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## Investigation of relationships between placental characteristics and kid birth weight in goats

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**Abstract:** The aim of this study was to determine the relationships among the kid birth weight, the placental, and cotyledon traits in Saanen goats. The effect of parity, birth type, and birth weight (BIRWT), on placental and cotyledon characteristics, were analyzed by the general linear model. Significant differences for birth type occurred on cotyledon length (CL) and cotyledon depth (CDEP). The means for cotyledon width (CWID) and cotyledon depth (CDEP) were 21.52 and 0.361 mm, respectively. The general means of total cotyledon surface area (TCSA), cotyledon number (CN), and cotyledon weight (CWE) were 67.27 cm<sup>2</sup>, 66.76 unit, and 153.51 g, respectively. There was negative correlation among placental weight (PW) and each of the cotyledon density (CD) ( $r = -0.321$ ,  $p < 0.01$ ), placental activity (PA), and cotyledon activity (CA) ( $r = -0.630$ ,  $p < 0.01$ ). The relationship among total cotyledon number (TCN), cotyledon weight (CWE), and total cotyledon surface area (TCSA) were significantly and negatively correlated ( $p < 0.01$ ). It has been found that there was a significant and negative relationship between cotyledon density (CD) and cotyledon width (CWID). There was a positive relationship between cotyledon length (CL) and cotyledon width (CWID) ( $p < 0.01$ ). Finally, it may enhance to conduct of further nutrition studies examining the relationship between gestation and total cotyledon surface area (TCSA).

**Key words:** Placental characteristics, cotyledon traits, Pearson correlation coefficients, Saanen goats

### 1. Introduction

The placenta is the most important fetal organ, providing a variety of physiologic functions for the fetus [1,2]. Placental traits are used as a parameter of the postpartum losses of offspring for farm animals [3,4].

In the past few decades, some new approaches have been developed to assess newborn animals for survivability. It is known that low survivability may be detrimental to animal health and welfare [5]. The small ruminants, which have a polycotyledon in their placenta structure, have an exchange between mother and offspring circulatory system [3,4]. Therefore, nutrient exchange capacity between mother and offspring depends on the placental characteristics and the number of placentom [6]. Maternal nutrition during pregnancy plays a crucial effect in the regulation of fetal and placental development in goats [7,8]. The growth of the placenta continues in various phases during pregnancy. It begins on the 14th day after fertilization and continues until the 60th day of pregnancy. The organ produces changes until the very end of gestation [7–9]. Besides, placental development during pregnancy has been significantly affected by the maternal factor, such as nutritional level [8,10,11].

In addition, some studies have shown a significant relationship between placenta weight and birth weight [8,12,13]. Dwyer et al. [14] reported that birth weight and placental traits were influenced by maternal age. Besides, Wallace et al. [15] suggested that nutritional requirements might change during pregnancy in goats. It is increased the growth of the fetal weight at the use of nutrient requirements for the uterus and mammary gland during pregnancy. Thus, the risk of a significant limitation of the placenta weight in goats' increases. It can cause a detrimental decrease in birth weight with high mortality rates [16]. Therefore, changing the placental development may change the placental development as well as birth weight changes due to placental traits in first kidding goats [17]. This study was done to determine the affected factors of placental, and cotyledon characteristics in Saanen goats.

### 2. Materials and methods

#### 2.1. The animals, study area, and data collection

It was performed on 48 Saanen goats of different ages and lactation with the normal breeding season in İzmir, Turkey (38°27'19.8"N 27°13'36.1"E). According to the parity and

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birth type in goats, data have shown in Table 1. In the study, since there were no animals in the first lactation, all animals with two and third parity were used as research material. The number of goats was 17 in the second lactation, 22 in the third lactation, and 9 in the fourth lactation or higher. Goats were allowed to graze for 3 h per day during pregnancy. 800 g/goat/day as a concentrate; 400 g/goat/day as good quality alfalfa; straw 200 g/goat/day and 2 kg corn silage were given to meet daily nutritional requirements during experimental pregnancy. Permission of the ethics committee regarding the animal experiments was received for this study (27.07.2016-064, Republic of Turkey Ministry of Agriculture and Forestry).

Data for birth weight and sex of goat kids were recorded within 1 h after kidding. Naturally, it was allowed to discard the placenta were collected immediately after parturition. Each placental weight was measured individually after the placental fluid was removed. Total

cotyledon numbers (TCN) and total cotyledon weights, which were removed from Chorioallantois (fetal placenta), were counted. Cotyledon length (CL), cotyledon depth, and cotyledon width (CWID) were measured with a digital caliper. Cotyledon density was calculated as the number of cotyledons per gram placental weight [3,4,8,18,19,20]. Cotyledon activity has defined the ratio of birth weight to the total cotyledon surface area. The placental activity was determined as the ratio of offspring birth weight to placental weight for goat. After measuring all cotyledons in each placenta as cm<sup>2</sup>, the total cotyledon surface area was calculated according to the following formula:

Cotyledon Radius Square:  $(((\text{CWID} + \text{CL})/4)^2) \times 3.14(\pi) \times \text{TCN}$  (Ocak et al. [19]; Şen and Önder [20]).

The area of the cotyledon and placenta was determined by screen digitization using ArcGIS 10.5 software [21]. For

**Table 1.** Least square means and standard errors ( $\bar{x} \pm s_{\bar{x}}$ ) on placental traits of Saanen goats.

Main effects	n	GBDW (kg) X ± S X	BIRWT (kg) X ± S X	TKBW (kg) X ± S X	PW (g) X ± S X	PA (%) X ± S X
Parity						
2	17	60.93 ± 2.791	3.49 ± 0.202	6.38 ± 0.365	425.44 ± 79.834	0.892 ± 0.171
3	22	65.65 ± 2.181	3.72 ± 0.153	7.06 ± 0.431	431.26 ± 51.283	0.959 ± 0.145
4>	9	63.71 ± 3.383	3.77 ± 0.238	7.22 ± 0.277	441.63 ± 67.736	0.989 ± 0.110
		NS	NS	NS	NS	NS
Birth type						
1	11	64.54 ± 2.482	4.22 ± 0.174 <sup>b</sup>	4.52 ± 0.315 <sup>a</sup>	310.00 ± 125.44 <sup>a</sup>	0.897 ± 0.192 <sup>a</sup>
2	32	65.91 ± 1.692	3.89 ± 0.118 <sup>b</sup>	7.59 ± 0.215 <sup>b</sup>	396.15 ± 58.468 <sup>a</sup>	0.959 ± 0.145 <sup>b</sup>
3	5	59.83 ± 5.262	2.87 ± 0.373 <sup>a</sup>	8.55 ± 0.677 <sup>b</sup>	592.12 ± 39.790 <sup>b</sup>	0.989 ± 0.110 <sup>b</sup>
		NS	*	*	*	*
Sex						
Male	17	64.20 ± 2.315	3.47 ± 0.163	7.05 ± 0.328	397.34 ± 54.660	0.936 ± 0.130
Female	31	62.66 ± 2.544	3.85 ± 0.181	6.72 ± 0.295	468.18 ± 60.749	0.957 ± 0.117
		NS	NS	NS	NS	NS
Reg. (Lin) Goat Body Weight			0.013 ± 0.009 NS	-0.004 ± 0.040 NS	0.003 ± 0.001 *	1.955 ± 0.300 *
General	48	63.43 ± 2.041	3.66 ± 0.145	6.82 ± 0.262	432.76 ± 48.610	0.946 ± 0.104

Means with different superscript in each column (a, b) differ significantly; NS: not significant; \* p < 0.05, \*\* p < 0.01.

GBDW: goat body weight; BIRWT: kid birth weight; PW: placental weight; PA: placental activity;

TKBW: total kid birth weight per goat.

images of the study, the placenta was opened and placed on 90–120 cm paper consisting of each centimeter square and each placenta was taken with a high-resolution camera and angle up to 150 cm<sup>2</sup>. From images, four different properties were examined; total placenta and cotyledons were covered. The measurements, which were measured with a program, have shown in Figure.

## 2.2. Statistical analysis

The effects of parity on, birth weight of offspring, placental and cotyledon traits, and the other variables were analyzed using a completely randomized design by the general linear model (GLM) procedure of the IBM SPSS10 (IBM Corporation, Armonk, NY, USA) package program. A univariate procedure of SPSS was used to check for normality. The goat body weight for placental traits was used as cofactors in this model. The buck effect has not been studied in this study as it is not included in some studies on placenta traits [3,4,8,20,21]. Statistical significance was considered at  $p < 0.05$  and  $p < 0.01$  [22]. The mean differences were tested by the Duncan test. Relationships between variable traits for discrete data were determined with Pearson correlation analysis at the 95% confidence interval.

## 3. Results

### 3.1. Placenta traits

This structure, which can last for about 5 months in goats, may reflect the general condition of mother or offspring acting as a transplanted organ for growth and development of enzyme factory, inner secretory organ, and offspring. Meanings and standard errors of placenta traits in Saanen goats have shown in Table 1. The average birth weight for offspring was  $3.66 \pm 0.145$  kg. The effect of parity and sex on birth weight was statistically insignificant, but the type of birth is significant ( $p < 0.05$ ). The lowest and the highest birth weight were  $2.87 \pm 0.373$  kg and  $4.22 \pm 0.17$



**Figure.** An example of a placenta made of screen digitization.

kg, respectively. The average placental weight was  $432.76 \pm 48.610$  g. The effect of birth type on total placental weight for goats was significant ( $p < 0.05$ ), while the effect of parity and sex for offspring was insignificant. Similarly, the effect of birth type on placental weight was found to be significant ( $p < 0.05$ ), while the effect of parity and sex for offspring was insignificant. In Saanen goats, the lowest placental weight was  $310.00 \pm 125.44$  g and the highest value was found in goats giving triple with  $592.12 \pm 39.790$  g. The effect of the parity and sex for offspring on placental activity was determined insignificantly but the type of birth is significant ( $p < 0.05$ ). Table 1 shows least square means and standard errors ( $\bar{x} \pm s_{\bar{x}}$ ) for placental traits of Saanen goats.

### 3.2. Cotyledons traits

In goat placenta, chorionic villi were found as a group in certain foci, which are called cotyledon. The cotyledon length for goats varied from  $35.70 \pm 1.329$  to  $46.19 \pm 2.851$  mm, while the overall average was  $41.17 \pm 1.105$  mm. While the effect of parity and sex for offspring was insignificant, the effect of type of birth on cotyledon length and cotyledon depth was significant ( $p < 0.01$ ). The means for cotyledon weight and cotyledon depth were  $21.52 \pm 0.636$  and  $0.361 \pm 0.149$  mm, respectively. The effect of investigated properties on the cotyledon weight was found to be insignificant. The average of cotyledon density was  $16.20 \pm 1.571$ , While cotyledon activity ( $p < 0.05$ ), cotyledon density, the total cotyledon surface area, cotyledon numbers, and total cotyledon weight were found to be significant ( $p < 0.01$ ), the effect of parity and sex for offspring was insignificant, the total cotyledon surface area, cotyledon numbers, and cotyledon weight were significant ( $p < 0.01$ ) in Saanen goats. General means of the cotyledon activity, total cotyledon surface area, cotyledon numbers, and cotyledon weight were  $6.66 \pm 1.078$ ,  $67.27 \pm 8.727$  cm<sup>2</sup>,  $66.72 \pm 8.233$  unit, and  $153.31 \pm 19.246$  g, respectively. The covariance effect on cotyledon properties of live weight in Saanen goats was not important. The least-squares mean and standard errors of cotyledon traits of goats have been shown in Table 2.

### 3.3. Relationships between traits

In parturition, the relations between weights, placenta, and cotyledon traits for goats and offspring are included in the properties affecting the husbandry. Phenotypic correlations of properties obtained for goats have shown in Table 3. The relationship between placenta and cotyledon properties of birth weight for goat kids was found to be significant only with placental activity ( $p < 0.05$ ). In goats' relations among total birth weight, total cotyledon weight, cotyledon length, and cotyledon width were found to be statistically significant ( $p < 0.01$ ). However, a negative relationship was found among birth weight for offspring on cotyledon density and cotyledon weight ( $p < 0.01$ ). There

**Table 2.** Least square means and standard errors ( $\bar{x} \pm S_{\bar{x}}$ ) on cotyledon traits for Saanen goats.

Main effects	n	CL (cm) X ± S X	CWE (g) X ± S X	CDEP (cm) X ± S X	CD (n/g) X ± S X	CA (g/cm <sup>2</sup> ) X ± S X	TCSA (cm <sup>2</sup> ) X ± S X	TCN (unit) X ± S X	TCWE (g) X ± S X
Parity							69.363 ± 9.207	61.715 ± 13.522	140.36 ± 26.818
2	17	40.27 ± 1.539	21.04 ± 1.045	0.353 ± 0.018	15.10 ± 2.579	6.06 ± 1.771	72.08 ± 12.161	67.64 ± 11.472	149.78 ± 20.304
3	22	40.55 ± 1.165	21.20 ± 0.671	0.357 ± 0.014	16.55 ± 1.657	6.93 ± 1.503	70.37 ± 14.333	70.80 ± 08.686	169.79 ± 31.608
4>	9	42.72 ± 1.814	22.31 ± 0.887	0.374 ± 0.021	16.95 ± 2.189	7.00 ± 1.138	NS	NS	NS
Birth type							45.19 ± 22.524 <sup>a</sup>	83.99 ± 9.903 <sup>b</sup>	112.62 ± 4.053 <sup>a</sup>
1	11	35.70 ± 1.329 <sup>a</sup>	19.87 ± 0.765	0.288 ± 0.034 <sup>a</sup>	11.44 ± 4.053 <sup>a</sup>	5.26 ± 0.883 <sup>a</sup>	59.81 ± 10.497 <sup>b</sup>	72.54 ± 6.739 <sup>b</sup>	124.97 ± 23.149 <sup>a</sup>
2	32	41.60 ± 0.904 <sup>b</sup>	21.83 ± 1.642	0.395 ± 0.011 <sup>b</sup>	14.20 ± 1.286 <sup>a</sup>	7.28 ± 2.783 <sup>b</sup>	96.64 ± 7.144 <sup>c</sup>	53.62 ± 21.248 <sup>a</sup>	222.33 ± 15.754 <sup>b</sup>
3	5	46.19 ± 2.851 <sup>b</sup> *	NS	0.400 ± 0.016 <sup>b</sup> *	22.96 ± 1.889 <sup>b</sup> **	7.46 ± 1.297 <sup>b</sup> *	**	**	**
Sex							66.09 ± 9.814	63.13 ± 10.289	163.74 ± 24.052
Male	17	39.69 ± 1.242	20.28 ± 0.716	0.348 ± 0.016	14.70 ± 1.963	6.23 ± 1.348	74.45 ± 10.907	70.31 ± 9.258	142.89 ± 21.641
Female	31	42.65 ± 1.380	22.75 ± 0.795	0.374 ± 0.015	17.69 ± 1.766	7.10 ± 1.213	NS	NS	NS
Reg. (Lin) Goat Birth Weight		0.647 ± 0.369	-0.510 ± 0.654	4.077 ± 28.001	-0.609 ± 0.464	-0.046 ± 0.435	-0.065 ± 0.085	0.177 ± 0.130	-0.034 ± 0.056
General	48	41.17 ± 1.105	21.52 ± 0.636	0.361 ± 0.149	16.20 ± 1.571	6.66 ± 1.078	67.27 ± 8.727	66.72 ± 8.233	153.31 ± 19.246

Means with different superscript in each column (a, b) differ significantly; NS: not significant; \* p < 0.05, \*\* p < 0.01.

CL: cotyledon length; CA: cotyledon activity; CWE: cotyledon weight; TCSA: total cotyledon surface area; CDEP: cotyledon depth; TCN: total cotyledon number; CD: cotyledon density; TCWE: total cotyledon weight.

was a negative relationship (p < 0.01) among placental weight, cotyledon density (p < 0.05), placental activity, and cotyledon activity (p < 0.01). The relationship among total cotyledon number, total cotyledon weight, total cotyledon surface area, cotyledon density, and cotyledon depth were significantly and positively correlated (p < 0.01). There was a significant and negative relationship between cotyledon density, cotyledon length, cotyledon width, and cotyledon weight. There was a significant and positive relationship between cotyledon length, cotyledon weight, and cotyledon width (p < 0.01). A positive and significant

relationship was also found between the placental activity and cotyledon activity (p < 0.01). The relationship of placental activity with cotyledon measurements; cotyledon length, and depth (p < 0.05), weight, and width (p < 0.01) was significant and negative.

#### 4. Discussion

It was carried out to define the effect of some environmental factors on placenta traits and birth weight for offspring in Saanen goats. In this study, it was found that the findings were an insignificant source of variation

**Table 3.** Pearson correlation coefficient of placental and cotyledon traits for Saanen goats.

	BIRWE	TKBWE	PW	TCN	TCWE	TCSA	CD	PA	CA	CL	CWID	CDEP
TKBWE	0.115											
PW	-0.013	0.265										
TCN	0.089	-0.116	0.605**									
TCWE	0.034	0.386**	0.929**	0.597**								
TCSA	0.049	0.301*	0.866**	0.617**	0.920**							
CD	0.091	-0.488**	-0.322*	0.518**	-0.288*	-0.177						
PA	0.408*	-0.140	-0.832**	-0.554**	-0.769**	-0.705**	0.244					
CA	0.212	-0.244	-0.630**	-0.571**	-0.668**	-0.679**	-0.034	0.755**				
CL	0.070	0.691**	0.493**	0.088	0.601**	0.567**	-0.419**	-0.365*	-0.425**			
CWID	-0.076	0.469**	0.641**	0.023	0.580**	0.524**	-0.571**	-0.478**	-0.398**	0.620**		
CDEP	0.034	-0.413**	0.328*	0.424**	0.163	0.155	0.136	-0.350*	-0.147	-0.274	-0.081	
CWE	0.036	0.582**	0.505**	-0.035	0.555**	0.499**	-0.544**	-0.423**	-0.379**	0.696**	0.711**	-0.136

Means with different superscript in each column (\*, \*\*) differ significantly; NS: not significant; \*:  $p < 0.05$ , \*\*:  $p < 0.01$ .

BIRWE: birth weight; TCWE: total cotyledon weight; CA: cotyledon activity; CWE: cotyledon weight; TKBWE: total kids birth weight; TCSA: total cotyledon surface area; CL: cotyledon length; PW: placenta weight; CD: cotyledon density; CWID: cotyledon width; TCN: total cotyledon number; PA: placenta activity; CDEP: cotyledon depth.

in all of the placental traits studied. The results were found in Ocak et al. [21] determined different genotypes of sheep. Also, Ocak and Önder [23] reported that there was no significant difference in placental weight, placental activity, and cotyledon weight in Saanen goats, German blackhead, and Shami goats. However, contrary to results of Saanen goats, Ocak, and Önder [23] stated that the effect of parity, type of birth, and sex was insignificant. Placenta traits were not significantly affected by fetal sex and this result was consistent with earlier researches in sheep [17,21,24]. There are some differences according to the sex of the goat for cotyledon number, weight, and placental weight. The influence of breed, parity, litter size and age in kid survivability may exert before birth by influencing placental development. Additionally, the placenta plays an important role, not only in ensuring good fetal growth in late gestation but also in the development of the fetal brain, with likely consequences for neonatal behavior and survival [14]. Some studies have also confirmed that fetal growth and the placental capacity for glucose transport are greatly influenced by the number of caruncles and the number of placentomes [4,25,26]. There have been previous studies suggesting that litter size and body weight affect placental weight and total cotyledon surface area [27]. The greater number of larger and heavier cotyledons in twin kids (Table 2) and the strong negative correlations between total cotyledon surface area, cotyledon activity, cotyledon density, and cotyledon depth (Table 3) attest to this finding.

It was determined that parity did not effect on birth weight of offspring, total birth weight per goat, placental weight, and placental activity. The effect of the live weight of the goat on placental weight and activity at birth was found to be significant as linear ( $p < 0.01$ ). Ocak et al. [4] reported that parity had no effect on birth weight for offspring, whereas Dwyer et al. [14] found that parity played an important role in the birth weight of the lamb. However, the average live weight of Saanen goats used in this study was  $63 \pm 2.041$  kg. It is concluded that goats having lower live body weight than the general average, have relatively low placental and cotyledon properties. The main reason for this; it can be concluded that goats with low live body weight cannot feed their offspring during pregnancy or the fetus has not developed enough because they use more nutrients for their own needs [15]. In other words, during the mating season in case of females have not sufficient live weight; the priority of feeding may be going to their development [2].

Placental weight and placental activity, total cotyledon number, and total cotyledon weight in Saanen goat have increased with parity whereas CD was increased. In goats giving birth to the first, the placental weight was light and had a few number and lightweight of cotyledons [23]. Similarly, Konyalı et al. [3] reported that goats giving birth to the first had a lower placental weight and more intense cotyledon, while the total cotyledon number in the placenta was relatively higher than in other lactating goats and this approach was consistent with other research

findings. Previous studies on this issue suggested that the reduction in placental weight and total cotyledon number might be related to a decrease in the rate of development of the fetus [26,28,29]. Therefore, differences in placental weight and total cotyledon number, total cotyledon weight depending on the parity, and goats giving birth to the first were relatively less than the other lactating animals. Histological studies are required to explain this in more detail. On the other hand, the reason for the decrease in the cotyledon number, which gives birth to the first, can be explained by the rate of growth in the fetus, which is a result of the nutritional level of the dam during pregnancy. Moreover, the total cotyledon weight was found to be lower than those were, which gave birth to the first and with larger cotyledons proportionally.

In previous studies of cattle and sheep, placental activity showed an increase with parity [14,30,31]. However, Konyalı et al. [3] and Ocak et al. [18] found that placental activity did not increase with parity in sheep and goats, and this approach was not compatible with our study. The main reason for the decrease in placental weight is low live weight and singleton births in a goat that gave birth for the first time. As it is known the increase in the live weight of goats causes the increase of birth weight and the placental weight, but it may lead to a decrease in the activity of the placenta especially in goats that give birth to the first. Dwyer et al. [15], reported that in animals giving birth to single and multiple lambing/kidding animals, insufficient feeding during pregnancy may affect the development of the placenta as well as the development of offspring, and it is stated that it may cause the formation of offspring with lower birth weight. Therefore, the increase in PW in the single or multiple lambing/kidding animals is due to the increase in the volumetric and vascular systems. This information suggested that nutrients are transported more effectively to the placenta in older animals than in young animals.

Studies of placental activity and cotyledon density in previous years have shown that the fetus does not have enough parameters to determine the actual role of the placenta in transporting nutrients [18,20]. However, Ocak et al. [4] stated that cotyledon activity determined by measuring the total cotyledon surface area was the more accurate approach in the interpretation of placental properties. Furthermore, the nutrient carrying capacity of the placenta is related to the magnitude of the change in a surface area [31]. Although it is very difficult to determine the change in the surface area of the placenta, the total cotyledon surface area in each placenta is a strong indicator of offspring-to-mother attachment and strengthens the estimation of what the fetus is doing by taking enough nutrients from the placenta until parturition.

The results of the present study demonstrated that increasing parity increased placental weight and cotyledon number. The placenta of the young doe was lighter and contained fewer amounts of cotyledons than those of older doe (Table 1). The opposite off, Konyalı et al. [3] and Ocak et al. [19] indicated that the second parity does have lower placental weight and placental activity, but total cotyledon number in per placenta were greater than the higher parity does in contrast to our study. The results of the study suggested that the exchange of nutrients in the placenta of young animals is slower than that of animals in advanced lactation. The most typical sign of this was that goat giving birth to the first where gave offspring with lower birth weight. It suggested that placental activity and cotyledon density were not sufficient to explain the change in placental activity. Therefore, cotyledon activity may be more accurate in determining the relationship between mother-offspring than placental activity. Some studies suggested that the correlations between the birth weight of offspring and placental weight were insignificant [18,20]. However, in this study, no significant relationship was found between the birth weight of offspring and total birth weight per goat and placental weight. This finding was similar to some previous studies in cattle and sheep [14,18,27]. The relationship between birth weight, placental, and cotyledon traits was not important (the negative relationship between cotyledon density and cotyledon activity, a positive relationship between cotyledon density and birth weight). The increase in placental weight led to an increase in the total number and weight of cotyledon in the birth weight of offspring [3,4,20,32].

In this study, it was found that parity; type of birth, and sex for offspring, especially parity had a significant effect on placenta and cotyledon traits. Therefore, it should be remembered that, besides placental traits, cotyledon activity is an important selection criterion. Further investigation is recommended to identify the relationship between placental traits and maternal parity or age for postnatal development and vitality of kids. In addition, it has been understood that the widespread use of digital screen digitization on cells and tissues will cause less time loss in practice and such studies.

#### **Contribution of authors**

All the authors commented on the early and final versions of the manuscript.

#### **Conflict of interest**

There are no conflict of interests to declare.

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