbes during coitus and parturition. Many methods are used for the diagnosis of endometritis including inspection of vaginal discharge, transrectal palpation, transrectal ultrasonography,

uterine bacterial culture, uterine biopsy and endometrial cytology [12]. Follicular fluid (FF) is composed partly of secretions from the follicle and partly of exudates from plasma. The composition of FF reflects changes in the secretory processes of the granulosa layer and theca interna, and alterations in the components of plasma due to physiological or pathological processes. Follicular fluid consists of various nutrients exudates of serum and is also partially produced locally, which are related to the metabolic activity of the follicular cells. The follicular fluid proteins are largely derived both from plasma and follicular cells and play an important role in several physiological and biological processes namely oocyte maturation, folliculogenesis, ovulation, oocyte transport and various other follicle regulatory processes related to reproduction. Metabolic activity and blood–follicle barrier properties change during the growth phase of the follicles, hence, a different biochemical composition of the follicular fluid in different-sized follicles could be expected in ruminant species [13].

Progesterone is the natural hormone produced by the corpus luteum (CL) of the ovary following ovulation and sustains pregnancy. Progesterone also known as P₄ (pregn-4-ene-3, 20-dione) is a C-21 steroid hormone involved in the female reproductive cyclicity. The corpus luteum (CL) releases progesterone, which acts on the endometrium to induce release of histotroph that supports the free-floating conceptus and prepares for epithelial-chorial placentation. Progesterone is the principle steroid secreted by the corpus luteum in many domestic animals [14]. Progesterone seems to be the primary ovarian steroid that governs the ability of the uterus to resist infections [9]. Progesterone has long been associated with uterine infections and it is considered that susceptibility to uterine infections are more during progesterone phase as compared to oestrogenic phase, probably due to reduced leukocytic activity [15]. Progesterone concentrations in the follicular fluid vary during different phases of the ovarian cycle with higher levels reported during the early luteal phase [16]. A decrease in the concentration of progesterone was observed with an increase in the follicle size [17]. The lower level of progesterone in the follicular fluid of preovulatory follicles has been attributed to an increase in prostaglandin production [18] or conversion to estradiol [19]. Studies indicated that there is a link between puerperal uterine infection and abnormal or atypical postpartum (pp) ovarian function, including abnormal folliculogenesis, and development of ovarian cysts, prolonged anoestrus and prolonged luteal phases [20, 21], which is due to the action of bacterial endotoxins mediated by prostaglandins [22]. In cattle and buffalo the studies on the diagnosis and therapeutic management of infertility have been conducted systematically and widely [23]. Perusal of available literature revealed that information on hormonal level in ewes suffering from uterine infections in relation to cyclicity is not available. Therefore it is important to know the incidence of infertility due to infectious or inflammatory conditions of the uterus along with their effect hormonal levels to understand the possible measures for its amelioration in animals. Keeping in view the above facts, this study was designed to study morphological and progesterone profile in cyclic and acyclic ewes with or without uterine infections.

2. Materials and Methods

The study was conducted from May to October 2013, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-K,

Shuhama. The study was conducted in female sheep that were brought for slaughter in the local abattoirs. The age, breed, body condition of the animal was recorded and clinico-gynaecological examination of the animal was performed to select the animals without any apparent systemic diseases. At the time of slaughter, blood was collected from 92 ewes in two parts. Female genitalia were procured from all ewes after slaughter at the local abattoir and transported to the P. G. laboratory of Division of Animal Reproduction Gynaecology and Obstetrics FVSc & AH, SKUAST-K, Shuhama in Phosphate Buffer Saline (PBS) in Ice Chest for further processing and diagnosis. Out of the total experimental ewes, 66 were subjected for further investigation in respect of functional structures of ovaries and progesterone profile. Upon reaching the laboratory, the genital tracts were examined for any gross abnormality and then opened longitudinally from the cervical end to the oviductal end of both horns with the help of scissors. The endometrial washing was collected and subjected to the following tests for diagnosis of uterine infection. The identification of tracts for uterine infection was done with the help of white side test, pH of uterine washings followed by endometrial cytology. The identified tracts were divided into two groups- positive (n=24) or negative (n=68) for uterine infection.

2.1 White Side Test: Those tracts which looked apparently normal were subjected to white side test for the diagnosis of sub clinical endometritis [24]. To perform this test, firstly a cut was given at the cervical end of each tract and then distilled water was infused at the cervical end through both the horns and the fluid was collected at the utero tubular end of both horns after cutting it. The collected fluid was mixed with an equal amount of 4% NaOH and heated up to the boiling point. Any change in colour was studied and graded accordingly. Samples that produced yellow colour on boiling were considered as positive while as no change in colour were considered as negative.

2.2 Endometrial Cytology: The sample was aseptically collected using a sterile cotton swabs and then a smear was prepared by rolling it on a clean glass slide [25]. The smears were then allowed to dry and stained with Leishman stain. The slide was examined for the presence of epithelial cells, PMNs and lymphocytes. Cytological assessment was done by determining the percent PMNs to provide a quantitative assessment of endometrial inflammation and the samples with a PMN ratio exceeding 5% were considered to be positive [26].

2.3 Processing and classification of ovaries: After dissecting from the tracts, adjacent tissues were removed from the ovaries and washed with normal saline. Then the ovaries were weighed and grossly examined for the presence or absence of functional structures. The reproductive stages of the collected genital tracts were evaluated on the basis of presence or absence of functional structures on the ovaries. The genital tracts with ovary having functional structures were classified under cyclic group and tracts without functional structures on their ovaries were classified as acyclic group. Ewes with (positive) or without (negative) uterine infection were further subdivided into follicular, luteal and acyclic groups.

2.4 Collection of follicular fluid (FF): The number of antral follicles was counted in each ovary of each tract and the diameter of the largest follicle was measured with the help of

Vernier calliper. Follicular fluid was collected from the antral follicles by aspiration technique and the aspirated fluid was centrifuged at 3000 rpm for 15 minutes to remove cellular debris. Supernatant fluid was transferred to a new set of tubes and the aliquots of FF was prepared for different parameters depending upon the required volume and stored at -20°C till further processing.

2.5 Enucleation of Corpus luteum (CL): Corpus luteum (CL) of ovaries from luteal cyclic groups whether positive or negative for uterine infection was enucleated from the ovaries. The enucleated CL was weighed and then homogenized in ice cold deionized distilled water to produce 10% (w/v) homogenate. The homogenate was centrifuged and the supernatant was stored at -20°C for biochemical and hormonal estimation.

2.6 Estimation of Progesterone: Progesterone was estimated in serum, follicular fluid and CL samples by “Ds-Eia-Steroid-Progesterone” Kit.

2.6.1 Principle: The “Ds-Eia-Steroid-Progesterone” kit method is a one step immunoassay to determine the amount of progesterone in serum using competitive microplate enzyme immunoassay. The microtitre wells are coated with an anti-Progesterone antibody directed towards an antigenic site on the progesterone molecule. Progesterone in serum samples, calibrators and control serum competes with a progesterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of progesterone in the sample. After addition of the substrate solution, the intensity of colour developed is inversely proportional to the concentration of progesterone in the sample.

2.6.2 Procedure

1. All the components in test kit and serum samples were brought to room temperature.
2. The microplate well for each calibrator, control serum and serum sample (in duplicate) were labelled accordingly.
3. A 25 µl of calibrator, control serum and each serum sample were taken with the help of eppendorf pipette (with disposable tips) into respective wells.
4. Then 100 µl of conjugate was added to each well and contents were mixed by gentle swirling. The microplate was covered with absorbant paper and incubated for 90 min at room temperature.
5. Then each well was washed 5 times with 300 µl of working washing solution and the plate was tapped firmly against absorbant paper to remove any residual volume.
6. After washing 100 µl of TMB-Substrate was added to each well and then incubated for 25 min at room temperature in the dark.
7. Then the reaction was stopped by adding 150 µl of stopping reagent into well and gently mixed for 5-10 sec.
8. Absorbance of each well was measured on the microplate reader at 450 nm within 20 min after the addition of

stopping reagent.

2.7 Statistical Analysis

The data for functional structures in the ovaries, leukocyte count, biochemical and endocrine profile were analyzed by one way ANOVA for comparison between groups using SPSS (14) version for windows. The data pertaining to a particular parameter for comparison between two groups in respect of corpus luteum were compared using independent sample T test. If a main effect was found significant, post hoc analysis was performed with LSD. The values were considered as significant at $P < 0.05$. The data are presented as Mean \pm S.E.M.

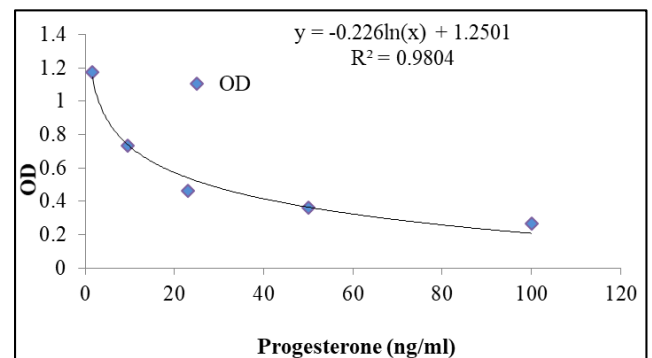


Fig 1: Standard curve for estimation of Progesterone

3. Results

The alterations in morphological and endocrine profile of the follicle, corpus luteum and blood (serum/plasma) as a result of uterine infection in ewes with special reference to cyclicity were investigated in the present study. The blood serum samples were used to study endocrine (progesterone) profile. The follicular fluid aspirated from antral follicles was investigated for progesterone level in order to reveal the overall changes that take place in the follicular compartment during uterine infection. The corpus luteum was examined immediately after enucleation for morphology (CL weight and size). The 10% homogenate of corpus luteum was used for estimation of progesterone.

A total of 92 genitalia were examined, out of which 24 (26.09%) were detected positive while 68 (73.91%) were found negative for uterine infection based on the white side test, pH of uterine washings and endometrial cytology. On the basis of the functional structures present on the ovaries, both infectious and normal ewes were further sub divided into Follicular infectious (FI), Luteal infectious (LI), Follicular normal (FN), Luteal normal (LN) and Acyclic normal (AC). The percentage of ewes in FI, LI, FN, LN and AC groups was 6.52%, 19.56%, 23.91%, 17.39% and 32.60%, respectively (Table 1).

3.1 Identification and classification of genitalia

The mucus and uterine secretions/washings collected from the genitalia were subjected to white side test for diagnosis of the genitalia with or without uterine infection (Plate 1 and 2). The white side test was interpreted based on the visible intensity of colour development ranging from various shades of yellow for positive and clear for negative samples (Plate 3).

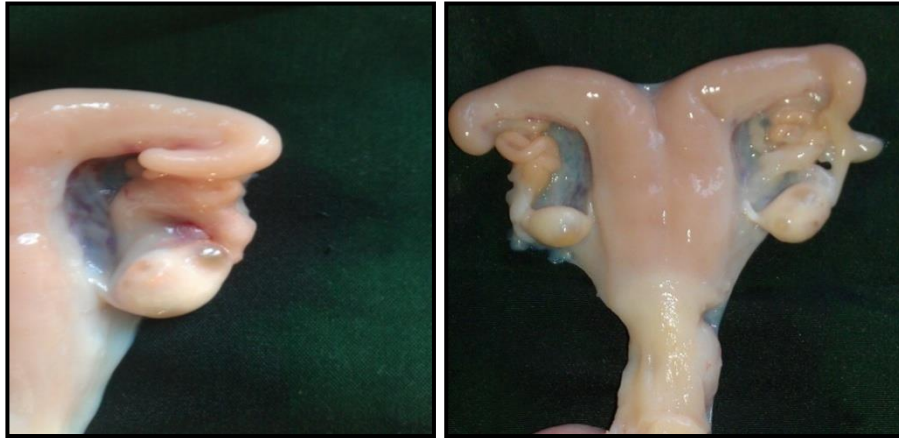


Plate 1: Cyclic group; presence of large antral follicle



Plate 2: Cyclic group; presence of mature corpus luteum



Plate 3: Acyclic group: Smooth Ovary

3.2 Biometry and functional structures of ovary

Left ovary: The mean ovarian weight was significantly higher ($P < 0.05$) in LN (1.12 ± 0.14 gm) and FN (1.04 ± 0.24 gm) group as compared to AC (0.39 ± 0.03 gm) group of ewes. The difference in mean of number of follicles between ovary bearing small and medium follicles of all the groups was not significant. The number of large follicles was significantly higher ($P < 0.001$) in FN (3.33 ± 0.61 gm) group than other groups. The total number of follicles was significantly higher ($P < 0.1$) in FN (14.83 ± 4.30) than FI (6.50 ± 1.89) and AC (6.16 ± 1.37) group (Table 3a; Fig 3a).

Right ovary: The mean ovarian weight was significantly higher ($P < 0.05$) in LI (1.34 ± 0.26 gm) and FI (1.17 ± 0.23 gm) group as compared to AC (0.45 ± 0.07 gm) group of ewes. The

mean number of medium size follicles was significantly higher ($P < 0.05$) in LN (6.66 ± 1.45) than FN (2.33 ± 0.61) group. The number of large follicles was significantly higher ($P < 0.1$) in FN (3.50 ± 1.14 gm) group than other groups. The ovarian weight after follicular fluid aspiration was significantly higher ($P < 0.05$) in LI (1.22 ± 0.23) and FI (1.02 ± 0.22) than AC (0.40 ± 0.06) group (Table 2b; Fig 2b).

3.3 Endocrine Progesterone (P_4) profile

The mean concentration of progesterone (ng/ml) in ewes with or without uterine infection is presented in Table 3 and Fig 3. Mean serum progesterone concentration was significantly higher ($P < 0.05$) in LN (15.71 ± 3.41) and LI (12.76 ± 2.47) as compared to FN (5.47 ± 1.31), FI (4.97 ± 2.29) and AC (0.38 ± 0.08) group of ewes. However the mean concentration

of progesterone was higher in cyclic normal ewes than the corresponding cyclic infectious group. The mean follicular progesterone concentration was significantly higher ($P<0.001$) in LI (31.45±2.43), LN (35.11±1.57) and FI (31.41±2.73) groups as compared to FN (19.36±1.63) and AC (23.04±1.27) group of ewes. Progesterone concentration of

corpus luteum did not differ significantly between infectious (31.51±1.46) and normal (31.49±0.90) group of ewes. The mean progesterone concentration was significantly higher in corpus luteum and follicular fluid than serum of LN and LI ($P<0.001$), FI and AC ($P<0.05$) and FN group of ewes ($P<0.1$) (Table 2).

Table 1: Distribution of experimental ewes

Total no. of samples N=92				
Infectious (n=24)		Normal (n=68)		
26.08%		73.91%		
FI (n=6)	LI (n=18)	FN (n= 22)	LN (n= 16)	AC (n=30)
6.52%	19.56%	23.91%	17.39%	32.60%

AC=Acyclic; FN= Follicular normal; FI= Follicular infectious; LN= Luteal normal; LI= Luteal infectious

Table 2a: Functional structures in the left ovaries of ewes with or without uterine infection

Group	Left Ovary								
	Weight Intact* (gm)	No. of Follicles			Total No. Follicles#	WAFFA (gm)	WACLE* (gm)	CL weight (gm)	CL Diameter (cm)
		Small	Medium	Large**					
AC	0.39±0.03 ^a	4.00±1.23	2.16±0.94	0.00 ^a	6.16±1.37 ^a	0.32±0.03	-	-	-
FI	0.80±0.15 ^{ab}	1.66±0.91	3.16±0.98	1.66±0.33 ^b	6.50±1.89 ^a	0.68±0.14	-	-	-
FN	1.04±0.24 ^b	8.00±3.49	3.50±0.71	3.33±0.61 ^c	14.83±4.30 ^b	0.76±0.20	-	-	-
LI	0.70±0.18 ^{ab}	2.66±0.33	4.50±0.34	1.00±0.35 ^{ab}	8.33±0.49 ^{ab}	0.56±0.17	0.06±0.06 ^b	0.54	0.48
LN	1.12±0.14 ^b	3.00±0.36	4.50±0.92	0.66±0.21 ^{ab}	8.16±0.87 ^{ab}	0.98±0.22	0.48±0.15 ^a	0.41±0.06	0.63±0.03

Means bearing different superscripts (a, b) within a column differ significantly * ($P<0.05$): ** ($P<0.001$): # ($P<0.1$)

AC=Acyclic; FN= Follicular normal; FI= Follicular infectious; LN= Luteal normal; LI= Luteal infectious, WAFFA= Weight after follicular fluid aspiration, WACLE= Weight after corpus luteum enucleation.

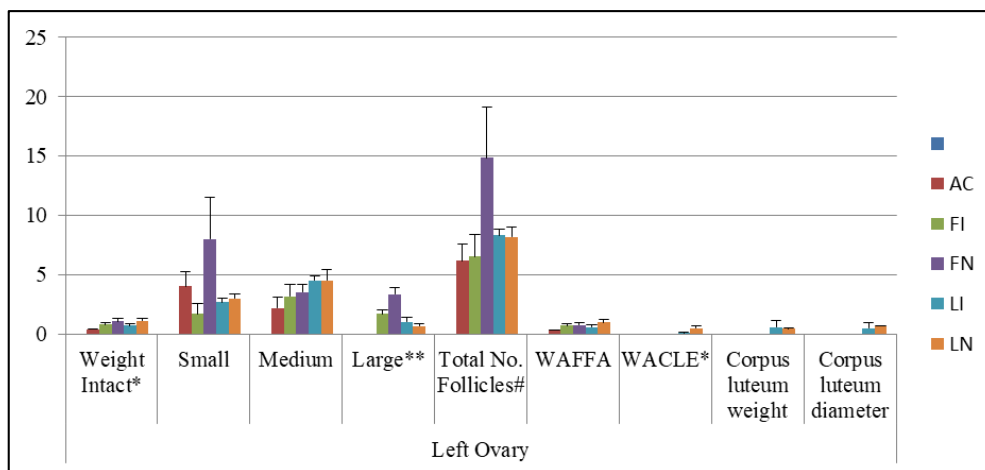


Fig 2a: Functional structures in the left ovaries of ewes with or without uterine infection

Table 3b: Functional structures in the right ovaries of ewes with or without uterine infection

Group	Right Ovary								
	Weight Intact (gm)	No. of Follicles			Total No. Follicles	WAFFA (gm)	WACLE (gm)	CL weight (gm)	CL diameter (cm)
		Small	Medium	Large					
AC	0.45±0.07 ^a	6.33±1.22	1.33±0.61 ^a	0.00 ^a	7.66±1.54	0.40±0.06 ^a	-	-	-
FI	1.17±0.23 ^b	1.16±0.30	4.16±1.30 ^{abc}	2.83±0.54 ^b	8.16±1.35	1.02±0.22 ^b	-	-	-
FN	0.99±0.21 ^{ab}	6.66±4.18	2.33±0.61 ^a	3.50±1.14 ^b	13.16±5.41	0.82±0.17 ^{ab}	-	-	-
LI	1.34±0.26 ^b	1.66±0.66	4.50±0.56 ^{bc}	1.80±0.37 ^{ab}	7.66±0.42	1.22±0.23 ^b	0.66±0.16	0.59±0.13	0.56±0.04
LN	1.02±0.13 ^{ab}	2.83±0.54	6.66±1.45 ^c	1.60±0.24 ^{ab}	11.00±2.06	0.89±0.13 ^{ab}	0.63±0.13	0.35±0.05	0.65±0.06

Means bearing different superscripts (a, b) within a column differ significantly ($P<0.05$)

AC=Acyclic; FN= Follicular normal; FI= Follicular infectious; LN= Luteal normal; LI= Luteal infectious, WAFFA= Weight after follicular fluid aspiration, WACLE= Weight after corpus luteum enucleation.

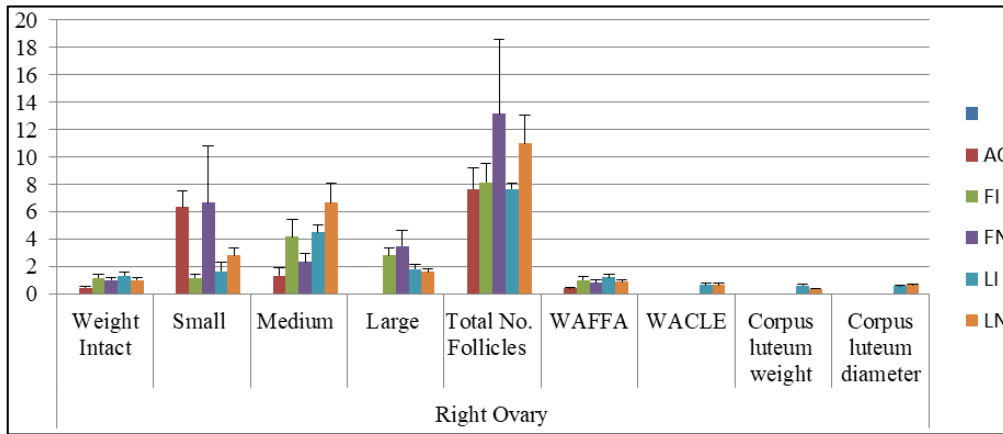


Fig 2b: Functional structures in the right ovaries of ewes with or without uterine infection

Table 3: Progesterone concentration (mean ± SEM) in ewes with or without uterine infections

Groups	P ₄ (ng/ml)		
	Serum*	Follicular fluid**	Corpus luteum
AC**	0.38±0.08 ^{aA}	23.04±1.27 ^{aB}	-
FI**	4.97±2.29 ^{aA}	31.41±2.73 ^{bB}	-
FN*	5.47±1.31 ^{aA}	19.36±1.63 ^{aB}	-
LI*	12.76±2.47 ^{bA}	31.45±2.43 ^{bB}	31.51±1.46 ^B
LN***	15.71±3.41 ^{bA}	35.11±1.57 ^{bB}	31.49±0.90 ^B

Means bearing different superscripts (a, b) within a column differ significantly; * ($P < 0.05$), ** ($P < 0.001$); (A, B) within rows differ significantly * ($P < 0.1$), ** ($P < 0.05$), *** ($P < 0.001$).

AC=Acyclic; FN= Follicular normal; FI= Follicular infectious; LN= Luteal normal; LI= Luteal infectious

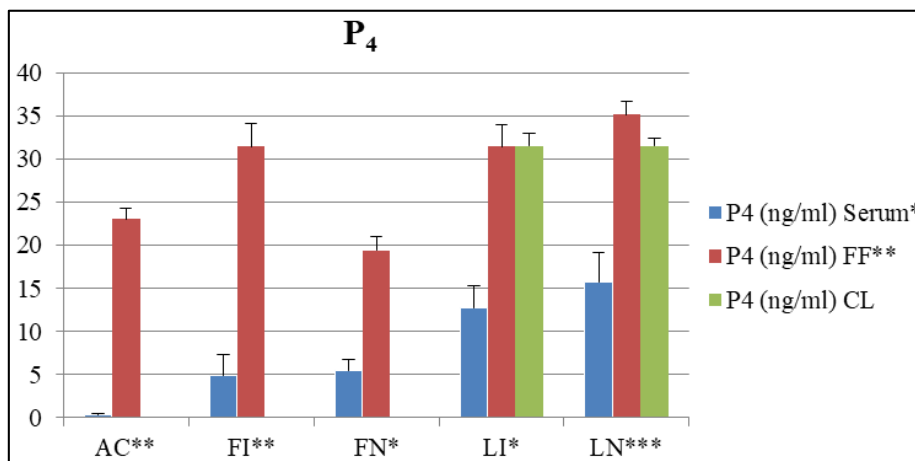


Fig 3: Progesterone concentration in ewes with or without uterine infections

4. Discussion

The uterine infection in ewes (26.08%) recorded in this study was in accordance with the previous reports in cattle and Buffalo [22, 27]. Greater incidence of uterine infection in cyclic and post partum animals than the present study has been reported on the basis of clinical observations and vaginal cytology in cattle [2] and Iraqi Buffalo [10]. However, the incidence of uterine infection was lower in clinical cases in goat and sheep [28] and abattoir based study in sheep [29]. The lower incidence reported by the previous worker might be attributed to the difference in diagnosis made by them only on the basis of clinical signs of the disease (purulent or mucopurulent secretions), whereas, in the present study thorough laboratory investigations in the form of white side test and endometrial cytology were performed; therefore, ewes suffering from subclinical endometritis could also be diagnosed. A higher number of endometritis and vaginitis due to *Salmonellosis* in case of ewes has been reported in

Himachal Pradesh [30]. The highest incidence of uterine infection in present study may take support from the findings of [30] who reported higher incidence in clinical cases of uterine infection on the basis of detailed etiological background. The higher percentage of infectious uterine condition recorded in this study warrants special investigation in ewes to ameliorating the infertility condition. This study showed higher incidence of acyclic ewes (32.60%) which is in line with the incidence of dry ewes reported by [31] in Corriedale ewes at an organized farm of Kashmir. However, the incidence of dry ewe percentage in crossbred Merino sheep in farmers flock of migratory sheep [32] and anestrus in Muzaffarnagari flock of ewes [33] was lower than the present study. The difference in incidence of uterine infection and acyclic ewes in the present study compared to earlier reports might also be attributed to the variations in species, age, breeds of animals, diagnostic procedures applied for the study and also agro-climatic conditions of the area of investigation.

The significantly higher ovarian weight recorded for the FN and LN groups in respect of left ovary and FI and LI group in respect of right ovary than AC group might be attributed to the presence of functional structures either in the form of follicle in follicular phase or in the form of CL in luteal phase. The significantly higher number of follicles in FI and FN group might be due to the ewes under the follicular phase of oestrous cycle where presence of medium and large follicle is evident. In contrary to this, previous study recorded greater number of small follicles in anestrus ewes than the ovaries of cyclic ewes [34]. The total number of medium and large follicles recorded in the cyclic ewes is comparable with the findings in ovaries of sheep [35] during different reproductive phases. The total number of follicles recorded in the cyclic normal ewes is in agreement with findings of [36] in the ovaries of healthy ewes collected from abattoirs. The higher weight of the right ovary compared to the left recorded in goats by [37] is partially comparable with the present study. However, the non significant difference found between left and right ovaries is in accordance with the findings of [38]. The weight of the ovaries recorded for cyclic ewes in the present study was higher than the report of [38] in goats. The much lower weight recorded for the acyclic ewes in this study indicates inactive ovaries and might be due to the absence of functional structures.

Progesterone concentration of in all body fluid/ tissue under observation (serum, follicular fluid and CL) was significantly higher in the luteal group of ewes (LN and LI) as compared to other group of ewes except the follicular fluid concentration in FI and AC group of ewes. The higher concentration of progesterone in the luteal group of ewes in all body fluids and tissue may be as a result of the active luteal stage of the ewes and their increased Mn and cholesterol concentration in the serum. The Progesterone is the principle steroid secreted by the corpus luteum in many domestic animals [39, 40]. The higher progesterone in the follicular fluid of FI group was in line with [41] who observed an increased level of progesterone in follicular fluid of buffalo cows suffering from endometritis. Both granulosa and theca cells of ovarian follicles produce large amounts of progesterone which serves as a precursor for androgen and subsequently estrogen production [42]. The higher progesterone in the follicular fluid of in the luteal group of ewes may be attributed to increased production of the hormones by the follicular cells in the follicular fluid – as the concentration of progesterone in follicular fluid vary during different phases of the ovarian cycle with higher levels reported during the early luteal phase than any other phases in buffalo [1]. The higher follicular progesterone in acyclic ewes compared to follicular normal ewes has also been reported by [43] in the follicular fluid of acyclic buffaloes compared to cyclic buffaloes and suggested that follicular development continues during acyclicity in buffaloes. The non-significantly higher serum level of progesterone in the healthy luteal group of ewes (LN) than the corresponding infectious group was in agreement with the report of [44] in buffaloes suffering from endometritis. However, no significant difference in luteal progesterone was observed between healthy and infectious luteal ewes, which are in contradiction to [17]. Higher follicular progesterone recorded in all group of ewes indicates important role of follicular progesterone in reproduction in ewes.

5. Conclusions

On the critical study, based on the findings, the following

conclusions could be drawn: As total number of follicles and number of large follicles were significantly higher in Follicular normal group than the other groups of ewes. The higher ovarian weight in the Follicular and Luteal group than the acyclic ewes indicates that the ovarian weight increases with the changes in functional structures. The increased follicular progesterone content in the follicular infectious group of ewes indicates disturbance in the follicular steroidogenesis.

6. References

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