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Results of Ultrasound Studies of the Growth Dynamics of Dominant, Subdominant Follicles and Determination of Estradiol Concentration in the Preovulatory Period in Cows

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ABSTRACT

The mechanism of natural selection of the dominant follicle and hormonal regulation of this process in cattle is still insufficiently studied. The purpose of the study was to study the dynamics of the growth of subdominant and dominant follicles in Holstein cows and optimize the technique of scanning the ovaries during the estrus cycle, measuring the parameters of follicle growth, analyzing the results of sonograms and determining the concentration of estradiol in the pre-ovulatory period. To study the growth dynamics of subdominant and dominant follicles, the method of scanning the ovaries of cows during the estrous cycle using PU2200Vet devices and the Mindray Z5 Vet ultrasound scanner equipped with a convex endorectal sensor with a frequency of 5.0-7.0MHz was used. Two or three waves of dominant follicle growth with a duration of 4 to 12 days were detected in the studied animals. The maximum area of dominant follicles ranged from 191.18 to 243.18mm^2. It is proved that the growth of dominant follicles in cows during the spontaneous estrous cycle is accompanied by an increase in the subdominant follicle population, per dominant follicle corresponds from 13.66 to 21.0 subdominant follicle. According to the results of the study, the growth rate of dominant follicle was higher compared to the growth of subdominant follicle, the growth rate of dominant follicle was from 0.9 to 4.43mm/day, this indicator for subdominant follicle was from 0.26 to 1.40mm/day. In the pre-ovulatory period, cows have a decrease in the amount of estradiol in the blood serum. The use of Doppler ultrasound scanning of the blood vessels of the corpus luteum allows determining the functional activity of the corpus luteum of the estrous cycle.

Key words: Follicle Growth, Follicle Atresia, Ovarian Ultrasound Scanning, Doppler Ultrasound, Estrous Cycle

INTRODUCTION

It is known that the process of follicle atresia, and follicle growth and ovulation, is a complex biological process, and follicle atresia is characterized by tissue remodeling (Silva et al. 2022). It is assumed that one of the enzymes of tissue remodeling is gelatinase, which plays an important role in this process. The concentration of the hormones luteinizing hormone (LH) and estradiol begins to increase before the onset of luteolysis (Baldrighi et al. 2022). There is a slight surge in follicle-stimulating hormone (FSH), which does not stimulate the follicular wave of growth of dominant follicles in the phase of luteolysis in cows (Ginther et al. 2013; Kotsiubenko et al. 2021).

The choice of the dominant follicle during the follicular wave is manifested by a deviation in diameter or a continuation of the growth rate of the largest follicle (F1) and a decrease in the growth rate of the next largest follicle (F2), usually the diameter of F1 reaches up to 8.5mm in cattle (Bevilaqua et al. 2022). The process of changing the diameter of the future dominant follicle begins approximately 12 hours before the deviation of the diameter F1 and involves an increase in granulosa cells (Ginther and Hoffman 2014). Thus, only F1 from the point of view of development is prepared to use the declining FSH and stimulate the release of FSH, respond to a short-term increase in LH to become dominant follicles (DF). The follicle that appears first can retain the F1 rating and become DF by reaching the critical stage of

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development first. However, an early size advantage is not a necessary component of the rejection process, as shown: F1 and F2 can change the diameter ranking. During the general growth phase; any follicle reaching 5mm regardless of the diameter rank can become DF; a subdominant follicle (SF) can become dominant when DF is removed; when F1 is removed with a diameter of 8.5mm, the next largest follicle that is larger than 7.0mm, or the first follicle, which subsequently reaches 7.0mm, becomes DF; after removing F1 with a diameter of 8.5mm, the concentration of insulin-like growth factor 1 (IGF1) and estradiol in the intra-follicular fluid increases (Ginther 2016).

There is information in the literature about the growth rate of dominant follicles during the first, second and third growth waves. Thus, the growth rate of F1 from 0 to 2 days (1.7±0.05mm/day) was greater ($P<0.0004$) than on days from 3 to 0 (1.4±0.1mm/day) and was greater ($P<0.002$) on days from 0 to 1 (1.8±0.1mm/day), than in days from 1 to 0 (1.5±0.1mm/day) (Abdelnaby et al. 2018; Sosa et al. 2021; Yan et al. 2021; Guo et al. 2022). The growth rate (mm/day) of each of the four follicles for the generally accepted classification differed from each other on days from -3 to 0, which is manifested in a gradual decrease in the growth rate among F1, F2, F3 and F4 follicles. Thus, the growth rate of follicle F1 during the first wave on days from -3 to 0 was 1.4mm/day, follicle F2 – 1.2mm/day, follicle F3 – 0.9mm/day, follicle F4 – 0.6mm/day (Ginther 2018; Alrabiah et al. 2021; Ginther 2021; Vieira et al. 2021).

Thus, the study of the growth dynamics of subdominant and dominant follicles is of great theoretical and practical importance. The role of the dominant follicle of the first wave in the effectiveness of oocyte fertilization during sexual hunting in cows is currently unknown. The study of the regularities of the growth of dominant and subdominant follicles allows theoretically substantiating the scheme of the use of ovulation induction in cows and heifers, for synchronization of the sexual cycle.

The aim of the study was to study the dynamics of the growth of subdominant and dominant follicles in Holstein cows and optimize the technique of scanning the ovaries during the estrous cycle, measuring the parameters of follicle growth, analyzing the results of sonograms and determining the concentration of estradiol in the pre-ovulatory period.

MATERIALS AND METHODS

All procedures performed in the study involving animal participants were in accordance with the ethical standards of the Kazakh National Agrarian Research University. A study was approved by Ethics Commission of the Kazakh National Agrarian Research University, No. A-186.

Experiments to study the growth dynamics of dominant and subdominant follicles were carried out on three cows (individual (ind) No. 1501, No. 265, No. 1697) of the Holstein breed of second lactation with a spontaneous estrous cycle of the breeding farm of Baiserke-Agro LLP (limited liability partnership) of Talgar district of Almaty region in the period from September 13 to October 4, 2018 using ultrasound scanning of the ovaries. Authors used an ultrasound device of the PU2200Vet brand equipped with a convex endorectal sensor with a frequency of 5.0-7.0MHz. The experiments were carried out in the conditions of the farm with an interval of 48 hours at a strictly fixed time from 14.00 to 18.00, after fixing the animal and pre-releasing the rectum from the fecal mass, ultrasound scans of the left and right ovaries were performed, respectively. Transrectal palpation of the ovaries was previously performed, then an ultrasound study was performed, while authors determined the number of subdominant and dominant follicles, measured the length and width of the follicles, the results were saved and transferred to a personal computer. The American-made PU2200Vet ultrasound device allows for two-dimensional measurement of the examined follicles, the measurement results were recorded in a work log indicating the location of the follicles (left or right ovary). The first ultrasound study was conducted on the day of the sexual hunt, followed by scans at intervals of 48 hours before the onset of the next sexual hunt. To measure the speed of blood flow, the degree of vascularization of the corpus luteum, a doppler ultrasound scanner of the Mindray Z5 Vet brand of Chinese manufacture was used, the length of the circle, the area of the dominant and subdominant follicles studied were determined.

As a material for determining the content of the hormone estradiol (E2) in the pre-ovulatory period, 18 blood serum samples from three Holstein cows of the breeding farm of Baiserke-Agro LLP were used. Blood for ELISA was taken from the tail vein of cows at intervals of 5 hours in the pre-ovulatory period, 12 samples of blood serum from four cows that were at different stages of the sexual cycle were used as control samples. To study the changes in the dynamics of the hormone estradiol (E2) in the blood of cows (inventory (inv) No. 9028, 9031, 82) in the pre-ovulatory period, blood samples were taken on November 9, 10 and 11, 2020 twice: in the morning at 9.30 am and in the afternoon at 14.30 pm. In animals of the control group (inv No. 5112, 6066, 5121, 13), blood samples were taken from randomly selected cows (at various stages of the sexual cycle) once, (November 9-11, 2020 at 14.30 hours).

Serum samples were stored at room temperature, and after 18 hours, the required amount of serum was separated using a dispenser. Then, aliquots of blood serum samples with a volume of 500µl were prepared, each tube was labelled and placed in a refrigerator (temperature +5-6°C). Authors determined the content of the hormone estradiol in the blood serum in the laboratory of "Genetic screening and cellular reproductive Technology" of the Department of Obstetrics, Surgery and Biotechnology of Animal Reproduction of the Kazakh National Agrarian Research University using an ELx808 immune enzyme analyzer (microplate reader) with a URIT-670 microplate mixer, a thermoshaker for a PST-60HL-4 tablet for ELISA diagnostics using the "ImmunoFA Estradiol" kit. The composition of the ELISA kit includes the following components: 96-well stripped tablet, estradiol conjugate with peroxidase, calibration samples, control serum, phosphate-salt buffer solution, substrate solution, stop reagent, film for sealing the tablet. The analysis of blood

serum samples of cows was carried out according to the protocol of the commercial kit "ImmunoFA Estradiol" Closed Joint Stock Company "HVO Immunotech", Russia.

RESULTS

Ultrasound scanning of the right and left ovaries in experimental cows was carried out at intervals of 48 hours, the length and width of the dominant follicle and subdominant follicles were measured, the localization sites of subdominant and dominant follicles were determined, the results of the ultrasound study were recorded in the form of a sonogram. Authors have identified subdominant follicles located in the cortical layer of the ovaries, the follicle shapes are often oval, the dominant follicle is well visualized, which gives an echogenic picture on the sonogram, in the form of a well-expressed darkphone (follicular fluid), the ovarian parenchyma on the sonogram has pronounced echogenicity (Fig. 1; 2).

The results of the ultrasound scan of the ovaries are presented in Table 1, the presumed dominant follicle (DF or F1) with the maximum size was detected in the right ovary on the 2nd day of the experiment with parameters 13.5x12.8mm, which, according to the results of the ultrasound study, was ovulated the next day. On the sonogram, the second dominant follicle was detected on the 5th day of the experiment in the right ovary with parameters 9.97x14.3mm (the first wave of subdominant and dominant follicle growth), the third dominant follicle (the second wave of subdominant and dominant follicle growth) was detected in the left ovary on the 17th day of the experiment with an interval of the second dominant follicle of 12 days, the size of the dominant follicle was 12.1x15.8mm.

An experiment to study the growth dynamics of subdominant and dominant follicles was conducted in three Holstein cows with a spontaneous estrous cycle during one sexual cycle. The results of the study indicate that the growth of the presumed dominant follicle is accompanied by an increase in the population of subdominant follicles. It is known that the growth of subdominant follicles is regulated by the ratio of the hormones FSH, estradiol and progesterone. Analysis of the data in Table 2 shows that an individual with ind No. 265 had two waves of dominant follicle growth during the estrous cycle, on the first day of the study, a dominant follicle was found in the right ovary, measuring 8.55-12.3mm (14.09.2018), according to the results of repeated ultrasound scanning, the ovulation process was detected in the animal (16.09.2018), i.e., image of the ovulation fossa (ultrasound, with transrectal palpation).

The dominant follicle of the first wave had parameters: length 15.3mm, width 10.2mm (Fig. 3). The dominant follicle of the second growth wave was detected in the left ovary after 10 days with parameters of 10.2-15.3mm, the third growth wave after 4 days in the left ovary with a size of 13.7-16.9mm (Fig. 4).

Three waves of dominant follicle growth were detected in cow ind No. 1697 during the estrous cycle, with the duration of the period of the first, second and third waves of follicle growth of 8, 5, 6 days, respectively (Table 3). All three dominant follicles were localized in the right ovary; the area of dominant follicles ranged from 137.76 to 243.18mm^2 (Fig. 5; 6). The sonogram of the left ovary was characterized by the growth of multiple populations of subdominant follicles, which were recorded during ultrasound scanning of the left ovary.

Table 4 shows that the number of subdominant follicles varies in the right and left ovaries, so the total number of subdominant follicles in the right ovary is 13 (2 dominant follicles, 14.09.2018 and 18.09.2018), and in the left ovary the number of subdominant follicles has reached 28 (1 dominant follicle, 30.09.2018). Analysis of the obtained results suggests that during the estrous cycle, two waves of dominant follicle growth were detected in a cow with ind No. 1501 with an interval between the first dominant follicle and the second dominant follicle for 4 days, between the second and third 12 days. Authors analyzed the number of dominant and subdominant follicles in cow ind No. 265 during the estrous cycle and found only 3 dominant follicles and 11 subdominant follicles in the right ovary, 30 subdominant follicles in the left ovary. A characteristic pattern has been identified that the growth of dominant follicles is accompanied by an increase in the population of subdominant follicles. During the estrous cycle, the maximum number of subdominant follicles were found in the cow ind No. 1697, 32 in the right ovary, 44 subdominant follicles in the left ovary. The analysis of sonograms shows that during the period from 8 to 17 of the estrous cycle, the growth of subdominant follicles in large numbers, from 8 SF to 13 SF, is observed.

In experiments, using ultrasound scanning of the ovaries of three Holstein cows during one spontaneous estrous cycle, two waves of follicle growth were recorded in two animals (No. 1501, No. 1697), three waves of dominant follicle growth in one cow (No. 265). The growth rate of DF ranged from 0.9 to 4.43mm per day, the growth rates of randomly selected SF ranged from 0.26 to 1.40mm per day (Table 5).

The measurement of blood flow velocity, the degree of vascularization of the corpus luteum of the sexual cycle on the 7 and 10th days of the estrous cycle was carried out using a Mindray Z5 Vet Doppler ultrasound scanner, while the speed of blood flow in the capillaries that formed the corpus luteum was determined (Fig. 7; 8).

Analysis of the sonograms obtained by Doppler ultrasound shows that on the 10th day of the estrous cycle, the blood flow rate increases, thereby activating the development of the corpus luteum of the sexual cycle. The results of determining the amount of the hormone estradiol in cows in the pre-ovulatory period, as well as in control animals are shown in Table 6. In cows in the pre-ovulatory period, the content of the hormone estradiol ranged on the first day from 99.169 to 93.315pmol/l, on the second day from 79.785 to 87.434pmol/L and on the third day from 96.879 to 99.422pmol/L. A similar dynamics of the content of the hormone estradiol in the pre-ovulatory period was observed in cows of the experimental group (inv No. 9031 and inv No. 82). However, in cows of the control group, the content of the hormone estradiol in the blood serum varies greatly, so the cow inv No. 5112 has a decrease in the concentration

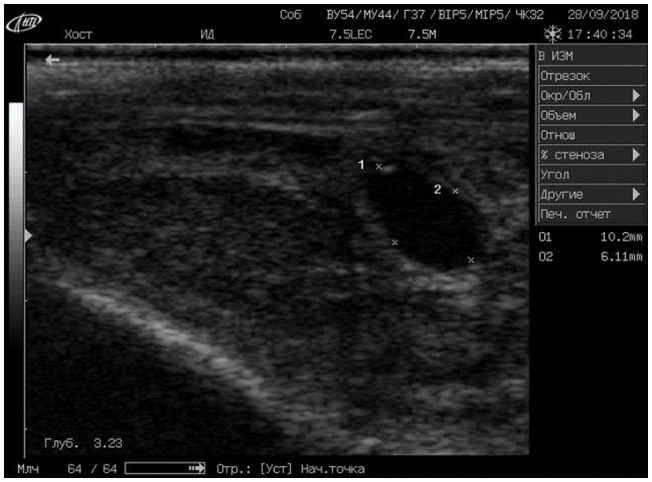

Fig. 1: Sonogram of the right ovary of a cow, ind No. 1501, 28.09.2018, dominant follicle, during the second wave of follicle growth, length 10.2mm, width 6.11mm.

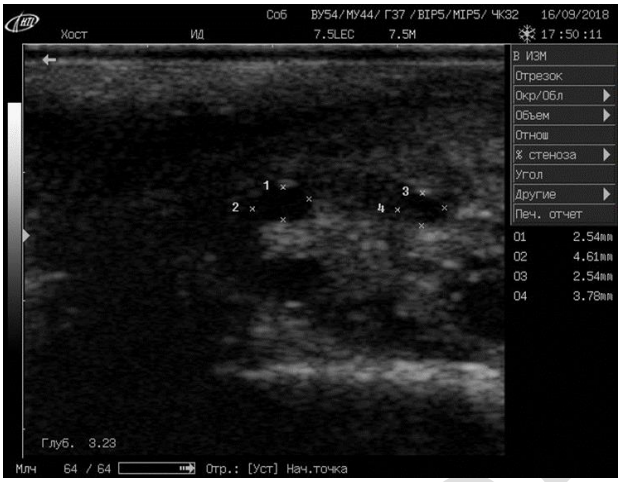

Fig. 2: Sonogram of the left ovary of a cow, ind No.1501 subdominant follicles located in the cortical layer of the ovary during the first wave of follicle growth, 16.09.2018, SF1 length 4.61mm, width 2.54mm, SF2 length 3.78mm, width 2.54mm.

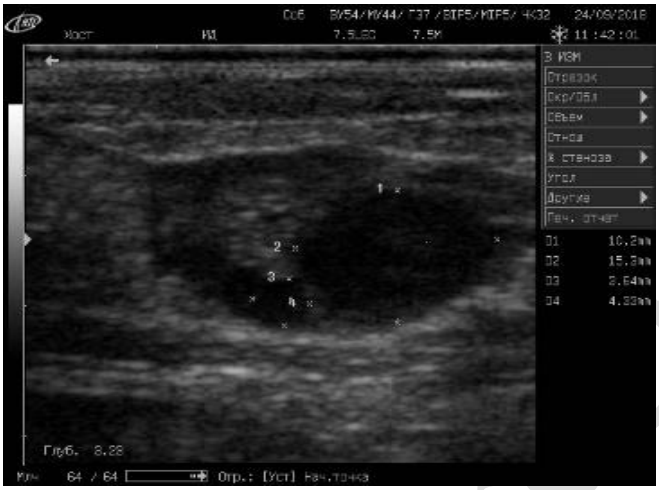

Fig. 3: Sonogram of the left ovary of a cow ind No. 265, 24.09.2018, dominant, subdominant follicles during the first wave of follicle growth, DF length 15.3mm, width 10.2mm, SF length 4.08mm, width 2.94mm.

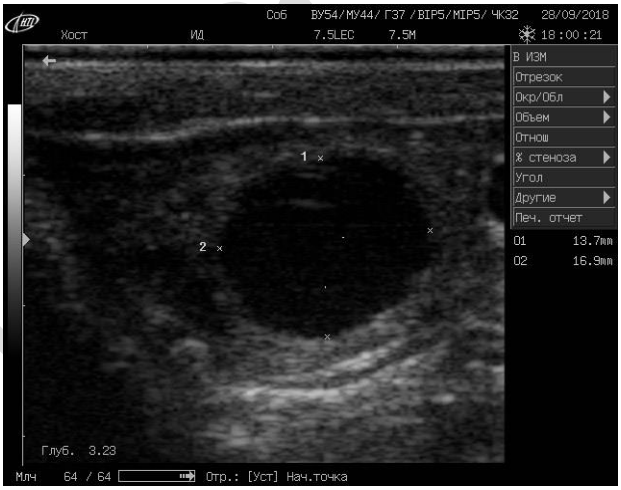

Fig. 4: Sonogram of the left ovary of the cow ind No. 265, 28.09.2018, dominant follicle during the second wave of follicle growth, length DF 16.9mm, width 13.7mm.

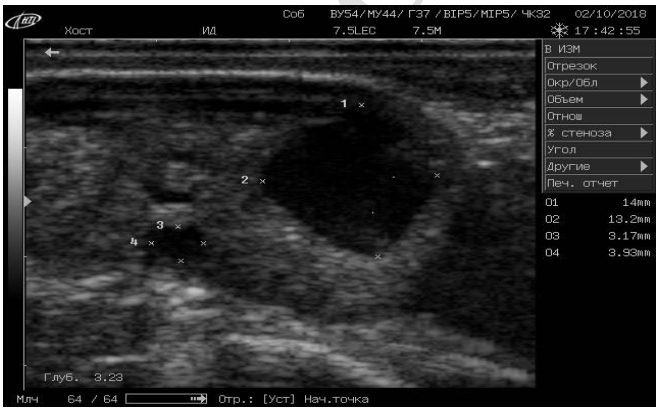

Fig. 5: Sonogram of cow ovary ind No.1697, 02.10.2018, dominant, subdominant follicles during the second wave of follicle growth in the right ovary, length DF 14.0mm, width 13.2mm, SF length 3.93mm, width 3.17mm.

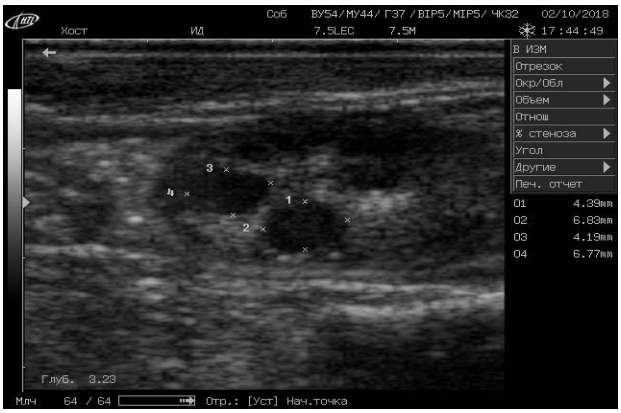

Fig. 6: Sonogram of cow ovary ind No. 1697, 02.10.2018, subdominant follicles during the second wave of follicle growth in the left ovary, SF1 length 6.83mm, width 4.39mm, SF2 length 6.77mm, width 4.19mm

Table 1: The results of ultrasound scanning of the ovaries of the cow ind No. 1501 during the estrous cycle

Date of ultrasound	Dimensions of DF and SF (mm) (right ovary)	The area of the follicle (mm^2)	Dimensions of DF and SF (mm) (left ovary)	Follicle area (mm^2)
13.09.18	12.5-11.4	142.5	5.93-6.55	38.8415
			3.56-4.31	15.3436
14.09.18	13.5-12.8	172.8 DF	5.39-7.8	42.042
			2.93-5.01	14.6793
16.09.18	6.76-13.9	93.694	3.16-4.53	14.3148
	3.29-6.62	21.7798	4.77-4.35	20.7495
			2.54-4.61	11.7094
			2.54-3.78	9.6012
18.09.18	9.97-14.3	142.571 DF	3.16-6.63	20.9508
	7.86-16.2	127.332	2.24-5.47	12.2528
			3.63-6.55	23.7765
			3-4.08	12.24
21.09.18	6.55-16.4	107.42	3.24-6.16	19.9584
			3-3.16	9.48
			4.79-5.62	26.9198
			4.31-6.7	28.877
24.09.18	12.6-7.7	97.02	3.54-4.01	14.1954
			3.08-2.08	6.4064
			5.01-6.47	32.4147
			4.79-3.1	14.849
26.09.18	6.16-12.5	77.0	4.62-5.0	(23.1)
			3.77-5.08	19.1516
28.09.18	4.89-9.17	44.8413	5.05-4.5	22.725
	10.2-6.11	62.322	3.51-4.32	15.1632
			10.0-16.01	160.1
			2.77-3.77	10.4429
30.09.18	3.47-4.57	29.6685	12.1-15.8	191.18 DF
			8.96-15.6	139.776
			2.7-3.47	9.369
02.10.18	5.86-8.55	50.103	2.85-3.95	11.2575
	4.25-7.03	29.8775	3.47-2.78	9.6466

Note: dominant follicle (DF), subdominant follicle (SF).

Table 2: The results of ultrasound scanning of the ovaries of the cow ind No. 265 during the estrous cycle

Date of ultrasound	Dimensions of DF and SF (mm) (right ovary)	Follicle area (mm^2)	Dimensions of DF and SF (mm) (left ovary)	Follicle area (mm^2)
14.09.18	8.55-12.3	105.165 DF	4.7-6.01	28.247
	9.02-11.4	102.828	2.77-4.24	11.7448
16.09.18	Ovulation DF		3.78-4.58	17.3124
			3.45-4.53	15.6285
			2.54-4.16	10.5664
			2.63-4.91	12.9133
18.09.18			3.54-7.48	26.4792
			4.86-2.95	14.337
			4.08-5.03	20.5224
			2.31-4.31	9.9561
			3.31-4.62	15.2922
			2-3.7	7.4
			2.36-4.24	10.0064
			2.85-3.78	10.773
21.09.18	4.47-4.16	18.5952	7.44-5.09	37.8696
	3.08-4.47	13.7676	5.36-7.76	41.5936
			5.32-5.54	29.4728
			3.93-6.16	24.2088
			4.41-5.16	22.7556
			4.93-6.24	30.7632
24.09.18	6.32-10.7	67.624	10.2-15.3	156.06 DF
	2.39-4.55	10.8745	3.64-4.33	15.7612
	5.63-8.71	49.0373	2.94-4.08	11.9952
	5.29-7.25	38.3525	3.08-5.01	15.4308
26.09.18			9.94-15.7	156.058
			3.76-3	11.28
28.09.18	3.16-4.0	12.64	11.9-19.1	227.29
			13.7-16.9	231.53 DF
30.09.18	4.01-4.24	17.0024	9.96-14.4	143.424
			5.01-8.31	41.6331
02.10.18	4.85-7.01	33.9985	9.56-10.6	101.336
			6.73-4.99	33.5827
04.10.18			9.11-9.75	88.8225
			7.9-5.5	43.45

Note: dominant follicle (DF), subdominant follicle (SF).

Table 3: The results of ultrasound scanning of the ovaries of the cow ind No. 1697 during the estrous cycle

Date of ultrasound	Dimensions of DF and SF (mm) (right ovary)	The area of the follicle (mm^2)	Dimensions DF and SF (mm) (left ovary)	The area of the follicle (mm^2)
13.09.18	11.2-14.3	160.16	6 follicles with a diameter of 1-2mm	-
	9.3-10.2	94.86		
	5.39-6.7	36.113		
14.09.18	5.54-10.8	59.832	6.57-8.16	53.6112
	3.23-4.93	15.9239	3.55-3.16	11.218
16.09.18	6.93-9.32	64.5876	6.55-9.29	60.8495
	5.39-7.06	38.0534	5.01-6.63	33.2163
			2.54-3.57	9.0678
			3.19-5.28	16.8432
18.09.18	10.0-13.7	137	7.16-11.4	81.624
	4.19-4.64	19.4416	4.47-5.01	22.3947
	2.3-4.85	11.115	3.01-4.78	14.3878
	2.63-2.32	6.1016	3.63-5.62	20.4006
21.09.18	12.6-19.3	243.18		
	2.63-2.31	6.0753	3.08-4.16	12.8128
	3.93-10.3	40.479	2.79-4.78	13.3362
	3.24-4.01	12.9924	3.8-4.8	18.24
	2.39-3.39	8.1021	2.47-3.7	9.139
	1.85-2.62	4.847	2.85-3.7	10.545
			2.47-3.31	8.1757
24.09.18	7.25-16.6	120.35	5.19-7.85	40.7415
	4.31-8.13	35.0403	5.55-8.11	45.0105
			2.78-4.25	11.815
			3.85-6.66	25.641
26.09.18	6.78-9.36	63.4608	5.56-6.86	38.1416
	4-4.72	18.88	6.62-6.78	44.1554
			4.25-5.81	24.6925
	4.26-5.25	22.365	3.01-4.01	12.0702
	8.2-16.8	137.76		
	6.93-12.8	88.704		
28.09.18	6.63.-11.5	76.245	3.7-4.93	18.241
	11.5-6.81	78.315	5.81-4.72	27.4232
	6.88-4.56	31.3728	4.39-6.57	28.8423
	3.39-6.93	23.4927	5.03-6.25	31.4375
			3.07-5.75	17.6525
			2.16-3.24	6.9984
			4.32-6.01	25.9632
			4.34-5.2	22.568
30.09.18	4.95-8.72	43.164	4.81-7.47	35.9307
	6.07-11.2	67.984	4.32-5.1	22.032
	7.55-9.41	71.0455	5.58-4	22.32
	3.1-4.7	14.57	3.29-4.43	14.5747
			3.2-4.43	14.176
			2.78-3.7	10.286
02.10.18	14.0-13.2	184.8	4.39-6.83	29.9837
	3.17-3.93	12.4581	4.19-6.77	28.3663
04.10.18	4.26-4.93	21.0018	6.28-6.66	41.8248
	3.62-4.48	16.2176	4.55-3.78	17.199

Note: dominant follicle (DF), subdominant follicle (SF).

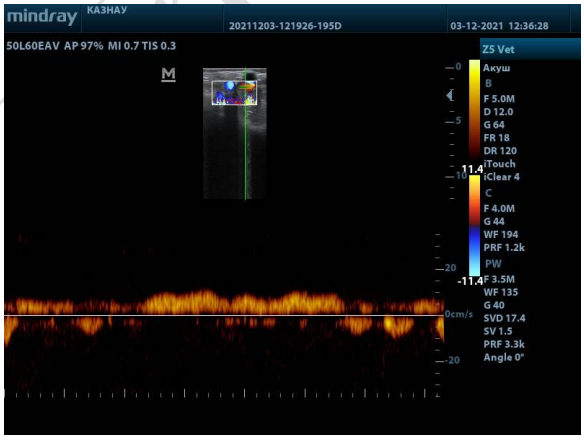

Fig. 7: Doppler ultrasound sonogram of the corpus luteum blood vessel (CL) on the 7th day of the estrous cycle of the cow.

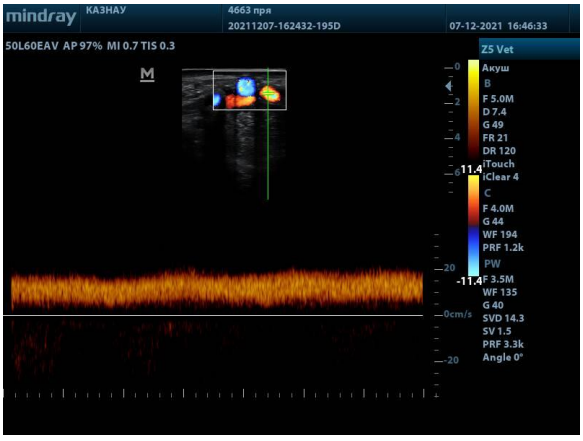

Fig. 8: Doppler ultrasound sonogram of the corpus luteum blood vessel (CL) on the 10th day of the cow's estrous cycle.

Table 4: The number of subdominant and dominant follicles detected by ultrasound scanning during the estrous cycle in experimental cows

The multiplicity of the study	Date of the study	Number of follicles	
		Right ovary	Left ovary
cow ind No. 1501			
1	13.09.18	1 SF	2 SF
2	14.09.18	1 DF	2 SF
3	16.09.18	2 SF	2 SF
4	18.09.18	1 DF + 1 SF	4 SF
5	21.09.18	1 SF	4 SF
6	24.09.18	1 SF	4 SF
7	26.09.18	1 SF	2 SF
8	28.09.18	2 SF	4 SF
9	30.09.18	1 SF	1 DF + 2 SF
10	02.10.18	2 SF	2 SF
Total		15	29
Cow ind No. 265			
1	14.09.18	1 DF + 1 SF	4 SF
2	16.09.18		3 SF
3	18.09.18		8 SF
4	21.09.18	2 SF	6 SF
5	24.09.18	4 SF	1 DF + 3 SF
6	26.09.18		2 SF
7	28.09.18	1 SF	1DF + 1 SF
8	30.09.18	1 SF	2 SF
9	02.10.18	1 SF	3 SF
10	04.10.18	1 SF	2 SF
Total		12	32
Cow ind No. 1697			
1	13.09.18	1 DF +2 SF	2 SF
2	14.09.18	2 SF	2 SF
3	16.09.18	2 SF	4 SF
4	18.09.18	4 SF	4SF
5	21.09.18	1 DF + 5 SF	6 SF
6	24.09.18	2 SF	6 SF
7	26.09.18	1 DF + 4 SF	4 SF
8	28.09.18	5 SF	8 SF
9	30.09.18	4 SF	6 SF
10	02.10.18	1 DF + 1 SF	2 SF
11	04.10.18	2 SF	2 SF
Total		36	44

Note: dominant follicle (DF), subdominant follicle (SF).

Table 5: The main parameters of the growth of dominant and subdominant follicles in experimental animals during the estrous cycle

Ind No. of animals	Maximum area DF	Minimum area DF	Growth rate DF (mm/day)	Growth rate SF (mm/day)	The number of SF per one FF
1501	191.18mm ^{2}	142.571mm ^{2}	1.2	0.35	21.0
			1.8	0.53	
			0.9	0.28	
265	231.53mm ^{2}	105.165mm ^{2}	4.11	0.26	13.66
			2.47	0.34	
1697	243.18mm ^{2}	137.76mm ^{2}	2.73	1.40	19.0
			4.43	1.10	

Note: dominant follicle (DF), subdominant follicle (SF).

Table 6: The results of determining the concentration of estradiol in the blood serum of cows of Baiserke-Agro LLP in the pre-ovulatory period and at various stages of the sexual cycle (pmol/L)

Inv No. of cows	Pre-ovulatory period					
	9.11.2020		10.11.2020		11.11.2020	
	9.30am	14.30 hour	9.30am	14.30 hour	9.30 am	14.30 hour
9028	99.169	93.315	79.785	87.434	96.879	99.422
9031	84.658	76.697	79.402	84.012	80.769	98.967
82	84.136	73.276	75.296	99.263	81.245	231.062
Inv No. of cows	Control group, % (at various stages of the sexual cycle)					
5112	256.251		207.334		94.647	
6066	752.164		131.326		602.759	
5121	90.650		79.298		92.922	
13	531.623		494.147		579.018	

of estradiol from 256.251pmol/L (1st day) to 94.647pmol/L (3rd day).

In two other cows (inv No. 6066, inv No. 13), there is an abrupt change in the content of the hormone estradiol, i.e., at first a high concentration, and then the amount of estradiol decreases and on the 3rd day there is a repeated increased secretion of this hormone. In the fourth animal (inv No. 5121), the dynamics of changes in the estradiol content is like that of cows in the pre-ovulatory period. The low estradiol content in cows in the pre-ovulatory period is probably associated with the completion of the growth of dominant follicles before ovulation. Literature data show that the growth of subdominant and dominant follicles is controlled by increased secretion of the hormone estradiol.

DISCUSSION

In three Holstein cows of the second lactation, the growth of dominant and subdominant follicles was monitored at 48-hour intervals during one spontaneous estrous cycle by ultrasound scanning. Two waves (No. 1501, 265) were detected in two cows, three waves of follicle growth were detected in one animal (No. 1697), the duration of the wave of growth of dominant and subdominant follicles was in animals (No. 1501, 265) 4, 12, 4, 10 days, respectively, in a cow (No. 1697) 5, 7 and 8 days. With ultrasound scanning of the ovaries to study the growth dynamics of dominant and subdominant follicles, it is difficult to identify dominant and subdominant follicles, since the only way to determine the corresponding follicles is to preserve sonograms and schematic images of follicles, respectively, in the right or left ovaries of the animal. The main parameters of follicle growth dynamics are the size of dominant follicles, ranking by the size of the maximum area of dominant follicles indicates that in experimental animals this indicator had the following numerical values: No. 1501 – 243.18mm^2, No. 265 – 231.53mm^2, No. 1697 – 243.18mm^2, the minimum area DF in cows No. 1501 – 142.571mm^2, No. 265 – 105.165mm^2, No. 1697 – 137.76mm^2. The results of the growth dynamics of dominant follicles obtained by us correspond to the results of the study of other authors (Mihm et al. 2000; Wathes et al. 2003; Ginther 2018; Pereryadkina et al. 2018), an important characteristic of the growth of subdominant and dominant follicles is the growth rate of follicles. Thus, the growth rate of dominant follicles was higher compared to the growth of subdominant follicles, the growth dynamics of DF ranged from 0.9mm/day to 4.43mm/day, this indicator for subdominant follicles ranged from 0.26mm/day to 1.40mm/day. Thus, according to scientists, the growth rate of F1 from 0 to 2 days was 1.7±0.05mm/day, from 3 to 0 days 1.4±0.1mm/day, on days from 0 to 1 day 1.8±0.1mm/day (Ginther 2018).

In the phase of follicular growth, subdominant follicles are growing and one of them becomes dominant, studies are currently being conducted to study the mechanism of selection of the dominant follicle (Ginther 2016). According to the results of research, it was found that the growth of dominant follicles is accompanied by an increase in the population of subdominant follicles, there are from 13.66 to 21.0 subdominant follicles per dominant follicle. The Doppler ultrasound method was used by scientists to study the hemodynamics of the reproductive system in cows during the estrous cycle (Abdelnaby et al. 2018). Authors used this technique to study the blood flow rate and the degree of vascularization of the blood vessels of the corpus luteum on the 7 and 10th days of the estrous cycle using an ultrasound scanner (Mindray Z5 Vet, China) and found that on the 10th day, compared with the 7th day, the speed and intensity of blood flow increases, which apparently is connected with the development and active secretory function of the corpus luteum of the sexual cycle. Thus, the use of ultrasound devices of the PU2200 Vet brand and Mindray Z5 Vet, allows studying the dynamics of the growth of dominant and subdominant follicles during the estrous cycle. According to scientists from other countries, the growth of dominant follicles is accompanied by an increase in the concentration of estradiol (Ryotaro 2019).

In the research by Khandoker et al. (2001), follicles 2-6mm in size were isolated, follicular fluid was divided according to the morphological appearance of cumulus-oocyte complexes. Gelatinase activity in the follicular fluid was analyzed using gelatin zymography. It was found that Pro-MMP-2 (Pro-Matrix metalloproteinases) was found in all normal and atretic categories of follicles. The active form ofmmP-2 (Matrix metalloproteinases) and the additional form of Pro-MMP-9 (Pro-Matrix metalloproteinases) were found only in atretic follicular fluid. Gelatinase activity was recorded both in granulosa cells (GC) and in theca cells (TCs) but was found in greater numbers in those follicles that had a thinned and partially detached granulosa layer.

Scientists have studied changes in the concentration of three hormones, FSH, LH and P4 (progesterone) relative to the growth dynamics of spontaneous and induced follicles during the interovulatory interval (IOI) (Mihm et al. 2000; Mihm et al. 2002; Wathes et al. 2003; Kaplunov and Gavrichenko 2017; Ginther 2018; Pereryadkina et al. 2018; Ryotaro 2019; Leonardi et al. 2020). The dynamics of follicle growth was determined using an ultrasound scanner SSD 3500 Aloka (Aloka America, Wallingford, Connecticut, United States of America), with a sensor, frequency 7.5MHz, the size of the follicles was determined by measuring the length and width of the follicles. The results of the study indicate that there is a correlation between the growth rates of spontaneous and induced follicles and with the concentration of hormones FSH and P4 (Gomez-León et al. 2018).

More detailed studies, in particular the hypothesis of organelle changes in cattle oocytes in different phases of follicle development were conducted by scientists from Canada. The authors collected cumulus-oocyte complexes from heifers of the Hereford breed by aspiration of follicles under the control of ultrasound (ultrasound examination) from DF in the growth phase (n=5; day 0 = ovulation), in the static phase (n=5), in the regression phase (n=7) of wave 1 and preovulatory follicles (n=5). Transmission electron micrography of the peripheral, perinuclear and central parts of the plasma was used to evaluate the oocytes. According to the results of the study, the volume of mitochondria occupied by lipid droplets was higher ($P<0.03$) in oocytes from regressing follicles

(193.0±10.4/1000microns^3 and 3.5±0.7%) than in the growth stage and in the preovulatory stage (118.7±14.4/1000microns^3 and 1.1±0.3%; 150.5±28.7/1000microns^3 and 1.6±0.2%, respectively). Thus, it was found that lipid droplets undergo spatial reorganization, i.e., from peripheral to uniform distribution during the growth phase and the mitochondrial lipid contact area increases as the follicle matures (Dadarwal et al. 2015; Fayezi et al. 2018; Mylostyvyi et al. 2021; Azevedo et al. 2022; Nascimento et al. 2022).

Conclusion

In the studied three cows, the duration of the growth period of dominant and subdominant follicles was 4, 5, 7, 8, 10, 12. For example, two cows had two growth waves, one cow had three growth waves of follicles. It was found that the growth of dominant follicles is accompanied by the growth of subdominant follicles, for one dominant follicle corresponds from 13.66 to 21.0 subdominant follicles. In the pre-ovulatory period, the concentration of estradiol in the blood serum of cows decreases, for a functional assessment of the activity of the corpus luteum, it is recommended to use the Doppler ultrasound scanning method of the blood vessels of the corpus luteum. The content of the hormone estradiol in the blood serum of three cows in the pre-ovulatory period ranged from 75.296 to 99.422pmol/L, in one animal the concentration of estradiol reached up to 231.062pmol/L. In the studied animals, there is no fluctuation in the concentration of estradiol during the day, hormone secretion is maintained at a certain level, a single abrupt increase in the concentration of estradiol in the cow ind No. 82 can probably be explained by an artifact. In the blood serum samples of randomly selected cows that were at different stages of the estrous cycle, the concentration of estradiol was high and ranged from 94.647 to 579.018pmol/L, only one animal (No. 5121) showed a decrease in the concentration of estradiol about 79.298pmol/L. The fact of a decrease in the concentration of estradiol in cows in the pre-ovulatory period can be explained by the hypothesis that the growth of dominant and subdominant follicles is accompanied by increased secretion of the hormone estradiol, as is known, the pre-ovulatory period in cows is characterized by a decrease in the concentration of estradiol.

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Author's Contribution

AT, KK, MA, ZhB and YeU contributed to the design and implementation of the research, the analysis of the results, and the writing of the manuscript. All authors have read and approved the final version of the manuscript.

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