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Original Paper

HISTOLOGICAL CHARACTERISTICS OF THE ENDOMETRIUM AND ITS AFFECTING FACTORS IN DAIRY CATTLE *(BOS TAURUS)* ON EMBRYO TRANSFER DAY

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In cows embryo transfer occurs on the 7th day of the oestrous cycle. Embryo survival and successful pregnancy depend on many factors, including early interaction between embryo and endometrium. Therefore, the aim of our research was to describe histo-morphological characteristics of endometrium in donor and recipient cows on obtaining and transfer day, respectively (7th day of the oestrus cycle). In addition, progesterone and oestradiol levels, the ipsilateral and contralateral corpus luteum, animal age, lactation number and days postpartum were determined. The results of the study revealed a significant difference in endometrial gland cell size, vacuolisation of glandular epithelium cytoplasm, and stromal oedema in the donor and recipient cows. However, these parameters were related only to the level of progesterone in the blood, and no other investigated factors in this study.

Keywords: *endometrium morphology, oestrous, multiple ovulation, progesterone.*

INTRODUCTION

Dairy farming plays an important role in the global economy; therefore, a lot of research has been done to improve dairy cattle productivity in connection to longevity, health, and especially on reproduction. Nowadays, embryo production *in vivo* (multiple ovulation and embryo transfer, MOET) and other assisted reproductive techniques, like oocyte aspiration and embryo production *in vitro* have continued to develop rapidly in recent years (Ferre *et al*., 2019; Lolatto *et al*., 2022). Embyo transfer (ET) is used to preserve certain rare cow breeds or highly productive cow individuals, to achieve genetic diversity, higher milk or meat productivity or to allow the creation of genetically optimal couples. It increases marketing opportunities with purebred cattle, and a higher number of genetically high-grade offsprings (Seidel and Seidel, 1991). Special attention should be paid to determine appropriate recipients (Pedersen *et al*., 2017). There are many factors influencing successful ET, the most important of which are: effective multiple ovulation in the donor, suitable synchronisation of the donor and recipient cows, embryo quality, and reproductive health of cows involved (Yesilkaya and Erdem, 2022). The uterine environment plays a significant role in embryo survival. Results from several studies indicate the importance of increasing circulating concentration of progesterone (P4) post-ovulation to enhance the uterine environment and conceptus development. P4 promotes the abundance of selected nutrients in the histotroph, which advances conceptus development and increases the survival of conceptuses (Song *et al*., 2005; Bazer *et al*., 2011). Consequently, interactions among embryos and the complex mixture of molecules secreted into the uterine lumen by endometrium glands and luminar epithelium are important factors that influence the survival, growth, development, and implantation of the conceptus. Heifers were used as embryo recipients on the 7th day of the oestrous cycle (Sįnchez *et al*., 2019). Poor uterine environment may lead to embryonic death before day 7 in an inseminated cow and causes the death of a transferred healthy embryo after day 7 (Hansen, 2020). The intrauterine environment is formed by secretion of the endometrial gland and luminar epithelium. It was found to have significantly greater endometrium superficial gland epithelial cell height than the deep gland during of oestrus cycle (Benbia *et al*., 2017). So far, no specific studies were found, which were dedicated to comparing endometrium morphological structures in cows with induced MO and cows with physiological oestrus cycle. However, histological differences in endometrium between the donor and recipient cows could impact the transferred embryo survival ability. Therefore, the aim of our research was to describe endometrial histopathological characteristics in cows with MO (donor cow) and without MO (recipient cow) on embryo transfer day (7th day of the oestrus cycle) depending on P4, and oestradiol (E2) levels, the presence of corpus luteum (CL) in ipsilaterally or contralaterally, animal age, lactation number and days postpartum.

MATERIALS AND METHODS

Animals. From January 2019 until December 2021, 22 clinically healthy dairy cows were selected from the Research and Study Farm "Vecauce" and Agrofarm "Tērvete" (Holstein and Latvian Brown cows). Cows were 4.6 ± 1.9 years old and in 2.4 ± 1.8 days of lactation. Their mean body condition score (BCS) was 4.13 ± 0.53 (5 points scale). Cows were 426 ± 208 days postpartum.

All animals were divided into two groups. The control group (CG) included ten cows without hormonal stimulation, and the multiple-ovulated cow group (MO) included 12 cows hormonally stimulated for MO.

An oestrus synchronisation and superovulation (multiple ovulation) protocols. The CG cows were synchronised using Ovarelin (Gonadorelin, Ceva Sante Animale, France) injections of 50 μg ml^{-1}·2 ml i.m. on the 0 and 10th day, and Enzaprost (Dinoprost, Ceva Sante Animale, France) injections 5 mg ml^{-1}·5 ml i.m. on the 7th and 8th day.

The MO cow group was synchronised using standard OvSynch protocol using prostaglandin F2α (PGF2α) (PGF Veyx, cloprostenol) and GnRH (Gonavet Veyx 50 μg/ml solution, gonadorelin) injections (Nowicki *et al.,* 2017). To induce MO, pregnant mare's serum gonadotropin (PMSG; Sergon, Bioveta) 2500 IU i.m. SID (10 day) was used (Abdel-Khalek *et al.,* 2018).

Ultrasonography examination. To evaluate ovarian activity and to confirm successful MO, by the presence of multiple follicles and corpus luteum (CL), 22 cows were examined rectally by palpation and transrectal reproductive ultrasonography (Easy-Scan, BCF Technology) on the 7th day of the oestrus cycle.

Sampling. Blood and endometrium biopsy samples were collected on the 7th day of the oestrus cycle from all animals. Blood samples was used to determine the level of P4, E2 and some blood morphological and biochemical parameters (RBC, Hbg, HTC, MCV, MCH, MCHC, WBC, PLT, ASAT, GGT, LDH, Urea, TP, Alb, Na, K, Ca, P, ketones, glucose). The blood haematological and biochemical tests were taken to evaluate animal health status, and P4 and E2 levels in blood to determine estrus stage and cow ovarian activity. Blood samples were collected from the caudal vein. Blood analyses were carried out in the accredited laboratory of the Institute of Food Safety, Animal Health and Environment "BIOR", Latvia (No. LVS EN ISO/IEC 17025:2017).

Two biopsy samples were taken from each animal's uterus: one sample from the left and one from the right medium part of the uterus horn using an endometrial biopsy instrument (Kruuse, Denmark). In total, 44 histological samples were obtained. To reduce physical pain and suffering caused by the biopsy procedure, epidural anaesthesia was provided using Procamidor 20 mg·ml^{-1} (Procaine hydrochloride, Richter Pharma).

Histological samples were prepared at the Faculty of Veterinary Medicine, Latvia University of Life Sciences and Technologies. The samples were fixed in 10% formalin solution and stored for 24 hours at room temperature. A Leica ASP200S tissue processor was used for dehydration and removal of fat. Then the samples were embedded in the paraffin blocks using a Medite TES Paraffin Embedding Centre. The samples were sectioned by microtome Leica RM2255 at 5-μm thickness and were placed on slides. The samples were stained with haematoxylin and eosin (HE) in a Medite TCA 44-720Tissue Stainer. Histological examination of the slides was performed at the Institute of Food Safety, Animal Health, and Environment "BIOR", Latvia. Endometrium morphology was evaluated and endometrial gland epithelium cells were measured using a light microscope Nikon ECLIPSE Ci-L complected with the data processing program NIS-Elements D 5.30.05. This equipment had undergone verification according to LATAK accreditation.

Histological samples evaluation. Histological samples of uterus horns endometrium were divided into four groups: 1) biopsy samples of uterus horns with the absence of CL in the ipsilateral ovarium or presence of CL in the contralateral ovarium of the cow without hormonal stimulation (CG with contralateral CL); 2) biopsy samples of uterus horns with the presence of CL in the ipsilateral ovarium of the cow without hormonal stimulation (CG with ipsilateral CL); 3) biopsy samples of uterus horns of the cow with unsuccessful hormonal stimulation (unsuccessful MO group); and 4) biopsy samples of uterus horns with the presence of bilateral CL in ovarium of the cow with successful hormonal stimulation (successful MO group).

There is no single golden methodology for biopsy examination, so the development of the methods to be used depends on the purpose of the study. Histological characteristics of each endometrium sample were assessed by the following parameters: 1) height of the epithelial cells (from the basement membrane to the apex of the epithelial cell), measuring randomly selected six cells from three endometrium glands, giving a total of 18 measurements from each histological sample; the number of cells was chosen to be statistically representative of the small biopsy; and 2) presence of vacuolisation of glandular epithelium cytoplasm (supranuclear and subnuclear vacuoles) (Fig. 1), endometrium stromal oedema, hemosiderin, and presence of polymorphonuclear neutrophils (PMN) in the whole slide (Ohtani *et al.*, 1993; Salleh *et al.,* 2013; Benbia *et al.* 2017; Fagundes *et al.,* 2019; Sivas *et al*., 2020; Lietaer *et al*., 2022; Pengjie *et al*., 2022). During vacuolisation of endothelium, at first basal cytoplasm of glandular epithelial cells becomes vacuolated, which correlates with the early secretory phase, and later vacuoles appear in the apical cytoplasm and release in the lumen of the gland; therefore, we described supranuclear and subnuclear vacuoles in the gland epithelium cytoplasm separately (Wang *et al*., 2007). To avoid subclinical endometritis and to determine the health status of the uterus, biopsy samples were evaluated on a 4-point scale: 0 – no evidence of inflammation; 1 – mild infiltration of lymphocytes and plasma cells, few fibrotic areas, cystic glandular degeneration, vascular changes; 2 – moderate inflammation showing prominent leukocyte, mild periglandular or blood vascular fibrosis, less cystic glandular degeneration; and 3 – severe inflammation, high infiltration of polymorphonuclear cells, uterine gland atrophy, cystic degeneration and necrosis, extensive fibrosis of blood vessels, neutrophil and macrophage infiltration of the endometrium, isolated sites of oedema, vascular congestion, haemorrhage, epithelial disruption or loss (Chapwanya *et al*., 2009).

Statistical analysis. Data were normally distributed and homogenous as assessed by the Shapiro-Wilk and Levene's tests. The independent-sample T-test was conducted to determine between the means of two independent groups. The dependent samples T-test was conducted to determine significant differences between the means of the two related groups. The Spearman's correlation test was used to test the association between two variables. To compare histological parameters (stromal oedema, hemosiderin, supranuclear vacuolisation, subnuclear vacuolisation and subnuclear vacuolisation) between groups, the chi-square test of homogeneity was used. Data are presented as means ± SD unless otherwise stated. Statistical data analysis was carried out using SPSS Statistics version 23 (IBM Corporation, Chicago, Illinois).

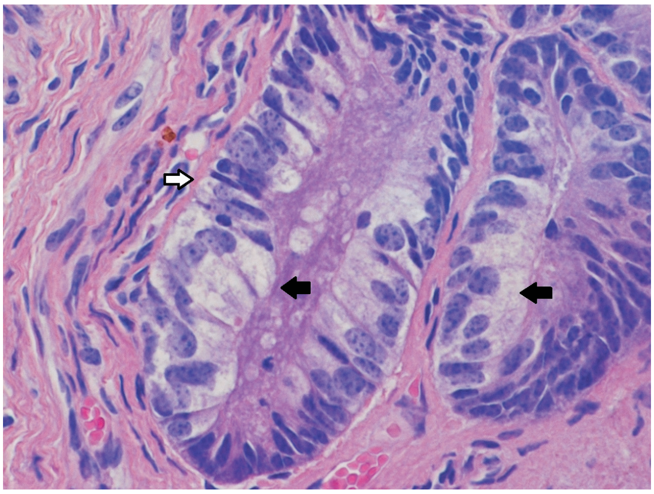

Fig. 1. Supranuclear (black arrow) and subnuclear (white arrow) vacuoles in the gland epithelium cytoplasm of uterus, ×600, HE.

Table 1. Mean score for blood test results for cows, for all together

Index and measure	Results, mean ± standard deviation	Norm (Flatland *et al.*, 2010; Guyot *et al.*, 2017)
RBC 10^6 /μl	6.50 ± 0.84	5.0–10.0
Hbg, g/dl	10.87 ± 1.24	8.0–15.0
HCT, %	30.05 ± 4.71	24.0–46.0
MCV, fl	47.79 ± 4.31	40.0–60.0
MCH, pg	15.38 ± 0.98	11.0–17.0
MCHC, g/dl	32.51 ± 1.09	31.0–34.0
WBC, 10^3/μl	7.34 ± 2.08	4.0–12.0
PLT, 10^3/μl	282.22 ± 107.70	100.0–800.0
ASAT, U/l	92.06 ± 17.60	42.0–115.0
GGT, U/l	18.07 ± 13.14	4.0–30.0
LDH, U/l	501.63 ± 62.39	308.0–938.0
Urea, mmol/l	4.75 ± 1.02	2.4–7.5
TP, g/l	71.98 ± 5.29	66.0–78.0
Alb, g/l	30.37 ± 5.45	21.0–36.0
Na, mmol/l	140.29 ± 8.48	135.0–157.0
K, mmol/l	3.9 ± 0.54	3.5–4.5
Ca, mmol/l	2.39 ± 0.13	2.2–2.9
P, mmol/l	1.92 ± 0.26	1.6–2.3
Glucose, mmol/l	3.08 ± 0.2	2.2–3.3
Keton bodies, mmol/l	0.48 ± 0.22	0–1.2

RESULTS

Blood test results of RBC, Hbg, HTC, MCV, MCH, MCHC, WBC, PLT, ASAT, GGT, LDH, Urea, TP, Alb, Na, K, Ca, P, ketones, glucose were in the normal range for animals (Table 1) (Flatland *et al.,* 2010). In our study, inflammation in endometrial biopsies was not detected, and all samples were evaluated by score 0 according to the scoring system of Chapwanya *et al*. (2009), and therefore subclinical endometritis can be excluded for all investigated animals. A minimal amount of PMN presents in different estruses periods (Ohtani *et al*., 1993). However, an increased PMN number leads to the development of endometritis (Pascottini *et al.,* 2016). Previously, there was no clear determined PMN number in the endometrium for a healthy cow. PMN number in endometrium was from 0.38 ± 1.06 to 0.5 ± 1.24 for CG and from 2.64 ± 3.41 to 4.0 ± 6.55 for the MO cow group (Fig. 2). Morphological characteristics of biopsy samples did not show a significant difference in the

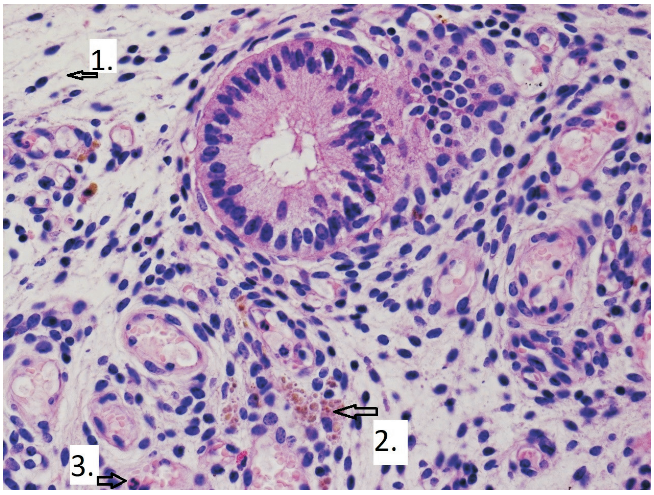

Fig. 2. Endometrium stromal oedema (1), hemosiderin (2), and presence of PMN (3) shown in the endometrium of uterus horn of successful MO group's cow, ×600, HE.

amount of PMN among the groups (Tables 2, 3, 4). Hormonal stimulation in the MO group occurred in 58% of cases (7 cows out of 12), which was confirmed by ultrasound examination of ovaries. On average there were 14 CL per animal (min 9 – max 19), and CL was presented bilaterally in the ovary. In 42% of cases (5 cows out of 12), MO was not observed. According to literature data, hormonal stimulation is unsuccessful, if ovaries did not develop any CL or contained 3 or fewer CL per animal (Jaśkowski *et al.,* 2021). In our study, cows with unsuccessful hormonal stimulation had 1 to 3 CL (Fig. 3). However, in all control group cows, CL was detected mainly in the unilateral ovary and for two cows CL developed in bilateral ovaries.

Level of P4 was significantly higher ($p < 0.05$) in the MO cow group with successful hormonal stimulation and multiple CL had developed in this group (215.21 ± 73.13 $nmol \cdot l^{-1}$), but not in CG animals, which did not show hormonal stimulation (12.30 ± 10.03 $nmol \cdot l^{-1}$), (Table 2).

Table 2. Histological parameters and steroid hormones in samples of the control and multiple ovulated groups

	CG with contralateral CL, M ± SD	Un-successful MO, M ± SD	*p*	d	CG with ipsilateral CL, M ± SD	Successful MO, M ± SD	*p*	d
PMN in the endometrium	0.38 ± 1.06	4.00 ± 6.55	0.115	NA	0.50 ± 1.24	2.64 ± 3.41	0.051	NA
Height of epithelial cells SG	17.48 ± 2.82	17.62 ± 2.55	0.922	NA	18.58 ± 2.38	22.49 ± 3.97	0.007	1.17
P4 nmol/l	15.39 ± 9.08	18.85 ± 30.71	0.655	NA	12.30 ± 10.03	215.21 ± 73.13	< 0.001	3.74
E2 pg/ml	13.04 ± 6.90	10.28 ± 3.36	0.348	NA	12.54 ± 5.95	11.78 ± 9.61	0.814	NA

CL, corpus luteum; CG, control group; MO, multiple ovulated group; P4, progesterone; E2, oestradiol; M, mean; SD, standard deviation; d, effect size; NA, not applicable

Table 3. Histological parameters and steroid hormones in the control and multiple ovulated groups

	CG				MO			
	with contralateral CL, M ± SD	with ipsilateral CL, M ± SD	*p*	d	successful MO, M ± SD	un-successful MO, M ± SD	*p*	d
PMN in the endometrium	0.38 ± 1.06	0.50 ± 1.24	0.818	NA	4.00 ± 6.55	2.64 ± 3.41	0.514	NA
Height of epithelial cells SG	17.48 ± 2.82	18.58 ± 2.38	0.376	NA	17.62 ± 2.55	22.49 ± 3.97	0.004	1.39
P4 nmol/l	15.39 ± 9.08	12.30 ± 10.03	0.492	NA	18.85 ± 30.71	215.21 ± 73.13	< 0.001	3.29
E2 pg/ml	13.04 ± 6.90	12.54 ± 5.95	0.864	NA	10.28 ± 3.36	11.78 ± 9.61	0.641	NA

CL, corpus luteum; CG, control group; MO, multiple ovulated group; P4, progesterone; E2, estradiol; M, mean; SD, standard deviation; d, effect size; NA, not applicable.

Table 4. Histological parameters comparing between animals of successful MO group and other groups

Histological parameters	Successful MO group and unsuccessful MO group	Successful MO group and CG with contralateral CL	Successful MO group and CG with ipsilateral CL
Stromal oedema	$p = 0.081$	$p = 0.050$	$p = 0.041$*
Hemosiderin	$p = 0.770$	$p = 0.512$	$p = 0.081$
Supranuclear vacuolisation	$p = 0.388$	$p = 0.021$*	$p = 0.005$*
Subnuclear vacuolisation	$p = 0.521$	$p = 0.030$*	$p < 0.001$*

* $p < 0.05$ is significant difference

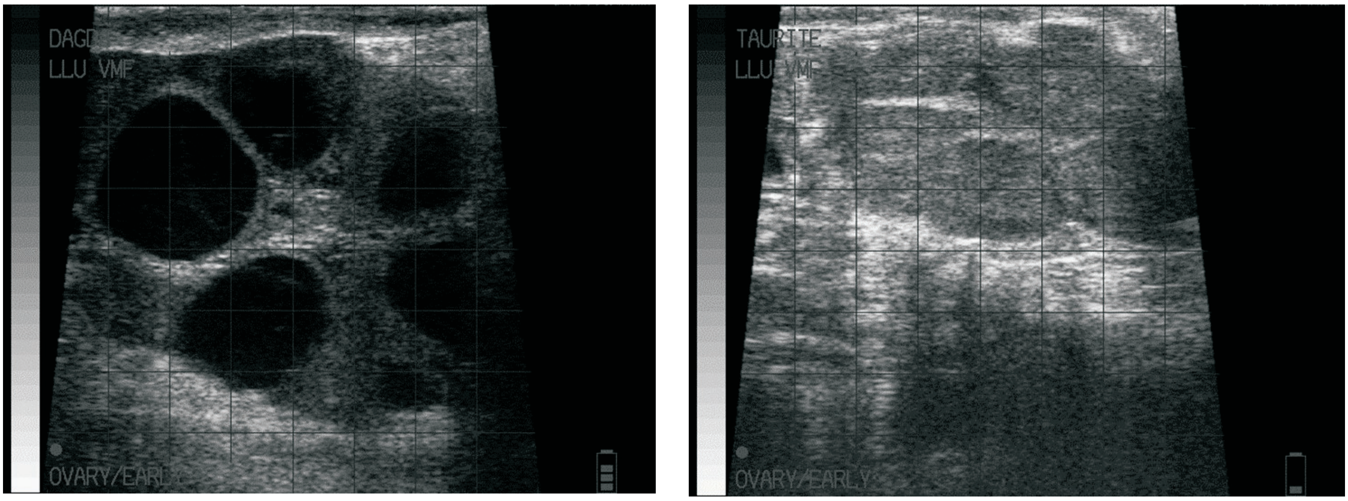

Fig. 3. Ultrasonography examination (Easy-Scan, BCF Technology). Ovarium with multiple follicles (on the left). Ovarium with multiple corpus luteum (CL) in cow with successful MO (on the right).

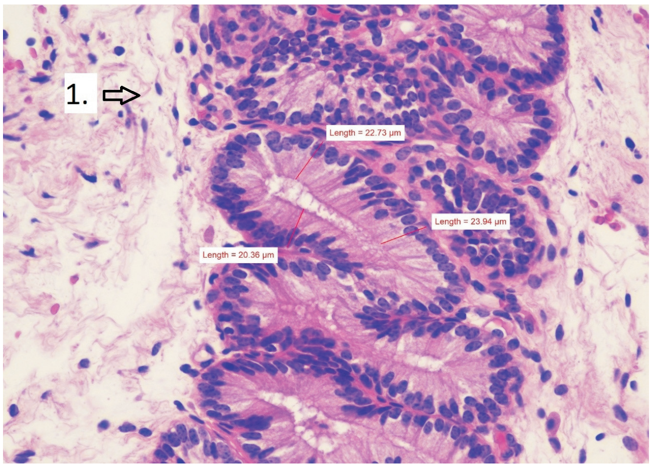

Fig. 4. Superficial endometrium gland of cow from successful MO group. Stromal oedema (1) and three measurements for glandular cell height, ×400, HE.

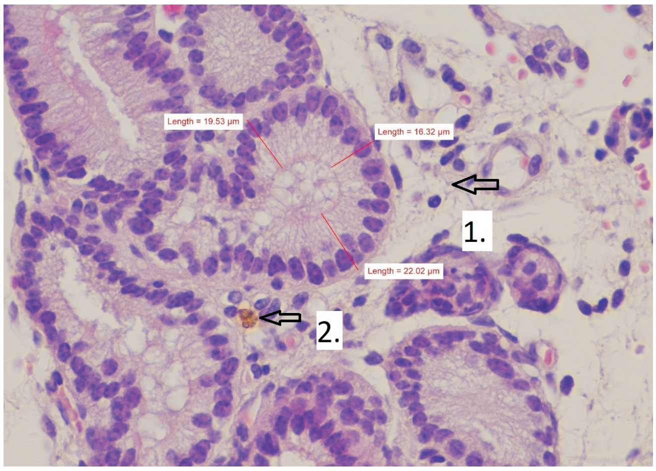

Fig. 5. Superficial endometrium gland of cow from CG with ipsilateral CL. Stromal oedema (1), hemosiderin (2), and three measurements for glandular cell height, ×600, HE.

There was no significant difference in the E2 level among groups (Tables 2, 3).

The size of glandular epithelium cells was significantly higher (22.49 ± 3.97 µm) in the MO cow group than in the CG cows that lacked hormonal stimulation and in cows of the unsuccessfull MO group (Figs. 4, 5, 6, 7). Animals of the MO group with unsuccessful hormonal stimulation and CG cows showed similar ($p > 0.05$) height of glandular epithelium cells in endometrium (17.62 ± 2.55 µm and 17.48 ± 2.82 µm, respectively) (Tables 2, 3). We did not find a statistically significant difference between endometrial glandular cell height depending on CL localisation in the control group cows (Tables 2, 3).

There was a statistically significant positive strong correlation between the P4 level and height of the glandular epithelial cells ($r = 0.548$, $n = 42$, $p < 0.001$). There was no correlation between glandular epithelial cell height and animal age or lactation number, or days postpartum ($p > 0.05$). However, there was a significant difference in the intensity of vacuolisation in glandular epithelium cytoplasm in cows

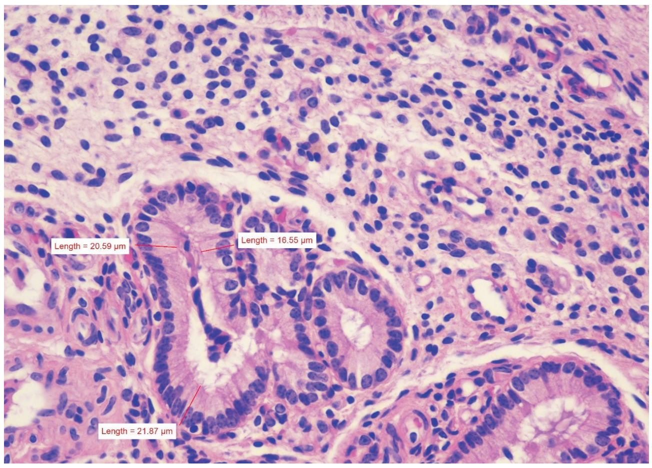

Fig. 6. Superficial endometrium gland of cow from CG with contralateral CL with three measurements for glandular cell height, ×400, HE.

that had successful MO and in CG animals (Table 4, Fig. 2). Endometrial oedema had significantly lower occurrence in the biopsy samples from CG cows with ipsilateral CL than in the MO animal group. It seemed that MO had no impact

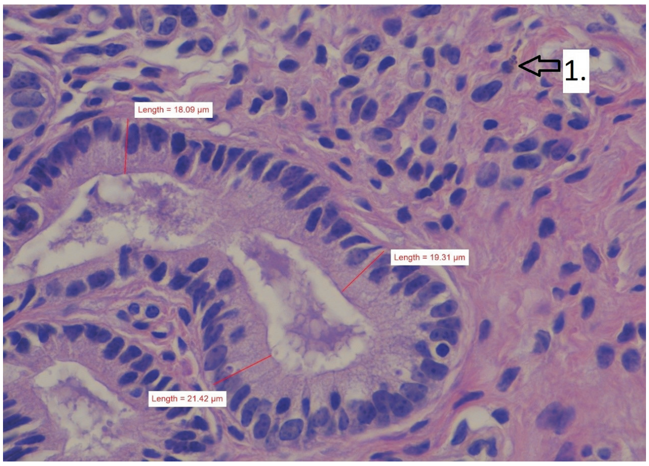

Fig. 7. Superficial endometrium gland of cow from unsuccessful MO group. Stroma present hemosiderin (1), absence of oedema and three measurements for glandular cell height, ×600, HE.

on the presence of the hemosiderin in the uterine endometrium (Table 4, Fig. 2).

DISCUSSION

Morphometry has been used to monitor pathological changes in the bovine endometrium. Particular changes in the cellular morphology of the bovine endometrium occur during the oestrous cycle and in early pregnancy: there is an increase in the frequency of mitoses in the epithelium and stroma during the proliferative phase, when the epithelial cells become increasingly ciliated, and an increase in the branching of endometrial glands. This branching reaches a peak with the cessation of cell proliferation during the early secretory phase when the basal cytoplasm of glandular epithelial cells becomes vacuolated with accumulating secretory material. In the secretory phase, this material appears in the apical cytoplasm and fills the lumen of the glands (Wang *et al*., 2007; Espejel and Medrano, 2017). The results of our research confirmed the above, as on the 7th day of the oestrus cycle, an increased P4 level stimulated endometrial gland secretory activity that manifested in significantly higher glandular epithelium cell size, as well as increased vacuolisation of glandular epithelium cytoplasm and stromal oedema in MO cow samples from the uterus horn ipsilateral to ovarium with CL, compared to CG samples. This indicates that in the case of ET, there is a significant difference in the endometrium gland activity between donor and recipient cows, which leads to the significant difference in the contents of histotroph. The uterine environment formed by histotroph affects the survival rate of transferred embryos.

Not every cow has a reproductive tract competent to support sperm survival, fertilisation, and development of the early embryo. Thus, even the most competent blastocyst transferred into a recipient cow may die if the uterine environment is inadequate to support embryo development (Hansen, 2020).

Uterine secretions in the lumen are not well defined in cattle, but there are complex mixtures of proteins, amino acids, sugars, lipids, and ions derived from genes expressed in the endometrium as well as selective transport of components (amino acids, glucose, and albumin and other proteins) from maternal blood. Endometrial epithelial secretions are particularly important for conceptus survival and growth (Geary *et al*., 2016).

In a similar study (Wang *et al*., 2007; Benebia *et al*., 2017), some additional endometrium characteristics were assessed, like morphometric parameters of deep endometrium glands and luminar epithelial cell size, which was not possible in our study, as in the previous study cow uteri were collected shortly after slaughter in an abattoir but in our case biopsy samples were taken from living farm animals. Therefore, in our study we were not able to assess some of the uterus endometrium histological characteristics discussed in the literature.

There was no significant differences among researched groups in the amount of hemosiderin and polymorphonuclear leukocytes in the endometrium, and their presence can be considered as representing physiological norms.

In our study, no correlation between glandular epithelial cell height and animal age or lactation number or postpartum days was found.

Results from several studies indicate the importance of the increase in circulating concentrations of P4 postovulation to enhance the uterine environment and conceptus development. P4 enhances the abundance of select nutrients in histotroph, which advances conceptus development and increases the survival of conceptuses (Song *et al*., 2005; Bazer *et al*., 2011). The concentration of P4 is low during the late follicular and early luteal phase, but after the formation of the CL its concentration increases dramatically (Benbia *et al*., 2017). Results of our study demonstrate that the P4 level was significantly higher in the MO group with CL caused by multiple bilateral CL. P4 concentration reaches its plateau in blood and stays high throughout pregnancy or starts to decrease with regression of CL in proestrus if pregnancy has not occurred. P4 action via the endometrium of the uterus is critical for conceptus growth and elongation. Dynamic changes in endometrial gene expression occur between days 7 and 13, which are regulated by P4 in nonpregnant and pregnant cattle (Geary *et al*., 2016).

MO is achieved by using follicle-stimulating gonadotropins, FSH and LH hormones to promote the development of subordinate follicles, which results in the development of multiple ovum and multiple CL in ovaries. However, there are many factors, such as environment, management, genetics, nutrition, applied hormones, and the scheme of insemination, that may influence how a particular cow responds to hormonal stimulations (Antane *et al.,* 2021). In our study, hormonal stimulation in the MO group was successful in 58% of cases (7 cows out of 12), confirmed by ultrasound examination of multiple CL in ovaries. In 42, or five of 12 cows, MO was not observed and ovaries contained 1–3 CL per animal. Significant elevation of P4 concentration in serum was detected in cows with a successful response to MO treatment (215.21 ± 73.13 $nmol \cdot l^{-1}$), compared to other animal groups. There was no significant difference in serum P4 concentration between cows with unsuccessful MO and CG. Therefore, we can conclude that animals with unsuccessful MO developed physiological ovulation, because 1–3 CL was confirmed by ultrasound examination. This explains why the gland cell size of endometrium was similar between cows of the unsuccessful MO group and CG cows.

In multiple ovulation embryo transfers it is crucial to start the synchronisation protocol so that both the donor and the recipients will be at day 7 of the oestrous cycle when flushing occurs. This will allow the fresh embryos to be transferred directly to a recipient that has a uterus ready for the establishment of pregnancy. If a recipient does not have a CL at the time of transfer, she should not be used. Pregnancy rate was approximately 65% when fresh embryos were transferred into recipients at the correct stage of the oestrous cycle (Hansen, 2020). Results of our study confirm the importance of CL presence in ovarium, and their role in providing a higher P4 level and consequent morphological changes in uterus endometrium.

We did not find a significant correlation between glandular epithelial cell height and animal age or lactation number or postpartum days. However, according to the literature, depending on the physiological status of the female, about 84% of inseminated beef heifers (Diskin and Sreenan, 1980; Carter *et al*., 2008) and 72% of dairy heifers (Sartori *et al*., 2002) would be pregnant at day 6 to 8 but only about 58% of lactating beef cows (Breuel *et al*., 1993) and 52% of lactating dairy cows. To increase the successful pregnancy rate after embryo transfer it has been suggested to avoid heat stress and repeat breeding (Hansen, 2020).

Low serum P4 during the development of the ovulatory follicle might be one of the critical factors leading to the increase in early embryonic death in lactating dairy cows. Fertilisation rates were not affected when different circulating concentrations of P4 were tested during the development of the ovulatory follicle, but low circulating concentrations of P4 and the subsequent increase in LH pulses can create persistent follicles and cause germinal vesicle breakdown, which leads to reduced fertility (Martins *et al*., 2018). There has been a suggestion to increase the recipient P4 level to improve successful pregnancy rate like in human medicine, whereas it is economically disadvantageous in cattle reproduction. Differences in endometrial tissue P4 concentration between different regions of the same uterine horn and between the horns ipsilateral and contralateral to the ovary bearing the corpus luteum (CL) during the luteal phase have been reported, indicating a local effect of the CL on P4 concentration in the bovine endometrium. There is a local counter-current transfer system through which steroid hormones may be transported from the ovarian vein to the ovarian artery, which is connected to a branch of the uterine artery by a prominent anastomosis in cattle supplying the oviduct and uterine horn. The uterine endometrium plays a key role in driving the elongation process via secretion and production of histotroph into the uterine luminal fluid (ULF). Embryo transfer studies established that the incidence of embryo loss is higher following transfer to the uterine horn contralateral to the ovary containing the CL, compared to transfer to the ipsilateral horn (Sanchez *et al*., 2019; Bagés-Arnal *et al*., 2020). Therefore, it is recommended to transfer the embryo in uterine horn ipsilateral to the ovary containing CL (Bagés-Arnal *et al*., 2020). However, many researchers have tried to find a scientific explanation for this. Some studies did not find any difference in survival of embryo transfer into the ipsilateral or contralateral horn to the CL in cattle, for example, foetal survival at day 42 was similar if embryos were transferred into the base of the ipsilateral horn, base of the contralateral, or tip of the contralateral horn, which was associated with similar in P4 concentration or in PGR, PGRMC1, or PGRMC2 mRNA expression in the middle part of both uterine horns (Newcomb *et al.,*1980; Takahashi *et al.,* 2016). Although differences in gene expression exist between the endometrium of uterine horns ipsilateral and contralateral to the CL in cattle, they do not impact conceptus survival or length between day 7 and 14 (Sánchez *et al.,* 2019). In our study, we did not find any significant difference in endometrial gland activity in ipsilateral or contralateral horn to the CL in recipient cattle (control group) at 7 day of oestrous. However, the bovine embryo modulates the biochemical composition of the uterine microenvironment as early as day 7 *in vivo*, and such modulation seems to be local, in cranial part of horn (Sponchiado *et al.,* 2019).

A limitation of our study was related to one biopsy from each horn, which does not provide all the information about the structure of the endometrium in the parts of the uterus, but it provides unique data about histological appearance of superficial endometrium of cow on 7 oestrus day with and without induced MO and factors, which could affect the histological appearance of superficial endometrium of cow.

CONCLUSIONS

Oestradiol level of serum, animal age, lactation number and days postpartum did not affect the endometrial histological structure of healthy dairy cattle at 7^{th} of oestrous without induced MO. Also, morphological differences in endometrial gland activity in ipsilateral or contralateral horn to the corpus luteum in these cows was not found. There were considerable differences regarding endometrial gland activity between multiple ovulated cows, and cows with natural, unstimulated ovulation, which could affect the possibility of the transferred embryo survival rate. Endometrial glandular activity significantly depends on the progesterone level in cows' blood.

ETHICS

The protocols and procedures were evaluated and approved by the local Animal Welfare and Protection Ethics Board of the Latvia University of Life Sciences and Technologies (the letter of opinion No. 17.2 from 22 June 2017).

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REFERENCES

Abdel-Khalek, A., El-Harairy, M. A., El-Bana, M. K., Shehab El-Din, A. M., Omar M. E. A. (2018). Impact of single or multiple doses of pregnant mare serum gonadotropin (PMSG) on superovulatory response of post-partum Friesian cows. *J. Animal Poultry Prod.,* **9** (7), 295–304. https://jappmu.journals.ekb.eg/. DOI: 10.21608/JAPPMU.2018.41112.

Antane, V., Lusis, I., Sematovica, I., Waldmann, A., Kanska, I., Mangale, M., Ringa-Osleja, G., Lidaks, M. (2021). Factors influencing multiple ovulation in dairy cows from Latvian brown, Latvian blue, and Danish red breeds at risk in Latvia. *Livestock Sci.*, **243,** 104379. DOI: https://doi.org/10.1016/j.livsci.2020.104379.

Bagés-Arnal, S., Sánchez, J., Fernandez-Fuertes, B., McDonald, M., Behura, S., Spencer T. E., Trudee F., Lonergan P. (2020). Location relative to the corpus luteum affects bovine endometrial response to a conceptus. *Reproduction*, **159** (5), 643–657. https://rep.bioscientifica.com/view/journals/rep/159/5/REP-19-0464.xml. DOI: https://doi.org/10.1530/REP-19-0464.

Bazer, F. W., Wu, G., Johnson, A., Kim, J., Song, G. (2011). Uterine histotroph and conceptus development: Select nutrients and necreted phosphoprotein 1 affect mechanistic target of rapamycin cell signalling in ewes. *Biol. Reprod.*, **85** (6), 1094–1107. https://academic.oup.com/biolreprod/article/85/6/1094/2530551. DOI: 0.1095/biolreprod.111.094722.

Benbia, S., Yahia, M., Letron, I. R., Benounne, O. (2017). Endometrial cells morphology depending on estrous cycle and histologic layers in cows: Morphometric study. *Global Veterinaria,* **18** (1), 68–73. https://www.idosi.org/gv/gv18(1)17/11.pdf. DOI: 10.5829/idosi.gv.2017.68.73.

Chapwanya, A., Meade, K. G., Doherty, M. L., Callanan, L. L., Mee, J. F., O'Farrelly, C. (2019). Histopathological and molecular evaluation of Holstein-Friesian cows postpartum: Toward an improved understanding of uterine innate immunity. *Theriogenology,* **71** (9), 1396–1407. https://www.sciencedirect.com/science/article/pii/S0093691X09000296. DOI: https://doi.org/10.1016/j.theriogenology.2009.01.006.

Espejel, M., Medrano, A. (2017). Histological cyclic endometrial changes in dairy cows: An overview. *J. Dairy Vet. Sci.*, **2** (1). https://juniperpublishers.com/jdvs/pdf/JDVS.MS.ID.555577.pdf. DOI: 10.19080/JDVS.2017.02.555577.

Estrada-Cortés, E. E., Oritz, W. G., Chebel, R. C. (2019). Embryo and cow factors affecting pregnancy per embryo transfer for multiple-service, lactating Holstein recipients. *Transl. Animal Sci.*, **3** (1), 60–65. https://academic.oup.com/tas/article/3/1/60/5373008. DOI: 10.1093/tas/txz009.

Fagundes, N. S., Rezende, A. L., Alvarenga, P. B., Magalhães, L. Q., Santos, R. M., Headley, S. A., Silva, M. J. B., Beletti, M. E.,. Saut, J. P. E. (2019). Short communication: Proinflammatory gene expression relative to the collection technique of endometrial samples from cows with and without subclinical endometritis. *J. Dairy Sci.,* **102** (6), 5511–5517. https://www.sciencedirect.com/science/article/pii/S0022030219303303. DOI: https://doi.org/10.3168/jds.2018-15212.

Ferre, L. B., Kjelland, M. E., Stobech, L. B., Hyttel, P., Mermillod, P., Ross, P. J. (2019). Review: Recent advances in bovine *in vitro* embryo production: reproductive biotechnology history and methods. *Animal,* 1–14. https://sci-hub.ru/10.1017/s1751731119002775. DOI: 10.1017/s1751731119002775.

Flatland, B., Freeman, K. P., Friedrichs, K. R., Vap, L. M., Getzy, K. M., Evans, E. W., Harr, K. E. (2010). ASVCP quality assurance guidelines: Control of general analytical factors in veterinary laboratories. *Vet. Clin. Pathol.,* **39** (3), 264–277. https://doi.org/10.1111/j.1939-165X.2010.00251.x. DOI: 10.1111/j.1939-165x.2010.00251.x.

Geary, T. W., Burns, G. W., Moraes, J. G. N., Moss, J. I., Denicol, A. C., Dobbs, K. B., Ortega, M. S., Hansen, P. J., Wehrman, M. E., Neibergs, H., O'Neil, E., Behura, S., Spencer, T. E. (2016). Identification of beef heifers with superior uterine capacity for pregnancy. *Biol. Reprod.*, **95** (2). https://dx.doi.org/10.1095%2Fbiolreprod.116.141390. DOI: 10.1095/biolreprod.116.141390.

Guyot, H., Detilleux, J., Lebreton, P., Garnier, C., Bonvoisin, M., Rollin, F., Sandersen, C. (2017). Comparison of various indices of energy metabolism in recumbent and healthy dairy cows. *PLOS ONE*, **12** (1), e0169716. https://pubmed.ncbi.nlm.nih.gov/28107442/. DOI: 10.1371/journal.pone.0169716.

Hansen, P. J. (2020). The incompletely fulfilled promises of embryo transfer in cattle: Why aren't pregnancy rates greater and what can we do about it? *J. Animal Sci.,* **98** (11). https://dx.doi.org/10.1093%2Fjas%2Fskaa288. DOI: https://doi.org/10.1093/jas/skaa288.

Jaśkowski, B., M., Bostedt, H., Gehrke, M., Jaśkowski, J., M. (2021). Ultrasound characteristics of the cavitary corpus luteum after oestrus synchronization in heifers in relation to the results of embryo transfer. *Animals* (Basel), **11** (6), 1706. *https://pubmed.ncbi.nlm.nih.gov/34200482/.* DOI: 10.3390/ani11061706.

Larson, R. L., Randle, R. F. (2014). The Bovine Estrous Cycle and Synchronization of Estrus. https://www.researchgate.net/publication/242520460_The_Bovine_Estrous_Cycle_and_Synchronization_of_Estrus.

Lietaer, L., Pascottini, O., B., Heirbaut, S., Demeyere, K., Vandaele, L, Meyer, E., Fievez, V., Opsomer, G. (2022). Quantitative and functional dynamics of circulating and endometrial polymorphonuclear leukocytes in healthy peripartum dairy cows. *Theriogenology,* **178**, 50–59. https://www.sciencedirect.com/science/article/pii/S0093691X21004064. DOI: https://doi.org/10.1016/j.theriogenology.2021.11.002.

Lollato, J., P., M., Souza, C., C., Silva, R., C., P., Marques, M., O., Crozara, A., S., Gonēalves, R., L., Cunha, L., S., Seneda, M., M., Morotti, F. (2022.) *In vivo* embryo production in bovine donors with low and high antral follicle counts superovulated with low and high FSH doses. *Livestock Sci.,* **262** (2022)104985. https://www.sciencedirect.com/science/article/pii/S1871141322001640. DOI: 10.1016/j.livsci.2022.104985.

Martins, J. P. N., Wang, D., Mu, N., Rossi, G. F., Martini, A. P., Martins, V. R., Pursley, J. R. (2018). Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. *J. Dairy Sci.,* **101** (11), 10505–10525. https://pubmed.ncbi.nlm.nih.gov/30197145/. DOI: 10.3168/jds.2018-14410.

Moraes, J. G. N., Behura, S. K., Geary, T. W., Spencer, T. E. (2018). Uterine influences on conceptus development in fertility-classified animals. *Proc. Natl. Acad. Sci. USA,* **115** (8). https://doi.org/10.1073/pnas.1721191115. DOI: 10.1073/pnas.1721191115.

Newcomb, R., Christie, W. B., Rowson, L. E. (1980). Fetal survival rate after the surgical transfer of two bovine embryos. *J. Reprod. Fertil.,* **59**, 31–36. https://pubmed.ncbi.nlm.nih.gov/7401043/. DOI: 10.1530/jrf.0.0590031.

Ohtani, S., Okuda, K., Nishimura, K., Mohri, S. (1993). Histological changes in bovine endometrium during the estrous cycle. *Theriogenology*, **39** (5), 1033–1042. https://www.sciencedirect.com/science/article/pii/0093691X9390004O. DOI: https://doi.org/10.1016/0093-691X(93)90004-O.

Pascottini, O., B., Hostens, M., Dini, P., Vandepitte, J., Ducatelle, R., Opsomer, G. (2016). Comparison between cytology and histopathology to evaluate subclinical endometritis in dairy cows. *Theriogenology,* **86** (6), 1550–1556. http://dx.doi.org/10.1016/j.theriogenology.2016.05.014. DOI: 10.1016/j.theriogenology.2016.05.014.

Pedersen, H. S., Mazzoni, G., Stroebech, L., Kadarmideen, H. N., Hyttel, P., Callesen, H. (2017). Basic and practical aspects of pregnancy establishment in cattle. In: *Proceedings of the 31st Annual Meeting of the Brazilian Embryo Technology Society.* https://www.animal-reproduction.org/article/10.21451/1984-3143-AR1001/pdf/animreprod-14-3-581.pdf. DOI: 10.21451/1984-3143-AR1001 (accessed 14 May 2022).

Ponomarjova, O., Sematovica, I., Piginka-Vjaceslavova, I., Vanaga, A. (2020). Cattle (*Bos taurus*) endometrium morphology on the seventh day of the estrus cycle, *Res. Rural Devel.,* 35, 140–145. https://llufb.llu.lv/conference/Research-for-Rural-Development/2020/LatviaResRuralDev_26th_2020-140-145.pdf. DOI: 10.22616/rrd.26.2020.021.

Pothmann, H., Prunner, I., Waganer, K., Jaureguiberry, M., de la Sota, R. L., Erber, R., Aurich, C., Ehling-Schulz, M., Drillich, M. (2015). The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. *Theriogenology,* **83** (8), 1249–1253. https://www.sciencedirect.com/science/article/pii/S0093691X1500014X?via%3Dihub. DOI: 10.1016/j.theriogenology.2015.01.013.

Salleh, N., Helmy, M. M., Fadila, K. N., Yeong, S., O. (2013). Isoflavone genistein induces fluid secretion and morphological changes in the uteri of post-pubertal rats. *Int. J. Med. Sci.,* **10**, 665–675. https://pubmed.ncbi.nlm.nih.gov/23569430/. DOI:10.7150/ijms.5207.

Sánchez. J. M., Passaro, C., Forde, N., Browne, J. A., Behura, S. K., Fernández-Fuertes, B., Mathew, D. J., Kelly, A. K., Butler, T., Spencer, T. E., Lonergan, P. (2019). Do differences in the endometrial transcriptome between uterine horns ipsilateral and contralateral to the corpus luteum influence conceptus growth to day 14 in cattle? *Biol. Reprod.,* **100** (1), 86–100. https://academic.oup.com/biolreprod/article/100/1/86/5076021?login=true. DOI: doi:10.1093/biolre/ioy185.

Seidel, G. E., Seidel, S. M. (1991). *Training Manual for Embryo Transfer in Cattle*. Animal Reproduction Laboratory, Colorado State University, Fort Collins. https://silo.tips/download/training-manual-for-embryo-transfer-in-cattle.

Sivas, M. C., Tapisiz, O. L., Ayik, R. T., Kahraman, D., Altinbas, S. K., Tekin, O. M. (2020). Effects of melatonin on uterine hypertrophy/hyperplasia: A preliminary experimental rat study. *Heliyon,* **6** (10), e05142. DOI: 10.1016/j.heliyon.2020.e05142

Song, G., Spencer, T. E., Bazer, F. W. (2005). Cathepsins in the ovine uterus: Regulation by pregnancy, progesterone, and interferon tau. *Endocrinology*, **146** (11), 4825–4833. https://academic.oup.com/endo/article/146/11/4825/2499993?login=false. DOI:10.1210/en.2005-0768.

Song, P., Liu, C., Sun, M., Liu, J., Lin, P., Wang A., Jin, Y. (2022). Oxidative stress induces bovine endometrial epithelial cell damage through mitochondria-dependent pathways. *Animals,* **12**, 2444. https://doi.org/10.3390/ani12182444.

Spencer, T. E. (2013). Early pregnancy: Concepts, challenges, and potential solutions. *Animal Frontiers,* **3** (4), 48–55. https://doi.org/10.2527/af.2013-0033. DOI: 10.2527/af.2013-0033.

Sponchiado, M., Gonella-Diaza, A. M., Rocha, C. C. G. Lo Turco, E., Pugliesi, G. L. M. R. Leroy, J., Binelli, M. (2019). The pre-hatching bovine embryo transforms the uterine luminal metabolite composition *in vivo*. *Sci. Rep.,* **9**, 8354. https://doi.org/10.1038/s41598-019-44590-9. DOI:10.1038/s41598-019-44590-9.

Takahashi, H., Haneda, S., Kayano, M., Matsui, M. (2016). Differences in progesterone concentrations and mRNA expressions of progesterone receptors in bovine endometrial tissue between the uterine horns ipsilateral and contralateral to the corpus luteum. *J. Vet. Med. Sci.,* **78** (4), 613–618. https://pubmed.ncbi.nlm.nih.gov/26782011/. DOI:10.1292/jvms.15-0366.

Thasmi, C., Siregan, T. N., Wahyuni, S., Aliza, D., Panjaitan, B., Nazaruddin, N., Sabila, F. N., Fallatanza, M. (2018). Anatomical and histological changes of uterine horn of Aceh cattle with repeat breeding. *J. Adv. Vet. Animal Res.*, **5** (4), 445–453. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6702913/. DOI: 10.5455/javar.2018.e297.

Wang, C., Robinson, R. S., Flint, A. P., Mann, G. E. (2007). Quantitative analysis of changes in endometrial gland morphology during bovine oestrous cycle and their association with progesterone levels. *Soc. Reprod. Fertility*, **134** (2), 365–371. https://rep.bioscientifica.com/view/journals/rep/134/2/1340365.xml. DOI: doi:10.1530/rep-06-0133.

Yesilkaya, O., F., Erdem, H. (2022). Multiple ovulation and embryo transfer in cattle. In: *Animal Health Perspectives*. Unique Scientific Publishers, Faisalabad, Pakistan. Vol. I, pp. 168–174. https://uniquescientificpublishers.com/wp-content/uploads/2022/ahp-v1/168-174.pdf. DOI: https://doi.org/10.47278/book.ahp/2022.21.

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PIENA ŠĶIRŅU LIELLOPU *(BOS TAURUS)* ENDOMETRIJA MORFOLOĢISKAIS RAKSTUROJUMS UN TO IETEKMĒJOŠIE FAKTORI EMBRIJU TRANSFERENCES DIENĀ

Embriju pārnešana notiek govs estrus cikla 7. dienā. Embriju izdzīvošanas spēja un veiksmīga grūsniecība ir atkarīga no daudziem faktoriem, tostarp agrīnas mijiedarbības starp embriju un dzemdes endometriju. Tāpēc mūsu pētījuma mērķis bija aprakstīt endometrija histoloģiskās pārmaiņas embriju donoru govīm un govīm recipientiem embriju pārnešanas dienā (estrus cikla 7. diena) atkarībā no progesterona un estradiola līmeņa, ipsilaterālā un kontralaterālā dzeltenā ķermeņa, dzīvnieka vecuma, laktācijas skaita un pēcdzemdību dienas. Pētījuma rezultāti parādīja, ka embriju donoru govīm un govīm recipientiem ir būtiskas atšķirības endometrija dziedzeru šūnu izmērā, dziedzeru epitēlija citoplazmas vakuolizēšanā un stromas tūskā, kas ir atkarīgas tikai no progesterona līmeņa, bet ne no pārējiem pētījumiem faktoriem.