



# Determination of Ovar Functionality in Cows Ending the Open Day Period, by Ultrasonography



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## Abstract

The objective of this study was to determine the efficiency in the use of ultrasound (US) for the identification of the functionality and presence of ovarian structures in dairy cattle. 69 multiparous Holstein and Jersey cows were examined, during the final period of the open days, in a range of 45 to 115 postpartum days, which are located in the milk cattle production module of the Zootechnical Post "El Salado", belonging to the Faculty of Veterinary Medicine and Zootechnics of the Benemérita Universidad Autónoma de Puebla; in Tecamachalco, Puebla, México. Before making the reproductive diagnosis, the reproductive card of each cow was evaluated to know its history; and his body condition was evaluated. Each of the females was handled individually by introducing them to the milking parlor. Transrectal evaluations of the reproductive system were subsequently carried out by means of a linear transducer of the MEDISON SA 600 V ultrasound equipment.

The priority was to evaluate both ovaries, know the functionality, the presence of structures; mainly follicles, luteal bodies and measure these structures; as well as identify functional alterations such as the presence of cysts. The evaluations were recorded in the ultrasound equipment (US), and subsequently the data were captured in a variance database, for analysis and interpretation. The results of the study show a significant difference ( $<0.05$ ), between the means of the groups (services / conception), that is, they have a greater number of services / conception when they have a body condition of 2 and decrease as Increase body condition. As the body condition increases, the open day's decrease, from a body condition of 2 to 2.75, going from 158 days to 106 days, with a significant difference of 52 days, and there is also a significant difference between the CC of 2.25 to 2.75, with 44 open days.

The use of ultrasound compared to rectal palpation is more effective and has more applications, since it allows to visually characterize the reproductive organs and their possible pathologies. The ovaries contain a lot of information that helps diagnose the reproductive state of the cow, and to select the most appropriate treatment or reproductive intervention. The following ovarian structures can be distinguished by ultrasonography: stroma, blood vessels, corpus luteum, follicles and cysts. One of the advantages of the ultrasound technique is that, if we perform serial ultrasound of a cow, extra information is obtained about the physiological state of the ovaries (follicles; dominant, subordinate, growing or regression; or the presence of bodies lute, easy to visualize). When we analyze the reproductive status of animals by ultrasound and images taken from a group of cows during the open day's period, to evaluate the follicular dynamics, the individual changes of follicles  $> 4$  mm present in each ovary must be monitored. The work routine consists of a thorough review of each ovary to measure the follicles and determine their reproductive activity and thereby maximize the effectiveness of the treatments, especially those of synchronization of cows specialized in milk production.

**Keywords:** Ultrasonography; Activity and ovarian structures

## Introduction

It is evident that the use of ultrasound and hormonal measurement have contributed to the research the basis to improve the knowledge of the reproductive cycle in cows and to be able to contribute new techniques applicable to routine control. Therefore, its cost justifies its use in reproductive aspects. However, many veterinarians who are not directly involved in the research should rely on their daily practice with techniques that improve the quality of service such as ultrasonography. In this case the farmer can already visualize what we explain on the ultrasound screen, there are no doubts or conjectures about an

early positive diagnosis by palpation, which in some cases when this is done by the skill of the veterinarian, resorption may occur that they can only be demonstrated because the ultrasound image reveals it. In other words, rectal palpation imposes a barrier on us and only our ability to mentally visualize what we feel will allow us more or less accurate diagnosis. However, with ultrasound an image is available on which it is possible to interpret and specify that diagnosis, it is finally a technique that allows us to overcome the barrier imposed by rectal palpation. The correct use of ultrasound implies earlier diagnoses of pregnancy, confirmations

of pregnancy and even sexing of the fetus. All of the above has a greater reproductive profitability of the farm. Finally, having an ultrasound device is a necessary investment for every doctor who is fully dedicated to reproduction [1].

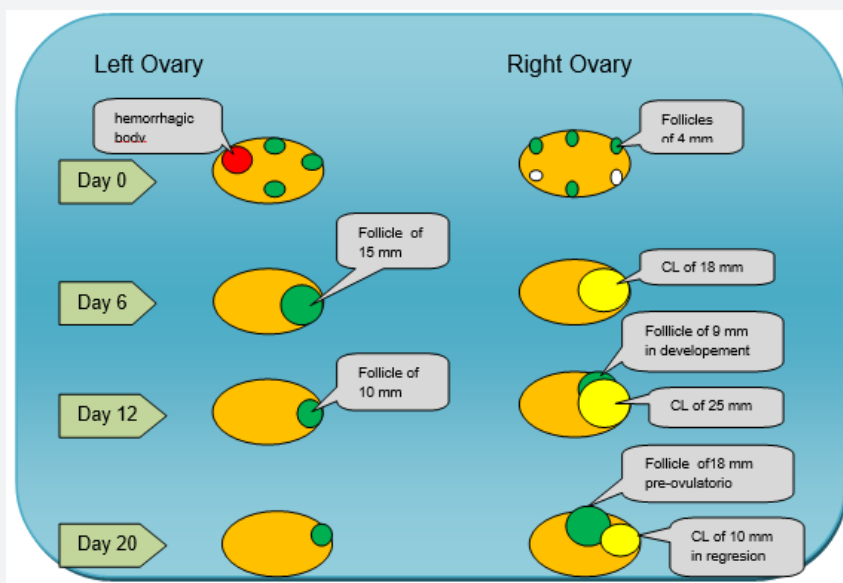
An important concept when referring to follicular dynamics during the beef estrous cycle is that of follicular waves. Folliculogenesis is defined as a series of recurring processes of recruitment, selection, growth, maturation and ovulation during the female estrous cycle, regulated by a combination of interactions between hormones, growth factors, cellular communication systems and genes [2]. Some researchers argued that follicular growth was continuous and independent of the cycle [3-5], but Rajakoski [6] introduced this new concept of follicular wave, stating that Population of follicles that emerge in the ovaries during the estrous cycle are characterized by a growth phase, followed by a static phase and finally ovulate or enter atresia, with different development patterns, the most frequent being those composed of two [7-10] and three follicular waves [9-12].

It is also possible to find estrous cycles with 1 or 4 follicular waves, although it is not the most frequent [13-15]. Until now, there were no works that characterized the follicular dynamics presented by repeating cows or repeat breeder cows (RBC) throughout their spontaneous estrous cycles, possibly due to the difficulty of getting a batch of cows with these characteristics without hormonally intervening on them. Cows with this syndrome are characterized by having repeated normal cycles of normal duration, failing pregnancy at least 3 times in a row, due to multifactorial causes [16-18], a dynamic structure, where antral follicles develop from primordial follicles, which remain dormant for many years, until they are recruited into a growth wave. In their growth, the follicles go through several stages, which are: primordial, primary, secondary, tertiary and DeGraaf follicles [19].

A wave of follicular growth involves the synchronous development of a group of follicles. This is characterized by the development of a large dominant follicle, several subordinates that invariably become aggressive. Some researchers have observed a preponderance of estrous 2-wave cycles, while others indicate a predominance of 3-wave cycles. A follicular wave usually has a duration of 8 to 10 days and the number of waves that occur during the estrous cycle depends on the period of life of the corpus luteum. They usually occur 2 to 4 during the estrous cycle, and approximately 75% of cows have 3-follicular waves [20]. The zero day of the cycle is the first day that the pre-follicle follicle disappears and coincides with the emergence of the first follicular wave. In 2-wave females, these begin on days 0 and 10 of the cycle; while in those of 3-waves, it begins on days 0, 9, and 16. The dominant follicle is ovulatory in the second wave in cows with 2-waves and in the third in 3-wave animals [21-26]. Follicular atresia is used to designate the normal process of follicular regression in the ovary and is applied to all follicles, whether cavitory or not [19].

**Materials and methods**

The present study was carried out during the months of August 2018 to November 2018, in the zootechnical post “El Salado”, belonging to the Faculty of Veterinary Medicine and Zootechnics of the Benémetita Universidad Autónoma de Puebla, México, in the milk module, with Holstein-Friesian cows and Jersey that end their period of open days, which had between 45 and 115 days postpartum, which were monitored by means of an ultrasonography device of the MEDISON S.A. 600 V brand, in order to check the functionality status of the ovaries as well as the Identification of ovarian structures and even their measurement to consider the time of ovulation and next fertilization. An evaluation of their body condition was also carried out and the reproductive cards of each cow were reviewed to have more accurate information on their reproductive activity [27-36].



**Figure 1:** Diagram of changes that occur in the ovaries during the estrous cycle. The changes that could be observed in cows with a 2-wave estrous cycle (day 0 = ovulation) are schematized.

To evaluate the follicular dynamics, the individual changes of follicles > 4 mm present in each ovary were monitored. The work routine consists of a thorough review of each ovary to measure the follicles and draw them on a spreadsheet according to their relative position. The form is individual for each animal, has a diagram of the right ovary and another of the left [37-40]. In addition to the position of each follicle, its diameter and the position and diameter of the CL are noted. To determine the position of each follicle, the transducer is placed on each ovary and rotates on its longitudinal axis. The diameter of the follicles can be measured with the electronic caliber of the equipment. When daily follicular development is monitored, the position and diameter of the follicles are compared with those of the previous days and in this way the follicles that do not grow, those that grow, those that return and the appearance can be individualized and differentiated of new ones (> 3mm) or the disappearance or ovulation of the dominant follicle of the second or third wave.

The technique of examination in transrectal ultrasonography, fecal matter is removed from the rectum before introducing the transducer, applying a lubricant (petroleum jelly) to facilitate its introduction through the anal sphincter and then cranially advancing on the floor of the rectum. The 5 MHz linear type transducer is suitable for monitoring the reproductive organs since their emitted waves reach a depth of 9 to 10 cm from their surface, allowing the operator an adequate examination of all parts of the genital tract, making a displacement from right to left allowing visualization of the uterus and ovaries (Figure 1).

**Table 3:** Although the anova notes that there is a difference between the different body conditions, the difference does not become significant, between the services / conception performed when you have a body condition of 2 and the services / conception when you have a body condition of 2.75.

Multiple Comparisons							
Variable dependiente: SERVICIOS/CONCEPCION							
Dependent variable: SERVICES / CONCEPTION							
	(I) CC	(J) CC	Mean differences (I-J)	Standard error	Sig.	95% confidence interval	
						Lower limit	Upper limit
HSD Tukey	2.00	2.25	.03333	.57700	1.000	-1.4900	1.5567
		2.50	.25000	.54739	.968	-1.1952	1.6952
		2.75	1.23333	.54303	.116	-.2003	2.6670
	2.25	2.00	-.03333	.57700	1.000	-1.5567	1.4900
		2.50	.21667	.48275	.970	-1.0579	1.4912
		2.75	1.20000	.47780	.068	-.0614	2.4614
	2.50	2.00	-.25000	.54739	.968	-1.6952	1.1952
		2.25	-.21667	.48275	.970	-1.4912	1.0579
		2.75	.98333	.44159	.127	-.1825	2.1492
	2.75	2.00	-1.23333	.54303	.116	-2.6670	.2003
		2.25	-1.20000	.47780	.068	-2.4614	.0614
		2.50	-.98333	.44159	.127	-2.1492	.1825

\*The difference in means is significant at the 0.05 level.

**Table 4:** As Sig > 0.05 there is no difference in variances within each corporate condition group for open days.

Levene statistic	df1	df2	Sig.
0.298	3	59	0.826

## Results

Using ultrasonography, it can be observed that two days after a wave is detected, there is a follicle (dominant follicle) that grows faster than the others (subordinate follicles). At 6-7 days from the beginning of the wave the dominant follicle has reached its maximum size (15-17 mm) and the subordinate follicles have undergone a process of atresia. At this time, the dominant follicle can ovulate or otherwise enters a stationary phase, which lasts approximately another 6 days and in which it maintains its size and ovulatory capacity. If the ovulation of this follicle has not occurred then, an atresia process begins and another 9 days later its size has already dropped below 4 mm (Tables 1-7) (Figures 2&3).

**Table 1:** Homogeneity test of variances.

Levene Statistic	df1	df2	Sig.
1.611	3	62	0.196

**Table 2:** Sig < 0.05, therefore the difference is significant between the means of the groups (services / conception), that is, it has greater services / conception when you have a body condition of 2 and decrease as the body condition increases.

	Sum of Squares	gl	Quadratic Mean	F	Sig.
Between groups	18.271	3	6.09	3.049	0.035
Within groups	123.85	62	1.998		
Total	142.121	65			

**Table 5:** The sig <0.05, therefore if there is a difference between the means of each body condition for the open days.

Homogeneity Test of Variances					
	Sum of squares	gl	Quadratic mean	F	Sig.
Between groups	24364.093	3	8121.364	4.070	.011
Within groups	117731.621	59	1995.451		
Total	142095.714	62			

**Table 6:** Contingency Variable.

Multiple Comparisons							
dependent variable: open-days							
	(I) CC	(J) CC	Mean differences (I-J)	Standard Error	Sig.	95% confidence interval	
						Lower limit	Upper limit
HSD Tukey	2.00	2.25	7.00000	18.83473	.982	-42.7951	56.7951
		2.50	19.80000	17.93012	.688	-27.6035	67.2035
		2.75	51.63158*	18.07597	.029	3.8425	99.4207
	2.25	2.00	-7.00000	18.83473	.982	-56.7951	42.7951
		2.50	12.80000	15.25787	.836	-27.5387	53.1387
		2.75	44.63158*	15.42899	.027	3.8405	85.4226
	2.50	2.00	-19.80000	17.93012	.688	-67.2035	27.6035
		2.25	-12.80000	15.25787	.836	-53.1387	27.5387
		2.75	31.83158	14.31071	.129	-6.0030	69.6661
	2.75	2.00	-51.63158*	18.07597	.029	-99.4207	-3.8425
		2.25	-44.63158*	15.42899	.027	-85.4226	-3.8405
		2.50	-31.83158	14.31071	.129	-69.6661	6.0030

\*The difference in means is significant at the 0.05 level.

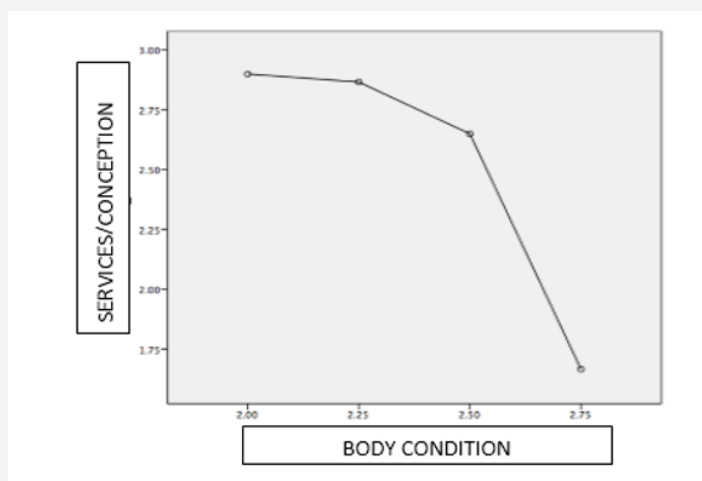
RIG- OVARY * LEF-OVARY cross tabulation															
Count															
DCL1		LEF_OVARY-													Total
		ICH	ICL1	ICL2	ICL3	ICLE	ICLQ	IE	IF10	IF15	IF5	IFQ	IQF		
RIG_OVARY	DCL1	0	0	0	3	0	0	0	3	1	0	0	0	0	7
	DCL2	1	0	3	0	1	0	0	2	2	0	2	0	0	11
	DCL3	0	0	0	0	0	0	0	0	1	0	0	0	0	1
	DCL3	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	DCLQ	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	DE	0	0	1	0	0	0	0	2	3	2	0	0	0	8
	DF10	0	1	1	1	2	0	2	2	0	2	5	0	0	16
	DF15	0	0	1	1	0	0	0	1	1	0	2	1	0	7
	DF5	0	0	1	1	0	1	0	1	1	0	1	0	0	6
	DFQ	0	0	0	2	0	0	0	1	0	0	1	0	0	4
	DQF	0	0	0	0	0	0	0	1	0	1	1	0	0	3
	DQL	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Total	1	1	7	8	3	1	2	13	9	5	14	1	1	66	

**Table 7:** Chi-square tests.

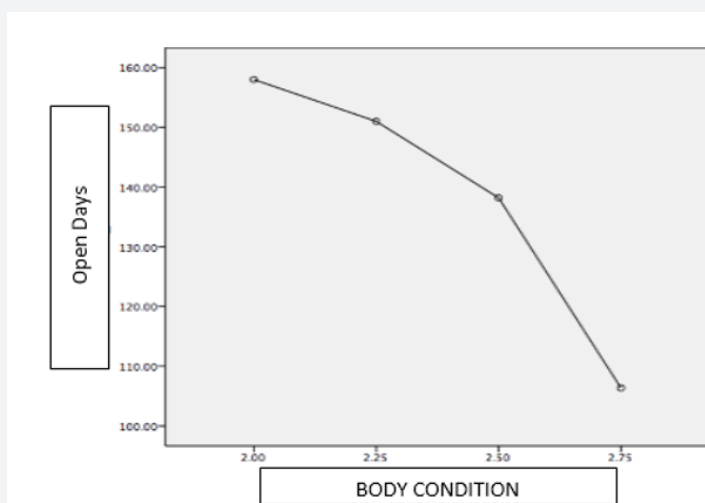
	Value	gl	Asymptotic Sig (2 faces)
Pearson's Chi-square	157.951 <sup>a</sup>	132	.061
Likelihood ratio	91.473	132	.997
N of valid cases	66		

a. 156 boxes (100.0%) have expected a count less than 5. The minimum expected count is .02.

Like the sig > 0.05, the variable “reproductive information of the right ovary” is independent of “reproductive information of the left ovary”. As well as the reproductive information of the ovaries with CC, Open days, Age, number of births and other variables is independent.



**Figure 2:** Since the variances are equal, Tukey is used to compare the means.



**Figure 3:** As the body condition increases, the open days decrease, from a body condition of 2 to 2.75, going from 158 days to 106 days, with a significant difference of 52 days, and there is also a significant difference between the CC of 2.25 to 2.75, with 44 days open.

## Discussion and conclusion

The knowledge of the waves of follicular development and its regulation has great practical importance and allows us to predict in a quite exact way the response of the animals to the different hormonal control methods of the cycle. In a physiological sexual cycle, the fundamental factor that determines the fate of the dominant follicle (ovulation or atresia) is the level of progesterone when this follicle ends its growth phase. Thus, when progesterone levels are high (luteal phase of the cycle), the dominant follicle regression occurs, in the follicular phase, without while phase of the cycle, without the “brake” of the progesterone, the fate of the dominant follicle is the ovulation. Throughout the estrous cycle, typically 2 or 3 waves of follicular development occur. In heifers and during the early postpartum of multiparous cows, ovarian cycles with 2 waves seem more frequent, while adult cows usually have 3-wave cycles. This difference is conditioned by the duration

of the corpus luteum cycle, logically less in a 2-wave cycle than in 3-wave cycles. Cycles with 4 follicular waves have also been detected and in these cases the duration of the cycle has been 24 days, with luteolysis occurring around day 20-21 of the cycle. Thus, the main factor that determines the duration of the cycle and therefore the existence of 2 or 3 waves per cycle, seems to be the life of the corpus luteum. In estrous cycles with 2 waves of follicular development, these can be detected on the day of ovulation (day 0) and on day 10 after ovulation.

This last follicle is the one that will ovulate, since the regression of the luteum corpus occurs on day 16-17 of the cycle, while the follicle that began its development on day 0 will normally undergo a process of atresia. In cycles with 3 waves these can be detected on days 0, 9 and 16 post ovulations, being the first two anovulatory because the luteal phase is maintained in these cases until day 19 of the cycle. Interestingly, this follicular dynamic is



maintained at least during the first 2-3 months of gestation, with the presence of periodic waves occurring every 9-10 days in pregnant cows. Logically, these follicles never get to ovulate due to the inhibitory effect of progesterone produced by the luteum corpus of pregnancy.

For now, the biological role and significance of the 2 and 3 wave cycles is unknown. It has been suggested that estrogen production by the dominant follicle of the first wave of the cycle would somehow regulate the transport of the egg to the uterus. In cycles of 2 and 3 waves, the follicles of the second wave would induce the formation of oxytocin receptors in the uterus, necessary for the synthesis and subsequent release of PGF<sub>2</sub>; by this organ. In any case, it has been shown that the dominant follicles of any wave of the cycle are capable of ovulating, and what is equally important, that the fertility subsequent to the ovulation of any of these follicles is very similar.

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