Determination of the effects of oxytocin and carazolol on uterine involution by pulsed-wave Doppler ultrasonography in Kıvırcık ewes

Sinem Özlem ENGİNLER[1,a,✉], Gamze EVKURAN DAL[1,b], Ali Can ÇETİN[1,c], Ahmet SABUNCU[1,d]
Kerem BAYKAL[2,e]

[1]İstanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, İstanbul, Türkiye; [2]İstanbul University-Cerrahpasa,The Institute of Graduate Education, İstanbul, Türkiye

[a]ORCID: 0000-0001-6522-285X; [b]ORCID: 0000-0002-9996-3290; [c]ORCID: 0000-0003-4617-8544; [d]ORCID: 0000-0001-7905-421X
[e]ORCID: 0000-0003-2200-403X

ARTICLE INFO

Article History
Received : 19.11.2021
Accepted : 26.09.2022
DOI: 10.33988/auvfd.1025621

Keywords
Carazolol
Oxytocin
Postpartum involution
Pulsed-wave Doppler ultrasonography
Sheep

✉Corresponding author
enginler@iuc.edu.tr

How to cite this article: Enginler SÖ, Evkuran Dal G, Çetin AC, Sabuncu A, Baykal K (2024): Determination of the effects of oxytocin and carazolol on uterine involution by pulsed-wave Doppler ultrasonography in Kıvırcık ewes. Ankara Univ Vet Fak Derg, 71 (1), 63-71. DOI: 10.33988/auvfd.1025621.

ABSTRACT

The aim of this study was to determine the effects of oxytocin and carazolol on uterine involution in postpartum Kıvırcık ewes. Thirty primiparous suckling Kıvırcık ewes were divided into 3 groups: carazolol, oxytocin, and control. The ewes underwent transcutaneous B-mode USG in the 1st week after birth, and the examinations continued via transrectal USG until the 28th day of postpartum (pp). The pulsatility index (PI), resistive index (RI), and systolic/diastolic (S/D) velocity ratio were recorded for uterine artery by Doppler USG. On postpartum 21st day, gravid horn diameter reduction was best achieved in the carazolol group. The PI values were found to be statistically significant (P<0.001) on days 14 and 21 pp. The mean RI values of uterine artery were statistically higher (P<0.01) in the oxytocin group. The highest RI value was detected on the 14th day of pp. The carazolol group had statistically higher (P<0.05) mean S/D values when compared to other groups. In conclusion, the most effective drug used to accelerate involution was found to be carazolol. The drugs used in this study appeared to have a contraction effect on the uterine arteries.

Introduction

The postpartum period includes many progressive changes in the genital tract during the transition to the normal, pregravid state. The completion of uterine involution and regeneration of ovarian activity are essential for the resumption of the normal estrous cycles, allowing for pregnancy to occur again (27, 38, 39). Uterine involution is completed by day 28 in ewes. During this process, the width of the endometrium and myometrium decreases gradually to the minimum amount (16). The size of the uterus decreases rapidly from the 3rd to the 10th day postpartum (27). Degeneration of the caruncular and uterine surface occurs at the end of the 16th day pp (27). According to this degeneration, lochia, a thick, brown-colored discharge composed of fluids and cells originates directly in the uterus after parturition (32, 35). Doppler USG is a noninvasive technique that provides information about blood flow and vascular perfusion (15). Uterine blood flow changes have been evaluated in many species, including women (19, 26), cows (22), mares (25), dogs (10), sheep, and goats (14) to observe the progressive changes in the uterus during puerperium. Elmetwally et al. (13) used color Doppler USG to detect uterine blood flow throughout gestation in small ruminants transrectally.

They used the urinary bladder as a landmark during transrectal examinations and they activated the color Doppler function of the ultrasound device to detect and visualize the uterine arteries.

Carazolol 1's chemical structure is (carbazol-4-yloxy)-3-isopropilamino-2-propanolol; it is a beta-adrenoreceptor blocker drug and an adrenaline analogue of catecholamine. It has a blocking capacity of approximately 12 hours (2). Carazolol was reported used in many animal species to cure stress-induced circulatory disorders, especially in pigs; reduce fetal deaths caused by poor uterine contractions; shorten the parturation time that gives birth to multiple offsprings, thereby decreasing the loss of offspring; collect semen from men; artificially inseminate females; and, in combination with other drugs, treat endometritis in cattle and both prevent and treat retained placenta, which is formed due to dystocia (8, 29). Carazolol increases the uterine contractions when used via iv injection in sows (30). Januszewski (20) reported that the uterine contractions of cows treated with carazolol were more potent and the uterine involution was more rapid than those of the control cows. Delayed uterine involution in the pp period can impair the excretion of the lochia, which is a beneficial medium for microorganisms that may lead to the formation of various types of metritis. According to one study, beta-adrenergic receptor blockers used in the pp period of cows hastens the uterine involution and reduce the risk of placental retention and endometritis (34). Oxytocin is widely used in domestic animals to stimulate myometrial contractions in various conditions, including the expulsion of fetuses from the uterus, to enhance or accelerate uterine involution, or to reduce the incidence of uterine disorders (7, 23). The effects of these drugs seem to decrease with the progressive involution of the uterus with their local action in the uterine blood flow rather than systemic circulation.

The purpose of this study is to investigate the involution of the sheep uterus in the pp period following the use of carazolol and oxytocin by pulsed-wave Doppler USG. The previous studies on pp uterus were mostly performed by B-mode USG. According to the author's knowledge, this is the first report that investigates these findings.

Material and Methods

Animals and management: Thirty primiparous suckling Kıvırcık ewes (2-3 years old, gave birth to singletons) were used without any uterine infection or metabolic disease in the study. No dystocia was detected in any of the ewes included in the study. The ewes were housed in a barn with access to outside runs. The animals had ad libitum access to hay, a mineral supplement, and water. The ewes were fed with a standard ration prepared by the Department of Animal Breeding, Veterinary Faculty, University of İstanbul-Cerrahpaşa, according to their nutritional status. Ethical approval for the study was obtained from the Istanbul University Animal Research and Ethics Committee (2018/47, 31.05.2018).

Study design: The ewes were divided into 3 groups randomly. A dose of 0.5 mg/sheep carazolol (Simpanorm, Fatro Italy, 100ml) according to the drug prescription, 10 IU/ sheep oxytocin (Teknovet Oxytocin, Vetaş, Türkiye, 50 ml), and 1 ml/sheep saline solution were administered i.m. to carazolol group, oxytocin group, and the control group, respectively. All of the medications were given bid on the first 3 days pp and no injection were performed after the 3rd day of pp. The first day of the delivery was considered to be day 1 of the study.

B mode USG: Ultrasonography was performed on days 1, 2, 3, 7, 14, 21, and 28, once per day. The ultrasonographic examinations were performed 30 minutes after the carazolol injection in group C and 10 minutes after the oxytocin injection in group O. The ewes underwent transcutaneous B-mode USG (Esaote Pie Medical MyLab Five Vet, 5-8 MHz microconvex transducer, Esaote Pie Medical, Genoa, Italy) in the first week after birth to better visualize the enlarged uterus. For examination by transcutaneous technique, hair in the inguinal and caudal abdomen region was fully clipped, and a coupling gel was applied. The examinations were continued by transrectal USG (Esaote Pie Medical MyLab Five Vet, 10-MHz linear transducer, Esaote Pie Medical, Genoa, Italy) thereafter. The gravid and nongravid uterine horn diameter, the caruncular diameter were measured by B-mode ultrasonographic examination. The presence of fluid in the uterus and the character of the discharge were recorded. The uterine lumen diameter measurements were taken on the largest uterine horn without discrimination of gravid or nongravid uterine horn diameter in all animals. The completion of uterine involution was assigned to each ewe as the day when the transversal diameter of the uterus returned to its original nonpregnant size (as in the oestrus cycle), ≤ 2 cm, and the uterine cavity was empty.

Pulsed-wave Doppler examinations for uterine artery in postpartum period of ewe: The uterine artery in the pp ewe was evaluated transrectally at the standing position, as described by Elmetwally et al. (13). The feces were evacuated manually, and then 10-15 ml of ultrasound gel was applied intrarectally and a rod fixed transducer was introduced into the rectum. The color flow mode was activated on the device to identify the localization of the uterine artery. When the uterine artery was found cranio-lateral to the bladder and close to the external iliac artery, a spectral mode was activated on the device, and the insonation angle was set to ≤ 60 (Figure 1). Cardiac cycles

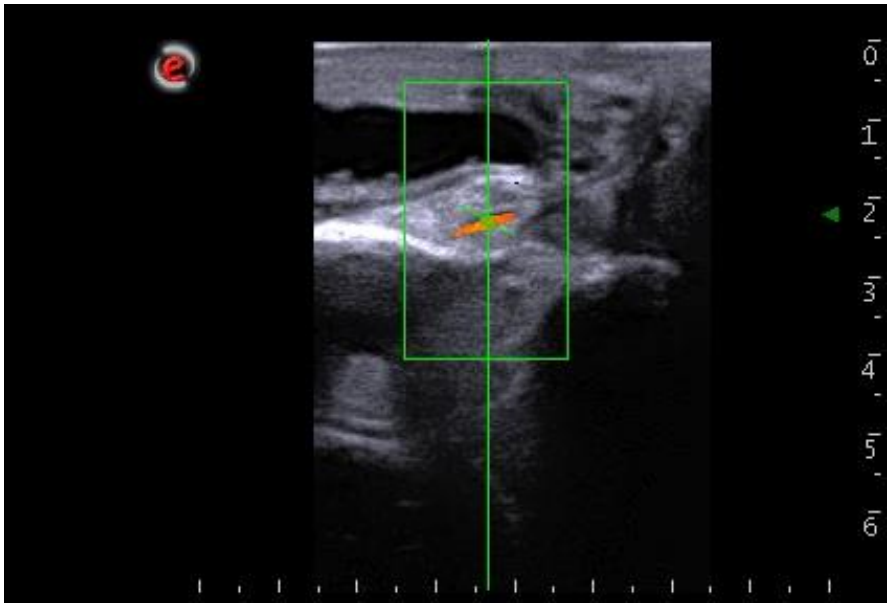

Figure 1. When the optimum color flow was achieved during ultrasonographic examinations, the pulsed-wave Doppler function was activated.

of the uterine artery have a characteristic diastolic notch. This notch is useful in demonstrating that the arteries of the sampling vessel belong to the arteria uterina (12). At least three consecutive cardiac cycles were taken in the same speed range. The hemodynamic parameters of the uterine artery, including the PI, RI, and S/D, were calculated automatically by the device.

Statistical analysis: Statistical analyses of the characteristics were conducted using the GLM repeated measures method between groups. The differences between the groups were compared with the Duncan method. The data were analyzed using an SPSS version 13.0 package program. For all statistical analyses performed, $P<0.05$ was accepted as significant.

Results

B mode USG results: The uterus could be visualised by transcutaneous USG during the first 3 days pp. However, the USG examinations were performed transrectally thereafter. The involution period was examined until the 28th day of pp in this study, and it was found to cease mostly on the 21st day, except in the 6 and 7 animals the oxytocin and control groups, respectively. In addition, involution ceased on the 14th day of pp in 1 ewe and 2 ewes from the oxytocin and carazolol groups, respectively. Thus, statistical analysis was conducted for all groups according to the 21st day of completion of the uterine involution in the study. The postpartum gravid horn diameter on the first day of pp was similar in all groups and was not statistically different between groups ($P> 0.05$) (Table 1). The gravid horn diameter decreased more rapidly in the oxytocin group than the carazolol group on day 2 pp and was statistically significant ($P<0.01$). The carazolol administration had a better effect on uterine involution on days 14 and 21 according to the pp gravid horn diameter and was found to be statistically significant on days 14 ($P<0.05$) and 21 ($P<0.001$) when compared to the other groups (Table 1). However, there were time-dependent significant differences ($P<0.001$) between all groups throughout the experiment days according to the pp gravid horn diameter results. The mean pp gravid horn diameter was 5.27±0.115 cm for all groups at the end of the 21st day of pp; gravid uterine horn diameter reduction was the best achieved (1.70±0.05 cm) in the carazolol group (Table 2). The diameter of the nongravid horn was lowest in the oxytocin-administered group compared to the other groups and was statistically significant on days 1, 2, and 3 ($P<0.01$, $P<0.001$, and $P<0.01$, respectively). But when the overall results were evaluated, carazolol was found to be more effective due to the decrease in the diameter of the pp nongravid horn on day 21 when compared to other groups and was statistically significant ($P<0.05$) (Table 1). All of the values tended to decrease throughout the experiment days, and there were time-dependent significant differences ($P<0.001$) between all groups. The thinnest mean diameter of the pp nongravid horn was that of the oxytocin-administered group compared with the other groups, and it was statistically significant ($P<0.001$). The mean pp nongravid horn diameter for all of the groups was 3.733±0.077 cm (Table 2). On day 2, the oxytocin group's pp caruncule diameters decreased more rapidly compared with the other groups, and it was statistically significant ($P<0.001$) (Table 1). The thinnest mean caruncule diameter was that of the oxytocin-administered group compared with the other groups, and it was statistically significant ($P<0.05$). All of the values tended to decrease throughout the experiment days, and there were time-dependent significant differences ($P<0.001$) between all groups (Table 2).

Table 1. The evaluation of the uterine measurements on experiment days according to the study groups.

Characteristics	PGHD (cm)			PNGHD (cm)			CD (cm)		
Groups	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**
Days	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err
1	8.78±0.24	8.34±0.18 NS	9.07±0.46	6.64±0.27^{a}	5.33±0.30^{b} **	6.96±0.48^{a}	2.25±0.09	2.07±0.12 NS	2.19±0.06
2	8.25±0.23^{a}	6.79±0.33^{b} *	7.68±0.57^{ab}	5.98±0.35^{a}	4.18±0.21^{b} ***	6.00±0.31^{a}	1.94±0.06^{a}	1.59±0.04^{b} ***	1.94±0.08^{a}
3	6.58±0.24	5.57±0.26 NS	6.13±0.42	4.64±0.39^{a}	3.28±0.16^{b} **	4.53±0.24^{a}	1.51±0.08	1.31±0.04 NS	1.48±0.10
7	3.94±0.18	4.17±0.17 NS	4.60±0.34	2.73±0.05	2.78±0.08 NS	2.96±0.14	1.04±0.04	1.07±0.02 NS	1.15±0.07
14	2.51±0.20^{b}	2.94±0.22^{ab} *	3.40±0.22^{a}	1.88±0.12	2.18±0.10 NS	2.29±0.13			
21	1.70±0.05^{b}	2.12±0.10^{a} ***	2.37±0.12^{a}	1.48±0.03^{b}	1.57±0.11^{b} *	1.80±0.14^{a}			

PGHD:Postpartum gravid horn diameter, PNGHD: Postpartum nongravid horn diameter, CD: Caruncule diameters.
NS: P>0.05, * : P<0.05, **: P<0.01, ***: P<0.001
$^{a, b, c:}$ the difference between the characteristcs indicated by different letters in the same line is significant.

Table 2. The results of the study and statistical analysis of the groups vs time.

Characteristics		PGHD (cm) Mean±Std. Error	PNGHD (cm) Mean±Std. Error	CD (cm) Mean±Std. Error	PI Mean±Std. Error	RI Mean±Std. Error	S/D Mean±Std. Error
Groups	Carazolol	5.296±0.199	3.891±0.134^{a}	1.686±0.052^{a}	2.170±0.078	0.814±0.08^{a}	12.705±0.215^{a}
	Oxytocin	4.990±0.199	3.221±0.134^{b}	1.511±0.052^{b}	2.052±0.078	0.815±0.08^{a}	11.516±0.215^{b}
	Control	5.541±0.199	4.088±0.134^{a}	1.690±0.052^{a}	1.944±0.078	0.779±0.08^{b}	9.943±0.215^{c}
		NS	***	*	N.S.	**	*
Time	1	8.730±0.182^{a}	6.311±0.209^{a}	2.171±0.054^{a}	1.824±0.079^{c}	0.798±0.007^{c}	5.833±0.156^{e}
	2	7.575±0.231^{b}	5.387±0.171^{b}	1.826±0.036^{b}	1.828±0.081^{c}	0.791±0.010^{bc}	9.443±0.250^{d}
	3	6.094±0.184^{c}	4.146±0.161^{c}	1.432±0.046^{c}	1.724±0.049^{c}	0.761±0.008^{d}	11.584±0.325^{c}
	7	4.236±0.141^{d}	2,820±0,057d	1.088±0.027^{d}	2.081±0.058^{b}	0.811±0.009^{bc}	12.688±0.288^{b}
	14	2.953±0.125^{e}	2.116±0.068^{e}		2.459±0.083^{a}	0.843±0.010^{a}	15.692±0.216^{a}
	21	2.064±0.053^{f}	1.619±0.060^{f}		2.414±0.088^{a}	0.812±0.005^{b}	13.085±0.193^{b}
		***	***	***	***	***	***
Group*Time		***	***	*	NS	NS	***
Overall Average		5.275±0.115	3.733±0.077	1.629±0.030	2.055±0.045	0.803±0.008	11.388±0.124

PGHD: Postpartum gravid horn diameter, PNGHD: Postpartum nongravid horn diameter, CD: Caruncule diameters.
NS: Non-significant, P>0.05, * P<0.05,**P<0.01, *** P<0.001
^{a,b,c,d,e,f}: The significance between the groups in the columns is shown in different letters

Pulsed-wave Doppler USG results for uterine artery in postpartum period of ewe: According to the Doppler ultrasonographic examinations, the PI values of the uterine artery were not statistically significant (P>0.05) between groups throughout the pp period (Table 3). In fact, the PI values fluctuated throughout the study period. The mean PI value for all groups was 2.055±0.045. The PI values on pp days 14 and 21 were statistically significant (P<0.001) when compared with the other examination days (Table 2). The mean RI value of the uterine artery was higher (0.815±0.08) in the oxytocin group and was statistically significant (P<0.01) when compared with the other groups. The highest RI value was detected on the 14th day of pp and was statistically significant (P<0.001).

The RI values fluctuated throughout the study period (Table 2). Although there was a fluctuation in the S/D values throughout the pp period in all groups, the carazolol group, compared to the oxytocin and control groups, had a higher mean of S/D values and was statistically significant (P<0.05) (Table 2). Specifically, the carazolol group had a higher mean S/D value and was statistically significant (P<0.001) between all groups on pp days 2., 3. and 21 (Table 3). Meanwhile, on pp days 7 and 14, the oxytocin group had a higher mean S/D, which was statistically significant (P<0.05 and P<0.001, respectively) between all groups (Table 3). The mean S/D of all the groups was detected as 11.388±0.124. The S/D values were statistically significant (P<0.001) on pp day 14 when compared to the other examination days (Table 2). There were significant time-dependent changes in all parameters (PI, RI and S/D) of the uterine artery throughout the study (P<0.001), (Table 2). The findings of uterine fluid accumulation and vaginal discharge throughout the study period are shown in Table 4.

Table 3. The evaluation of the hemodynamic changes of the uterine artery on experiment days according to the study groups.

Characteristics	**PI**			**RI**			**S/D**		
Groups	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**	**Carazolol**	**Oxytocin**	**Control**
Days	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err	Mean ± Std Err
1	1.98±0.14	1.82±0.178	1.67±0.057	0.82±0.009^{a}	0.81±0.016^{a}	0,77± 0,008^{b}	6.08±0.26	5.80±0.26	5.62±0.29
		NS			*			NS	
2	2.09±0.19	1.73±0.14	1.66±0.14	0.83±0.02^{a}	0.79±0.01^{ab}	0.75±0.007^{b}	12.34±0.54^{a}	8.04±0.36^{b}	7.94±0.38^{b}
		NS			**			***	
3	1.83±0.05	1.700±0.13	1.63±0.011	0.76±0.01	0.77±0.02	0.75±0.01	13.61±0.68^{a}	11.61±0.47^{b}	9.54±0.52^{c}
		NS			NS			***	
7	2.23±0.14	2.03±0.83	1.97±0.48	0.81±0.002	0.83±0.001	0.79±0.008	13.17±0.38^{a}	13.46±0.56^{a}	11.43±0.53^{b}
		NS			NS			*	
14	2.30±0.23	2.63±0.08	2.45±0.006	0.83±0.02	0.88±0.01	0.82±0.02	16.78±0.32^{a}	16.85±0.46^{a}	13.45±0.32^{b}
		NS			NS			***	
21	2.57±0.24	2.39±0.09	2.28±0.06	0.83±0.009^{a}	0.82±0.01^{ab}	0.79±0.005^{b}	14.25±0.44^{a}	13.33±0.19^{a}	11.67±0.33^{b}
		NS			*			***	

NS: P>0.05, * : P<0.05, **: P<0.01, ***: P<0.001

$^{a, b, c:}$ the difference between the characteristcs indicated by different letters in the same line is significant.

Table 4. The presence of uterine fluid during ultrasonographic examinations and determination of bloody vaginal discharge throughout the involution period.

	Carazolol Group										**Oxytocin Group**									
	Bloody vaginal discharge										**Presence of Uterine fluid**									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 3	+	+	-	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
Day 7	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	Oxytocin Group										**Oxytocin Group**									
	Bloody vaginal discharge										**Presence of Uterine fluid**									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day 3	+	+	-	+	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+
Day 7	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 4. The presence of uterine fluid during ultrasonographic examinations and determination of bloody vaginal discharge throughout the involution period (continued).

	Control Group																			
	Bloody vaginal discharge										Presence of Uterine fluid									
Ewes ID	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day 1	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
Day 2	+	+	-	-	+	-	+	-	-	-	+	+	-	+	+	+	+	+	-	+
Day 3	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-
Day 7	-	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-
Day 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Discussion and Conclusion

There are very few reports on postpartum uterine involution in small ruminants, and most of them focus on postmortem evaluations (11, 17, 22); moreover, the previous studies on the postpartum uterus were mostly performed only by B-mode USG (1, 3, 18, 38). This study was designed to investigate, by pulsed-wave Doppler sonography, the involution of the sheep uterus in the postpartum period following the delivery of medications that contract the uterus. In a study, the effect of prostaglandins (PGF2α) and oxytocin on postpartum uterine involution of Awassi ewes were evaluated (3) which the postpartum gravid horn diameter was found as 6.50±0.45 and 6.45±0.25 in the PGF2α and oxytocin groups, respectively, on the 7th day after parturition. On the 21st day of pp, Ahmed et al. (3) found the diameter to be 2.92±0.43 and 3.45±0.13 in the PGF2α and oxytocin groups, respectively. The pp gravid horn diameter decreased more rapidly in the current study than in Ahmed et al.'s (3) study. In addition, carazolol (3.94±0.18 cm) was found to have a more effective reduction than oxytocin (4.17±0.17 cm) on the 7th day of pp. The involution period was completed on the 21st pp day in this study; on the other hand, Ahmet et al. (3) reported the involution period as ending on the 28th day pp. This difference could be attributed to the different medications used in the studies. The mean pp gravid horn diameter (1.70±0.05 cm) decreased the best to the lowest diameter in the carazolol group on pp day 21, compared with 2.12±0.10 cm and 2.37±0.12 cm for the oxytocin and control groups, respectively, in this study. The gravid horn diameter was found to be smaller on the first day of pp in Elmetwally and Bollwein's (14) study than in this one. However, this parameter ended up being much smaller at the end of the current study compared with their study. Although the drugs used in this study cause the uterus to contract, these differences may have resulted from the greater gravid horn diameter measurement at the begining and the different breed (German Merino vs. Kıvırcık) of ewes used in both of the studies. Bademkiran and Horoz (6) compared the effect of carazolol and cloprostenol on the involution of cows and found that carazolol had shortened the completion of the involution compared with cloprostenol, but the difference in the involution time between these groups was not statistically significant. Hauser and Bostedt (18) measured a linear reduction of the caruncule size throughout the involution period in ewes, with an initial value of 2.02±0.16 cm on day 1 pp. They found a 1.24±0.17 cm caruncule diameter on day 8 pp and stated that ultrasonographic differentiation and accurate measurements of the caruncules were almost impossible to be carried out after day 8 pp. In the current study, similar results were achieved. On day 1 pp in unmedicated; control group, a caruncule diameter of 2.19±0.06 cm was recorded, and the caruncules were undetectable on the ultrasound screen after day 7 pp. The mean caruncule diameter was 1.15±0.07 cm on the last examination on day 7 pp, and no other measurements could be achieved for caruncule diameters on pp days 14, 21, and 28 of this study, ultrasonographically. Krajnicakova et al. (21), on the other hand, detected relatively measurable caruncles on the endometrium on day 17 pp, and the caruncles were barely visible on day 25 in their study of the microscopic examination of tissues obtained from the animals postmortem. The difference in the study plans may explain the different time points at which the caruncles disappeared. In a study that investigated the vascular changes of the uterine artery during pregnancy and the postpartum period, uterine artery S/D, PI, and RI were found to increase significantly after parturition (pp day 1). The maximum levels detected at PI and RI on the 15th day pp can be associated with the morphological regression of the uterus in the ewes (37). The onset of uterine involution is evident by a decrease in uterine blood supply. The cessation of circulating hormone concentrations due to the expulsion of fetal

membranes, vasodilation effect on uterine artery was found to be disappeared indeed decreasing of local blood perfusion leads to placental detachment (28). An acute reduction of uterine vasculature during the pp period is essential for uterine involution and endometrial repair (37). In this study, the same results were achieved for PI and RI values (2.459±0.083 and 0.843±0.010, respectively) for the uterine artery 14 days after lambing. The increase in PI and RI observed in this study is compatible with the decreased perfusion and decreased blood supply to the tissue. Elmetwally and Bollwein (14) reported constantly increasing PI values throughout the involution period in ewes, especially on day 6 pp, and their study's PI values ranged between approximately 0.5 (min) and 1.5 (max) during the 21 days of pp. In this study, the PI values ranged between 1.63 (min) and 2.63 (max) in different groups on different days of the study throughout the involution period. This difference may be a result of the decreased blood flow volume caused by medications used in this study that led to the contraction of the uterus. Birth was inducted with a progesterone antagonist 41 hours prior to the estimated date of lambing in Veiga et al.'s (37) study; the mean RI was ≅ 0.55, and the mean PI was ≅ 0.80 on pp day 1. In this study, the mean RI was 0.82±0.09 in the carazolol group, 0.81±0.016 in the oxytocin group, and 0.77±0.08 in the control group, respectively on pp day 1. The mean PI was 1.98±0.14 in the carazolol group, 1.82±0.178 in the oxytocin group, and 1.67±0.057 in the control group, respectively on pp day 1. The mean values of PI and RI were clearly higher than those of Veiga et al.'s (37) study. This difference may be due to the different protocols and the administration of different hormones in the studies. The RI values found in the present study were higher than Veiga et al.'s (37) results. The higher resistance detected in this study can be an indication of the decreased uterine artery's diameter and lower blood flow. In this study, when we investigated the average RI values of all the groups, we found that the highest mean RI value belonged to the oxytocin group, and the highest value was 0.843±0.010 on the 14th day of pp, so the blood flow may have decreased due to oxytocin use. This finding supports the finding of oxytocin receptors in vascular smooth muscle by Chen et al. (9) and Miller et al. (24). Furthermore, Vedernikov et al. (36) found that oxytocin can contract the uterine artery via its own receptors. Additionally, in this study, the carazolol results for this parameter (mean RI values) were very close to the oxytocin results, both of which were statistically significant ($P<0.01$) compared to the control group's results. Veiga et al. (37) detected an increased PI and RI and a peak S/D ratio 15 days after lambing, and they reported that this increase may be attributed to the decrease in the diameter and blood flow volume of the uterine artery, which was associated with the morphological regression of the uterus; this ratio tended to decrease on the 30th pp day in their study. Our study results regarding the S/D ratio are in line with Veiga et al.'s (37) study, and the same ratio tended to decrease in this study on day 21 pp. The highest mean S/D ratio was detected in the carazolol-administered group, and it was statistically significant ($P<0.05$) when compared with the other groups. The highest S/D ratio was 15.692±0.216 on day 14 pp. Elmetwally and Bollwein (14) reported the completion of the clearance of lochia occurring between days 12 and 15 pp. In the current study, we determined that lochia was not seen in most animals past the 7th day. This difference can be attributed to the medications used in this study. Ahmed et al. (3) observed uterine fluid accumulation on pp days 4–7 days in Awassi ewes. Ababnef and Degefa (1) observed the accumulation of fluid and tissue debris during the first 4 days pp in Balady goats. Badawi et al. (5) reported uterine fluid accumulation during the first week pp in Nubian goats. In this study, bloody vaginal discharge and uterine fluid accumulation had disappeared on the 7th day pp, except for two animals that received carazolol. In the oxytocin-administered ewes, as well, bloody discharge had disappeared on the 7th day of pp, except for two ewes, and fluid accumulation ended on day 7 pp in the oxytocin group, except for 3 ewes. Bloody vaginal discharge and uterine fluid accumulation disappeared on the 7th day pp, except for 3 animals, in the control group. The results of all these studies were similar to our study, but the differences between the studies might depend on the differences in animal breed and the species used in the experiments. Marnet et al. (23) reported that a lower dose of oxytocin (2 IU in total dose) was more effective for increasing rhythmic uterine contractions than higher doses. Tian and Noakes (33) and Sheldon et al. (31) reported that none of the hormonal treatments, including prostaglandin $F_{2\alpha}$, ostradiol-17 β, or an oxytocin analogue that was used shortly after lambing, had any effect on the increasing rate of uterine involution in sheep. Assali et al. (4) measured uterine blood flow with an electromagnetic flowmeter and suggested that the nonpregnant uterus was more responsive to the vasopressor hormone than to the oxytocic fraction. However, a contrary result exists for oxytocin in the pregnant or early pp uterus. They found that the decrease in the uterine blood flow induced by oxytocic drugs was due to its local action on uterine vessels. This action could be a result of direct vasoconstriction or a mechanical constriction of the vessels produced by contracting uterine musculature.

In conclusion, the most effective drug used to accelerate involution was found to be carazolol, according to the pp gravid horn diameter regression in this study. The

increased RI and PI values may reflect the decrease in blood flow volume and uterine artery diameter. In this study, carazolol and oxytocin administration led to an increase in PI and RI values, respectively; while carazolol was found to be more effective in increasing the S/D ratio, which leads to a decrease in vessel blood flow volume. Hence, Doppler ultrasound imaging can be used successfully to evaluate hemodynamic changes in the uterine vasculature during the pp period and suggests that the drugs used in this study had a contraction effect on the uterine artery. It is thought that involution can be accelerated depending on the use of drugs that increase uterine contractions in ewes whose postpartum involution is delayed.

Acknowledgements

We want to thank to Prof. Dr. Ömür Koçak for his valuable contributions for statistical analysis of this study.

Finacial Support

This research received no grant from any funding agenct/sector.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

SÖE, GED, AS conceived and planned the experiments. SÖE, GED, ACÇ, AS, KB carried out the experiments. GED, ACÇ, KB contributed to sample preparation. AS, GED, ACÇ contributed to the interpretation of the results. SÖE took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

Ethical approval for the study was obtained from the Istanbul University Animal Research and Ethical Committee (2018/47, 31.05.2018).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **Ababneh MM, Degefa T** (2005): *Ultrasonic Assessment of Puerperal Uterine Involution in Balady Goats.* J Vet Med A, **52**, 244–248.
2. **Abshagen U, Möllendorff EV** (1980): Pharmakokinetik oder Wirkungskinetik von Carazolol? Therapierelevante Daten. 255–258. In: Symposium Uber den Beta-Rezeptorenblocker Carazolol. Excerpta, Amsterdam.
3. **Ahmed MN, Ynzeel JH, Majeed AF** (2016): *Ultrasonographic study of uterine involution in of Awassi ewes in Iraq.* Al-Anbar J Vet Sci, **9**, 16-21.
4. **Assali NS, Dasgupta K, Kolin A** (1959): *Measurement of Uterine Blood Flow and Uterine Metabolism, VI. Effects of Oxytocic, Vasopressor, and Vasodepresscw Drugs on the Blood Flow to the Postpartum Uterus in Unanesthetized Sheep.* Am J Obst and Gynec, **78**, 313-321.
5. **Badawi ME, Makawi SEA, Abdelghafar RM, et al** (2014): *Assessment of postpartum uterine involution and progesterone profile in Nubian goats (Capra hircus).* J Adv Vet Anim Res, **1**, 36-41.
6. **Bademkiran S, Kaya HH** (2006): *İneklerde güç doğuma bağlı şekillenen retensiyo sekundinaryum'un engellenmesinde beta blokörlerin (carazolol) ve PGF 2a'nin etkilerinin karşilaştirilmasi.* J Fac Vet Med Istanbul Univ, **32**, 69-79.
7. **Bajcsy AC, Szenci O, van der Weijden GC, et al** (2006): *The effect of a single oxytocin or carbetocin treatment on uterine contractility in early postpartum dairy cows.* Theriogenology, **65**, 400–414.
8. **Ballarini G, Guizzardi F** (1981): *Wirkung des beta-blockers carazolol bei transportkrankheit oder stress-syndrom des Schweines.* Tierarztliche-Umschau, **36**, 171-180.
9. **Chen YL, Shepherd C, Spinelli W, et al** (1999): *Oxytocin and vasopressin constrict rat isolated resistance arteries by activating vasopressin V1A receptors.* Eur J Pharmacol, **376**, 45-51.
10. **Cunha Barbosa C, Barbosa de Souza M, Azevedo de Freitas L, et al** (2013): *Assessment of uterine involution in bitches using B-mode and Doppler ultrasonography.* Anim Reprod Sci, **139**, 121-126.
11. **Degefa T, Ababneh MM, Moustafa MF** (2006): *Uterine involution in the post-partum Balady goat.* Vet Arhiv, **76**, 119-133.
12. **Dickey RP** (1997): *Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy.* Hum Reprod Update, **3**, 467–503.
13. **Elmetwally M, Rohn K, Meinecke-Tillmann S** (2016): *Noninvasive color Doppler sonography of uterine blood flow throughout pregnancy in sheep and goats.* Theriogenology, **85**, 1070–1079.
14. **Elmetwally M, Bollwein H** (2017): *Uterine blood flow in sheep and goats during the peri-parturient period assessed by transrectal Doppler sonography.* Anim Reprod Sci, **176**, 32–39.
15. **Fielder FD, Baker RF** (1969): *Diagnostic applications of Doppler ultrasound.* Ultrasonics, **7**, 36–38.
16. **Gray CA, Stewart MD, Johnson GA, et al** (2003): *Postpartum uterine involution in sheep: histoarchitecture and changes in endometrial gene expression.* Reproduction, **125**, 185–198.
17. **Greyling JPC, Van Niekerk CH** (1991): *Macroscopic uterine involution in the postpartum Boar goat.* Small Rumin Res, **4**, 277-283.

18. **Hauser B, Bostedt H** (2002): *Ultrasonographic Observations of the Uterine Regression in the Ewe under Different Obstetrical Conditions.* J Vet Med A, **49**, 511–516.
19. **Jaffa AJ, Wolman I, Har-Toov J, et al** (1996): *Changes in uterine artery resistance to blood flow during puerperium – a longitudinal study.* J Matern Fetal Investig, **6**, 27–30.
20. **Januszewski T** (1985): Zur Wirkung des Beta – Adrenozeptorenblockers Carazolol nach tierarztlicher Geburtshilfe beim Rind auf den Abgang dea Nachgeburt. Praca dokt. Monachium.
21. **Krajnicakova M, Bekeova E, Maracek I, et al** (1996): *Morphological and functional uterine changes in the puerperium of ewes.* Folia Veterinaria, **40**, 21–24.
22. **Krueger L, Koerte J, Tsousis G, et al** (2009): *Transrectal Doppler sonography of uterine blood flow during the first 12 weeks after parturition in healthy dairy cows.* Anim Reprod Sci, **114**, 23-31.
23. **Marnet PG, Laurentie MP, Garcia-Villar R, et al** (1986): *Route of administration and uterine response to oxytocin in the ewe.* J Vet Phannacol Therap, **9**, 439-441.
24. **Miller ME, Davidge ST, Mitchell BF** (2002): *Oxytocin does not directly affect vascular tone in vessels from non-pregnant and pregnant rats.* Am J Physiol, **282**, 223-228.
25. **Mortensen C, Kelley D, Warren L** (2011): *Supplemental l-arginine shortens gestation length and increases mare uterine blood flow before and after parturition.* J Equine Vet Sci, **31**, 514–520.
26. **Mulic-Lutvica A, Eurenius K, Axelsson O** (2007): Longitudinal study of Doppler flow resistance indices of the uterine arteries after normal vaginal delivery. Acta Obstet Gynecol Scand, **86**, 1207–1214.
27. **Noakes DE** (2001): The puerperium and the care of the newborn. 189–202. In: DE Noakes, TJ Parkinson, GCW England (Eds), Arthur's Veterinary Reproduction and Obstetrics. Saunders, Edinburgh.
28. **Regassa F, Noakes DE** (1999): *Acute phase protein response of ewes and the release of PGFM in relation to uterine involution and the presence of intrauterine bacteria.* Vet Rec, **144**, 502–506.
29. **Retzer R** (1987): Zur medikamentellen behandiung der wehenschwache bei der geburt des rindes. Vet Med Diss, Muenchen.
30. **Rudloff PR, Borstedt H** (1984): *Effect of beta blockader carazolol (Suacron) on parturition in sows.* Tierarztl Prax, **12**, 443-449.
31. **Sheldon IM, Noakes DE, Bayliss M, et al** (2003): *The effect of oestradiol on postpartum uterine involution in sheep.* Anim Reprod Sci, **78**, 57–70.
32. **Sheldon IM, Dobson H** (2004): *Pospartum Uterine Health in Cattle.* Anim Reprod Sci, **82–83**, 295–306.
33. **Tian W, Noakes DE** (1991): *A radiographic method for measuring the effect of an exogenous hormone therapy on uterine involution in ewes.* Vet Rec, **129**, 463–466.
34. **Twardon J, Dejneka GJ, Błaszkowszowska M, et al** (2005): *The influence of simpanorm (carazolol) application on puerperal period course in dairy cows.* Fatro Vetup Dates, 11.
35. **Van Wyk LC, Van Niekerk CH, Belonje PC** (1972): *Involution of the postpartum of the ewe.* J S Afr Vet Ass, **43**, 19-26.
36. **Vedernikov YP, Betancourt A, Wentz MJ, et al** (2006): *Adaptation to pregnancy leads to attenuated rat uterine artery smooth muscle sensitivity to oxytocin.* Am J Obstet Gynecol, **194**, 252–260.
37. **Veiga GAL, Angrimani DSR, Silva LCG, et al** (2018): *Hemodynamics of the uterine and umbilical arteries during the perinatal period in ewes.* Anim Reprod Sci, **198**, 210-219.
38. **Zdunczyk S, Milewski S, Wojciech B, et al** (2004): *Postpartum uterine involution in primiparous and pluriparous polish longwool sheep monitored by ultrasonography.* Bull Vet Inst Pulawy, **48**, 255-257.