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# Relationship of single and double ovulations with serum progesterone concentrations and fetal numbers in Mamasani goats

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#### Abstract

Determining the effect of single versus double ovulations on progesterone concentration and luteal tissue volume is so important for better reproductive management in goats. The aim of the present study was to establish the relationship between ovulation number, luteal tissue volume and progesterone concentrations in Mamasani goats. For this purpose, the uteri and ovaries of 192 goats were collected in a slaughterhouse during the breeding and non-breeding seasons. Before slaughtering, blood samples were taken and analyzed for assaying progesterone concentrations. Study groups in the non-breeding season were: 1-single ovulation, 2-unilateral double ovulations and 3-bilateral double ovulations of non-pregnant goats. The groups in the breeding season were: 1-non-pregnant single ovulation, 2-non-pregnant unilateral double ovulations, 3-non-pregnant bilateral double ovulations, 4pregnant single ovulation with one fetus, 5-pregnant single ovulation with two fetuses, 6-pregnant unilateral double ovulations with one fetus, 7-pregnant unilateral double ovulations with two fetuses, 8-pregnant bilateral double ovulations with one fetus, and 9-pregnant bilateral double ovulations with two fetuses. Results showed that the right ovary had a pivotal role in ovulation during the non-breeding and breeding season. In both pregnant and nonpregnant gravid goats, the total volume of corpora lutea (CLs) as well as the serum progesterone concentration was higher after double ovulations. The total volume of CLs and the concentrations of serum progesterone in goats carrying two fetuses with single ovulation was significantly higher than that of animals with single ovulation and one fetus. It was concluded that although a higher concentration of progesterone in goats is associated with the total volume of luteal tissue, this level is not a reliable predictor for the number of fetuses per goat.

Key words: Corpus luteum, double ovulations, twin pregnancies, progesterone, Mamasani goat

#### Introduction

Size, structure and the steroid production of corpus luteum (CL) are different at various stages of the estrous cycle, as well as pregnancy period (Fields & Fields, 1996). In goats, the maintenance of pregnancy is dependent on the secretion of progesterone by CL (Khan & Ludri, 2002). It is reported that the plasma progesterone

concentration is affected by the embryo or the number of fetuses in pregnant ewes and goats (Kulcsar et al, 2006).

It is important to know the number of fetuses in pregnant goats and ewes for their better health management. The goats carrying twins need extra attention to prevent pregnancy toxemia, optimizing

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kids' birth weight and reducing perinatal mortality (Karen et al, 2006). The numbers of fetuses are commonly determined by ultrasonography or by the serum progesterone measurement (Medan et al, However, 2004). variable plasma progesterone levels exist due to individual factors, such as the differences in blood flow, the mass of luteal tissues and tissue capacity for progesterone production (Niswender et al, 2000). Moreover, it has been reported that after single and double ovulations in cattle (Mann et al. 2007; Voelz al. 2015) et and ewes (KaramiShabankareh al. 2009: et KaramiShabankareh et al, 2010) the serum progesterone levels are changed. Lopez et al in 2005, reported that double ovulations increased CL volume in dairy cows. However, some reports did not confirm the aforementioned claims in lactating cows (Voelz et al, 2015) and non-lactating cows (Mann et al, 2007). Gur et al (2011), reported that double ovulations and twining increased serum progesterone concentration compared to single ovulation in ewes.

There are limited data about the function of the CL in goats and the effect of ovulation numbers and CL volume on serum progesterone concentrations. We hypothesized that a relationship may be present between the total volume of CLs and serum progesterone concentration in goats. Therefore, we resorted to evaluate the effect of single and double ovulations on CL volume, serum progesterone concentration and number of fetuses in Mamasani goats during the breeding and non-breeding seasons.

## Materials and Methods Animals and experimental groups

This study was carried out in Kohgiluyeh and Boyer-Ahmad Province, southwest of (30°41′N latitude and 51°33′E longitudes). The reproductive organs of 48 Mamasani goats (2–3 years) were collected from a local abattoir. First, samples from non-pregnant goats with single and double ovulations were collected based on the counting of CL between May and June (non-breeding season). Then. experimental groups with 16 replicates per each were determined as follows: 1- single ovulation, 2- unilateral double ovulations and, 3- bilateral double ovulations (Figure 1).

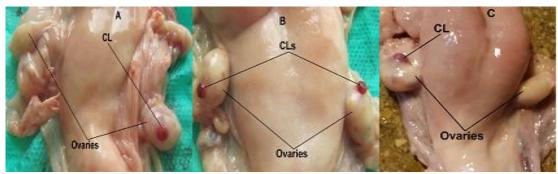


Figure 1: Reproductive tracts of non-pregnant goats. A and B represent single and bilateral double ovulations in non-breeding season; C = Single ovulation during breeding season. CL = Corpus luteum

Then the samples were collected during the breeding season (September to December) from 144 Mamasani goats (2–3 years), at a local abattoir. In non-pregnant goats, reproductive organs which had ovaries possess CL, were selected (Figure 1) and in pregnant goats the reproductive

organs possess similar fetuses age were selected (Figure 2 and 3).

Immediately after sample collection, the right and left ovaries were carefully detached and were transport in a 0.9% saline solution supplemented with procaine penicillin G 2 x 10<sup>6</sup> IU and dihydrostreptomycin 2 g to the laboratory of

Animal Physiology in Yasouj University (Iran). In the laboratory the numbers of actual or putative CL in the right and left ovaries of each animal were recorded. Furthermore, the presence of unilateral or bilateral CL and the single or twins' pregnancy was recorded for each goat. The volume of CLs was approached by considering them as a sphere (Bartlewski et al, 1999). In this stage of the study, nine experimental groups with 16 replicates were as follows: 1- non-pregnant single

ovulation, 2- non-pregnant unilateral double ovulations, 3- non-pregnant bilateral double ovulations, 4- pregnant single ovulation with one fetus, 5- pregnant single ovulation with two fetuses, 6- pregnant unilateral double ovulations with one fetus, 7- pregnant unilateral double ovulations with two fetuses, 8- pregnant bilateral double ovulations with one fetus, and 9-pregnant bilateral double ovulations with two fetuses.

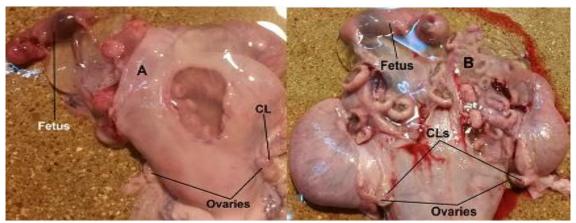


Figure 2: Reproductive tracts of pregnant goats during breeding season. A = Single ovulation with one fetus; B = Bilateral double ovulations with one fetus. CL = Corpus luteum

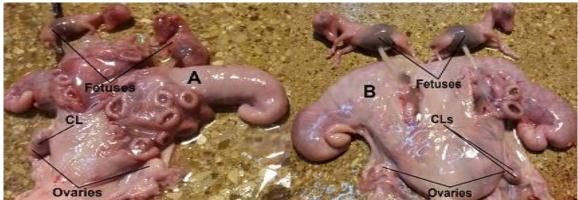


Figure 3: Reproductive tracts of pregnant goats during the breeding season. A = Single ovulation with two fetuses; B = Unilateral double ovulations with two fetuses. CL = Corpus luteum

#### **Blood sampling and analysis**

Before slaughtering the goat, blood samples were collected from jugular vein in 10 ml plain tubes and transported to the laboratory. Blood samples centrifuged at 2500 rpm for 20 min. and kept at -20°C before analysis. Serum progesterone

concentration was measured using a commercially available ELISA kit (ELA-4825-300, Monobind, Inc. Lake Forest, CA (92630) USA). Sensitivity of the assay was 0.105 ng/ml and intra- and inter assay coefficient of variation were 4.5% and 9.4%, respectively.

#### **Statistical analysis**

Incidence of single and double ovulations and its in the right or left ovaries were analyzed by chi-square test. Luteal characteristics and serum progesterone levels in single and double ovulation cases were analyzed by ANOVA procedure of SAS (Statistical Analysis Systems Institute, 1998, SAS Institute Inc., Cary, NC). A correlation coefficient Pearson calculated to determine the correlation between the total volume of CLs and serum progesterone level in non-pregnant goats during the non-breeding season, and also in non-pregnant and pregnant goats during breading season. Means difference for the volume of CLs was obtained by Student's ttest and Duncan's multiple range test.

#### **Results**

Ovulations, corpora lutea and fetuses

During the non-breeding season, the last single ovulation had occurred more frequently in the right ovaries than in the left ones (75% vs. 25%, P<0.05) (Table 1). Moreover, the right ovaries had more unilateral double ovulations in comparison to the left ovaries (88% vs. 13%, P<0.05). Similarly, the prevalence of single and double ovulations in non-pregnant goats during breeding season showed that single ovulation was higher in right ovaries than in left ones (81% vs. 19%, P<0.05) (Table 2).

Table 1: Distribution of single and double ovulations on ovaries during out-breeding season

Ovary	Ovulation (½)	Single ovulation (n)	Double ovulations (n)
Right ovary (unilateral)	54.2	12 a	14 <sup>a</sup>
Left ovary (unilateral)	12.5	4 <sup>b</sup>	2 b
Both (bilateral)	33.3	-	16 a
Total	100	16	32

Different letter (a, b) in the same column indicates significant differences (P<0.05).

Table 2: Distribution of single and double ovulations on ovaries in non-pregnant goats during breeding season

Ovary	Ovulation (½)	Single ovulation (n)	Double ovulations (n)
Right ovary (unilateral)	56.3	13 <sup>a</sup>	14 <sup>a</sup>
Left ovary (unilateral)	10.4	3 b	2 <sup>b</sup>
Both (bilateral)	33.3	-	16 <sup>a</sup>
Total	100	16	32

Different letter (a, b) in the same column indicates significant differences (P<0.05).

Table 3 shows that when two fetuses existed in uterus, it was only due to one single ovulation and this matter was seen mostly in right ovaries than left ovaries (94% vs. 6%, P<0.05). This could due to have homozygous derived fetuses. Thus, right ovaries appear to predispose the condition of developing homozygous twins. Some pregnant goats had unilateral double ovulations resulting in the presence of only one fetus. In 63% of these goats two corpora lutea were on right ovary. In unilateral

double ovulations with two fetuses, right ovaries were involved in 88% of the goats.

We observed 74 CLs on right ovary in 50 goats and 22 CLs on left ovary in 14 goats. In total, all 74 putative ovulations resulted in 79 fetuses in goats with right ovary ovulation and 17 fetuses in goats with left ovary ovulation. Twinning rate estimated to be 91% in both single and double ovulations in the right ovary goats however, twinning rate estimated to be 3% and 6% for single and double ovulations in the left ovary, respectively.

Table 3: Distribution of single and double ovulations and fetus number in pregnant goats during breeding season

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CL Fe		Fetus	Fetus Ovulation	Single ovulation (n)		Double ovulations (n)	
Ovary	(n)	(n)	(%)	One-	Two-	One-	Two-
					fetuses	fetus	fetuses
Right ovary	74 <sup>a</sup>	79 a	78.1	11 a	15 a	10 a	14 a
Left ovary	22 b	17 <sup>b</sup>	21.9	5 a	1 <sup>b</sup>	6 a	2 b
Total	96	96	100	16	16	16	16

Different letter (a, b) in the same column indicates significant differences (P<0.05).

# **CL** volume and serum progesterone concentration

The results of CL volume and serum progesterone concentration in non-pregnant goats are shown in Table 4. The mean volume of individual CL was larger (P<0.05) after single ovulation than double ovulations, both in breeding and non-breeding seasons. Furthermore, as expected the total volume of CLs in single ovulations was significantly smaller (P<0.05) than double ovulations. The breeding season had a clear effect on CL volume, since both the volume of individual CL and the total volume of CLs were larger (P<0.05) in

comparison to non-breeding season. There was no significant difference in total CL volume after unilateral or bilateral double ovulations. During the breeding season, the total volume of CL after unilateral double ovulations was larger than in the nonbreeding season. In non-pregnant goats, serum progesterone concentration after double ovulations was significantly (P<0.05) higher than after single ovulation, during both breeding and non-breeding seasons. There was a significant (P < 0.05) of the breeding season progesterone levels (Table 4).

Table 4: Luteal characteristics and serum progesterone concentration in single and double ovulations in non-pregnant goats during out-breeding and breeding seasons (mean±S.D)

Season	Out breeding season			Breeding season		
CL	Single	Double		Single	Double	
Position	-	Unilateral Bilateral		-	Unilateral	Bilateral
CL volume (mm³)	136.11±2.38 b	130.94±1.54 <sup>d</sup>	129.76±1.62 <sup>d</sup>	138.04±1.74ª	132.85±1.79°	132.82±1.38°
Total CL volume (mm <sup>3</sup> )	136.11±2.38 °	261.88±3.08 <sup>b</sup>	259.53±3.24b	138.04±1.74°	265.70±3.57 a	265.65±2.75 <sup>a</sup>
Serum P4 (ng/ml)	3.14±0.25 <sup>d</sup>	4.04±0.30 b	3.91±0.27 <sup>b</sup>	3.52±0.33 °	4.75±0.47 <sup>a</sup>	4.56±0.33 a

Different letters (a, b, c, d) in the same row for single and double ovulation indicate significant differences (P < 0.05).

In the pregnant goats as a whole the mean volume, of individual CL after single ovulation was larger (P<0.05) than after double ovulations. On the other hand, the total volume of CL and serum progesterone concentration after double ovulations were both larger (P<0.05) than after single ovulation. The total volume of CL and serum progesterone concentration was

higher (P<0.05) in goats after single ovulation resulting in two fetuses compared to goats with single ovulation and one fetus. This also applied for goats with double ovulation and one fetus compared to goats with single ovulation and one or two fetuses. Also, our results indicated that the total volume of CL and serum progesterone concentration was not affected by unilateral

and bilateral double ovulations. In addition, the results notably indicated that the total volume of CL and serum progesterone

concentration was not affected by double ovulations (unilateral and bilateral double ovulations) and a number of fetuses (Table 5).

Table 5: Luteal tissue volume and serum progesterone concentration in single and double ovulating

pregnant goats during breeding season (mean±S.D).

Ovulation	Cin	ala	Double			
Ovulation	Single		Unilateral	Bilateral	Unilateral	Bilateral
Fetus (n)	1	2	1	1	2	2
CL volume (mm³)	141.28±2.41 <sup>b</sup>	189.62±2.55 <sup>a</sup>	135.94±1.37°	136.82±1.26°	136.88±0.42°	136.44±0.53°
Total CL volume (mm <sup>3</sup> )	141.28±2.41°	189.62±2.55 <sup>b</sup>	271.88±2.74 <sup>a</sup>	273.63±0.88 <sup>a</sup>	273.78±2.53 <sup>a</sup>	272.89± 1.05 <sup>a</sup>
Serum P4 (ng/ml)	4.17±0.33 <sup>d</sup>	4.88±0.23 °	5.26± 0.24 ab	5.11±0.25 bc	5.38±0.20 a	5.32± 0.16 ab

Different letters (a, b, c, d) in the same row for single and double ovulation indicate significant differences (P<0.05).

The volume of individual CL in double ovulations was compared between breeding and out-breeding season and/or non-pregnant and pregnant goats in Table 6. Results showed that the CL volume in unilateral double ovulations of non-pregnant goats during the non-breeding season was minimum and bilateral double ovulations in pregnant goats with two fetuses was maximum (No 1; group 1) (P<0.05). However, the mean volume of corpora lutea No 2 (group 2) in unilateral double ovulation of pregnant goats with two fetuses was significantly (P<0.05) higher

than the remaining ovulation condition. However, this parameter in bilateral double ovulations of non-pregnant goats during out-breeding season was significantly (P<0.05) smaller than the remaining ovulation condition.

Serum progesterone concentration and total luteal tissue volume had positive correlation (r=0.722, P<0.0001) in non-pregnant goats during the non-breeding season as well as in non-pregnant and also over pregnant goats during breeding season (r = 0.846; P<0.0001: r = 0.828; P<0.0001).

Table 6: Volume of paired corpora lutea after double ovulations (mean±S.D)

	Volume of corpus luteum 1 (mm <sup>3</sup> )	Volume of corpus luteum 2 (mm <sup>3</sup> )		
Double ovulations (1) †	134.25±2.32 ° *	127.63±1.81 dc		
Double ovulations (2)	135.74±2.30 <sup>de</sup> *	123.79±1.91 °		
Double ovulations (3)	136.26±2.59 <sup>d</sup> *	129.44±2.25 <sup>b</sup>		
Double ovulations (4)	139.41±2.02 ° *	126.24±2.32 <sup>d</sup>		
Double ovulations (5)	140.32±1.64 ° *	131.56± 1.79 a		
Double ovulations (6)	142.44±2.09 <sup>b</sup> *	131.19±1.35 <sup>a</sup>		
Double ovulations (7)	141.12±1.49 bc *	132.65±1.48 <sup>a</sup>		
Double ovulations (8)	144.35±1.61 <sup>a</sup> *	128.54±1.68 bc		

<sup>† 1</sup> and 2 = Unilateral and bilateral double ovulations in non-pregnant goats during out-breeding season, respectively; 3 and 4 = Unilateral and bilateral double ovulations in non-pregnant goats during breeding season, respectively; 5 and 7 = Unilateral double ovulations in pregnant goats with one and two fetuses, respectively; and 6 and 8 = Bilateral double ovulations in pregnant goats with one and two fetuses, respectively.

Different letters (a, b, c, d) in the same column for volume of corpus luteum 1 and 2 indicate significant difference (P<0.05) per each position ovulation (n=16 cases in each groups).

<sup>\*</sup>Significant differences in the same row for the volume of corpus luteum 1 and 2 (P<0.01).

#### **Discussion**

The characteristics of double ovulation in goats are not well understood and reported. Therefore, this study was conducted to clarify more details on the aspects of single and double ovulation and progesterone concentrations in goats. In agreement with Pramod et al (2013) report, the present study showed that the right ovary is more active than left ovary in single and double ovulations during the breeding as well as in the non-breeding season. Dominancy of the right ovary was also reported for other ruminant species such as ewes and heifers (Shorten et al, 2013; Ginther & Hoffman, 2016). However, some studies challenge this concept, since no difference were observed in the numbers of ovulations between left and right ovaries (Chacur et al, 2015; Rezagholizadeh et al, 2015). Regassa et al (2007), reported that a higher proportion of single ovulations occurs in right ovaries, while there is no significant difference in proportion of double or more ovulations between both ovaries. The reason for discrepancies between studies may be related to species and environmental conditions.

Local paracrine and or autocrine factors and differences in lymphatic drainage of right and left ovaries may contribute to the observed variation in their activities (Alosta et al. 1998). Differences in vascularization blood flow could be another explanation (Morales et al, 1998). According to Cushman et al (2005), the increased functional activity of the right ovary in cattle is probably caused by a local temperature effect on the left ovary due to the nearness to the rumen.

The results of this study showed that both single and double ovulations in Mamasani goats occurred considerably more often in the right ovary than the left one. Furthermore, it seems that ovulations in the right ovary yielded more twin pregnancies. Nevertheless, in one-third of the non-pregnant population bilateral ovulations were occurred. Finally, there are some

indications from the results of the present study that show the homozygous twins also appear to come more often from the oocytes of the right ovary.

KaramiShabankareh et al (2009),reported that unilateral double ovulations were only observed in the right ovaries. Similarly, Kusaka et al (2017), showed that all unilateral double ovulations in first estrus postpartum in dairy cows occurred on the right side. The unequal activity of ovaries may cause differences in the development potential of oocytes. Moreover, in previous study it has shown a significant difference between the right and left ovaries regarding the recovery rate and the maturation of oocytes (Majeed & Adel, 2015). Fritzsche et al (2000), showed that oocytes originating from the right ovary led to pregnancy more often than oocytes originating from the left ovary. In the present study we showed that follicular activity of the right ovary resulted in more twins. Also, it is demonstrated that after superovulation by eCG, the response of the right ovary was stronger than the left one (Kermani-Moakhar et al, 2010; Martinez et al, 2007). Lehloenya et al (2008), showed that there were more CLs in the right ovary Boer goats after superovulation compared to the left ovary. KaramiShabankareh et al in 2015, showed that the dominance of the right ovary can be atresia by induction of luteolysis, which causes a greater proportion of ovulations in the left ovary.

Spell et al (2001), showed a relationship between corpus luteum size and plasma progesterone concentrations during the development of CL. Since the production of progesterone by CL only is mandatory to maintain pregnancy in goats (Islam et al, 2013), we studied the relationship between the volume of luteal tissue and progesterone level in more detail. The results showed that the total volume of luteal tissue is moderately / strongly correlated with serum progesterone levels in Mamasani goats

during the breeding and non-breeding season. The volume of single CLs appeared larger than those developed after double ovulation, but the total volume of CLs was always larger than after single ovulations, which eventually resulted in a higher mean progesterone. concentration of mechanism also occurs in cattle (Bech-Sabat et al, 2008). Our results are in contrast with those described in previous studies in which similar plasma progesterone concentrations were recorded for nonpregnant and pregnant cows with a single CL and two CLs (Starbuck et al, 2004; Mann et al, 2007). Other studies confirmed that there was a positive correlation between the total volume of luteal tissue and the progesterone concentration in cyclic and pregnant sheep (Kaulfuss et al, 2003). In addition, Manalu et al in 1997, have argued that increasing the number of CLs results in more progesterone secretion in pregnant sheep and goats bearing more than one fetus.

The most important finding in the current study for goat fertility management was the correlation between the serum progesterone concentration and the number of fetuses in pregnant goats. This study showed that in twin pregnancies coming from single ovulation, the volume of resulting CL develops more and produces more progesterone compared to a singlet pregnancy. Furthermore, goats that had

double ovulations but carried a single fetus had a higher total volume of CL tissue and corresponded to higher serum progesterone concentration than goats with two fetuses after single ovulation. This finding shows that the serum levels of progesterone in pregnant Mamasani goats is not a reliable predictor for single or twin pregnancy. Although a previous study (Boscos et al, 2003), showed a significant positive progesterone correlation between concentration and the number of kids born, this relation is not strong enough to reliably diagnose the numbers of kids in the individual goat. Our data did not support the previous observations in goat and ewes (Gur et al, 2011; Nawito et al, 2015), which reported that animals bearing more than one fetus had higher levels of progesterone compared to those bearing a single fetus. However, in goats during the last three weeks of gestation, maternal serum progesterone concentrations positively correlated with the number of corpus luteum and fetus (Khan & Ludri, 2002).

In summary, our results showed that progesterone levels depend on the total volume of luteal tissue and this volume is not always correlated with the number of fetuses. Therefore, for the individual goat an elevated progesterone level cannot be used to accurately predict twin pregnancy.

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#### **Conflict of interest**

The authors have no conflicts of interest.

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# ارتباط تخمکگذاریهای منفرد و دوتایی با غلظت پروژسترون سرم و تعداد جنبن در بزهای ممسنی

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### چکیده

تعیین تأثیر تخمکگذاریهای منفرد در مقابل دوتایی بر غلظت پروژسترون و حجم بافت لوتئال برای مدیریت بهتر تولیدمثل در بزها بسیار مهم است. هدف از این مطالعه بررسی ارتباط تعداد تخمکگذاری، حجم بافت لوتئال و غلظت پروژسترون در بزهای ممسنی بود. بدین منظور رحم و تخمدانهای ۱۹۲ رأس بز تازه کشتار شده در کشتارگاه طی فصول تولیدمثل و غیر تولیدمثلی جمع آوری شد. پیش از کشتار، نمونههای خون گرفته شد و غلظت پروژسترون آنالیز شد. گروههای مورد مطالعه در فصل غیر تولیدمثلی شامل: ۱- تخمکگذاری دوتایی یکطرفه و ۳- تخمکگذاری دوتایی یکطرفه و به تخمکگذاری دوتایی یکطرفه و به تخمکگذاری دوتایی یکطرفه (غیر آبستن)؛ ۳- تخمکگذاری منفرد (غیر آبستن)؛ ۲- تخمکگذاری دوتایی یکطرفه با دو جنین؛ ۷- تخمکگذاری دوتایی یکطرفه با دو جنین؛ ۷- تخمکگذاری دوتایی دوطرفه با دو جنین؛ ۷- تخمکگذاری دوتایی دوطرفه با دو جنین و ۹- تخمکگذاری دوتایی دوطرفه با دو جنین؛ ۷- تخمکگذاری دوتایی دوطرفه با دو جنین نقش تعیین کنده دارد. در هر دو مورد بزهای آبستن و غیر آبستن، حجم کل جسم زرد و همچنین غلظت پروژسترون سرم در بزهای با تخمکگذاری منفرد و دو جنین نسبت به بزهای به طور معنیداری بیشتر بود. حجم کل جسم زرد و همچنین غلظت پروژسترون در بز، با حجم با تخمکگذاری منفرد و یک جنین به طور معنیداری بیشتر بود. نتیجهگیری شد که اگر چه بالاتر بودن غلظت پروژسترون در بز، با حجم با حسم زرد مرتبط است اما این سطح یک پیشبینی قابل اعتماد برای تعیین نمودن تعداد جنین در هر بز نیست.

کلمات کلیدی: جسم زرد، تخمکگذاری دوتایی، آبستنیهای دوقلو، پروژسترون، بز ممسنی

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