



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(9): 362-367

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www.veterinarypaper.com

Received: 22-08-2025

Accepted: 25-09-2025

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Study the effect of type of birth on some physiological parameters in sheep

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i9f.2585>

Abstract

The aim of this study was to determine whether birth type-single versus twin has effects on some of the physiological measurements performed in Awassi ewes. Ten lactating ewes with live lambs were used; they were selected randomly and divided into two groups according to the birth order: five ewes with single lambs and, five ewes with twin lambs. Blood samples were taken three days post parturition for hematological (WBC, RBC, Hb, PLT), biochemical (cholesterol, triglycerides, HDL, LDL, VLDL) and hormonal (FSH, LH, progesterone, estrogen) parameters determination. Between a single and a twin group, no significant differences in hematological findings were observed. However, biochemistry assays showed significant differences in triglycerides, HDL, and VLDL levels, indicating possible metabolic differences associated with the birth mode. Nevertheless, levels of all reproductive hormones did not differ between groups. Generally, although hematological and hormonal parameters were some comprising predictors. Significant attempts to determine normal ranges have been proved in animal models and in male athletes.

Keywords: Reproductive system, male athletes, hormonal parameters, physiological parameters

Introduction

Reproductive efficiency in sheep production systems is closely tied to maternal health and physiological adaptation during pregnancy. One critical factor influencing maternal physiology is the type of birth, defined by the number of fetuses a ewe carries-singleton, twins, or multiple (triplets or more). The gestational burden increases with fetal number, leading to significant physiological, metabolic, and endocrinological changes in the ewe. These alterations are particularly evident in Complete Blood Count (CBC), lipid metabolism, and reproductive hormonal dynamics, all of which have implications for maternal welfare, reproductive success, and perinatal outcomes.

Take hematological parameters as an example: Pregnancy will make major changes in these values. Whether or not a ewe carries twins is critical to like having hemodilution. It's easy for ewes with multiple fetuses to become hemodiluted because their plasma volume increases disproportionately relative to red blood cell mass. This naturally means lower hemoglobin (Hb), hematocrit (HCT), and red blood cell (RBC) counts and hence decreased O₂ supply both in maternal tissues and to the developing fetuses. Multiparous pregnancies may also increase maternal requirements for iron, folic acid and vitamin B12-essential nutrients that are crucial to erythropoiesis. Failure to meet these needs could result in subclinical or overt anemia sapping the vigour from her body, and various complications such as uterine inertia during birthing. Normal adaptive physiologic changes during pregnancy may lead to increases in White Blood Cell (WBC) counts. This can be a consequence of physiological stress or inflammatory responses related to larger foetal burdens.

Behaviour: Pregnancy is a long period of metabolic adaptation which develops gradually. The ultimate goal is to satisfy the growing foetus and promote mammary development. In the last part of gestation, lipid metabolism alters significantly for ewes who bear multiples. If the foetus puts increased demands on the mother and her stomach is hindered by crowded conditions, then a negative energy equilibrium will always emerge.

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As a result, when lipolysis is accelerated, one outcome is an increase in plasma concentrations of non-esterified fatty acids (NEFA) and Beta-Hydroxybutyrate (BHB) which serve as indicators for fat mobilization and provide information on deficiencies in energy. Studies have shown that serum cholesterol, triglycerides, low and high density lipoprotein (LDL and HDL) levels are all susceptible to fluctuations based on litter size and maternal condition. This reflects the metabolic strain placed upon the ewe (Abd El-Wahab *et al.*, 2020) [1]. Those shifts, if left unchecked, can ultimately lead to pregnancy toxemia in ewes-particularly during late gestation.

The endocrine picture of reproduction can also be seen as a response to conditions of space. Ewe litter size will profoundly affect her reproductive system as well. Progesterone is secreted by the corpus luteum at first, and later by the placenta. This hormone is indispensable if a ewe is going to remain quiescent and hold the fetus in its uterus. In multiple-birth pregnancies, progesterone concentrations in the serum are generally high during mid-pregnancy. This is roughly correlated to an increase of placental mass and steroidogenic work load. However, during late pregnancy of multiple-birth ewes, it is possible that the decline in serum progesterone levels may occur sooner and then signal an earlier start to labor. Estradiol concentrations usually increase as parturition approaches so that they promote myometrial contractions and cervical dilatation. Also, hormones such as relaxin and prolactin have litter-size-specific patterns of secretion which not only affect the ewe's behaviour but also affect lactogenesis. Ewes bearing larger litters often require enhanced endocrine adaptation to ensure proper mammary gland development and postpartum maternal care.

Materials and Methods

Animals: This study was conducted on the local breeds of the sheep of Al-Awasi, Awassi ewes were randomly chosen from the private sheep location near Babylon city, Iraq to investigate the effect of birth type on some hematological biochemical and hormonal study.

Blood samples were collected from ten sheep of Al-Awasi and distributed as follows

- The first group included five sheep with single births.
- The second group: Included five sheep's with twin births.

Sample collection

Blood samples were collected from the jugular vein of animals using a 5ml syringe after cleaning the jugular vein area and sterilizing the area with the alcohols in the morning after three days of birth using a medical syringe. Blood samples were divided into two portions: 2 ml in an EDTA anticoagulant tube for hematologic parameters and 3 ml was placed in a clean bottle test tube free of coagulant and left for 20-15 minutes at the temperature of the temperature (MA) and then placed in the centrifuge at a speed of 3000 / minute for 15 minutes to separate the serum and then isolate the serum through a precise mechanical pipette It was placed in new plastic tubes and kept at 20 degrees Celsius for the purpose of conducting Lipid profile and hormonal test.

Hematological parameters

(WBC, RBC, HB, PLT) by using DYMIND advice

Biochemical parameters

Chol (GIESSE KIT, Italy) TG, (Giesse KIT, Italy) HDL (GIESSE KIT, Italy) LDL: Chol (HDL+TG/5) VLDL: TG/5 Hormonal test (FSH, LH, PRL, Estrogen and Progesterone) by using Tosoh Kit Japan.

Statistical Analysis

The results were subjected to statistical analysis with the aim of identifying the significant differences between the rates of the studied standards in the study groups. The significant differences were determined at the level p where the significant differences of the two groups were tested using LSD analysis.

Results

The study investigated the influence of birth type (single or twin) on key physiological parameters in sheep, focusing on hematological indicators such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), and platelet count (PLT), lipid profile and reproductive hormones. Data were analyzed to identify potential differences between single-born and twin-born individuals, using statistical methods to determine the significance of observed variations. Results are expressed as mean \pm standard deviation, and statistical significance was considered at the $p < 0.05$ level.

Table 1: Effect of type of birth on some blood parameters of Awassi ewes

Blood Parameter	Type of Birth		P-Value
	Single	Twin	
RBC ($\times 10^6$ corpus) NS	11.122 \pm 0.82	11.058 \pm 0.75	0.84
WBC ($\times 10^3$ cells) NS	186.67 \pm 13.25	199.08 \pm 3.49	0.19
HB (gm) NS	5.62 \pm 0.29	5.58 \pm 0.35	0.81
PLT NS	315.6 \pm 83.26	285.8 \pm 56.58	0.60
No of sample	5	5	

NS-Non-significant differences among groups

*Significant differences among groups p -value < 0.05

**highly significant differences among groups p -value < 0.01

This table depicts the comparison of some hematological parameters of two groups of Awassi ewes, one group born as single and the other as twin. Samples are taken in five groups are RBC, WBC, Hb, and PLT are tested in each group.

Red Blood Cell Count (RBC)

- **Single:** 11.122 \pm 0.82 (10^6 corpuscles)
- **Birth of twins:** 11.058 \pm 0.75 ($\times 10^6$ corpuscles)

The average RBC count is slightly greater in single born ewes, but the difference is not significant ($p > 0.05$).

White Blood Cell Count (WBC)

- **Single birth:** 186.67 \pm 13.25 ($\times 10^3$ cells)
- **Twin:** 199.08 \pm 3.49 ($\times 10^3$ cells)

Twin-born ewes have an increased WBC count compared with single-born ewes, which may indicate an increase in

immune response or physiological stress. But again, there is NO-significant difference NS

P-Value (0.19)

Hemoglobin (Hb)

- **Single birth:** 5.62 ± 0.29 gm
- **Twin birth:** 5.58 ± 0.35 gm

There is very little difference between the two groups in terms of hemoglobin levels but there was no difference (NS) between type of birth in the Hb concentration for these ewes p-value (0.81)

Platelet Count (PLT)

- **Single birth:** 315.6 ± 88.26
- **Twin birth:** 285.8 ± 56.68

The mean platelet count is higher in single born ewes, but the large standard deviations and shared superscripts indicate no significant difference p-value (0.60).

So All the mediators tested are similar, in single and twin born Awassi ewes, as shown by the absence of statistical difference in blood parameters: RBC, WBC, Hb and PLT. These data indicate that there is no influence of birth type on the hematological baseline of these animals.

Table 2: Effect of type of birth on biochemical blood parameters of Awassi ewes

Blood Parameter	Type of Birth		P-Value
	Single	Twin	
Cholesterol NS	76.000 ± 3.52	71.400 ± 3.50	0.08
Triglycerides**	5.620 ± 0.50	3.220 ± 0.03	0.015
HDL*	40.000 ± 1.64	46.000 ± 2.53	0.007
LDLNS	21.200 ± 5.12	26.400 ± 6.09	0.19
VLDL**	11.200 ± 0.37	5.140 ± 0.42	0.0015
No of sample	5	5	

NS Non-significant differences among groups.

*Significant differences among groups p-value < 0.05

**highly significant differences among groups p-value < 0.01

Cholesterol (mg/dL)

There is a small decrease in cholesterol levels in twin-born ewes, but the difference is not statistically significant

- **Single-born:** 76.000 ± 3.52
- **Twin-born:** 71.400 ± 3.50
- P-value ≈ 0.08 (non-Significant)

Triglycerides (mg/dL)

Blood levels of triglycerides are much higher in singleton ewes than in twins, which may imply a potential metabolic difference related to the mode of delivery.

- **Single-born:** 5.620 ± 0.50
- **Twin-born:** 3.220 ± 0.03
- P-value ≈ 0.015 (* $p < 0.05$)

HDL (mg/dL)

Compared with single-born, twin-born ewes have higher levels of HDL ("good cholesterol"), implying potentially a better lipid profile or cardiovascular status.

- **Single-born:** 40.000 ± 1.64
- **Twin-born:** 46.000 ± 2.53
- P-value ≈ 0.007 (Very Highly Significant at $p < 0.01$)

LDL (mg/dL)

The value of LDL is somewhat elevated in twin-born ewes, however, the difference is not significant.

- **Single-born:** 21.200 ± 5.12
- **Twin-born:** 26.400 ± 6.09
- P-value ≈ 0.19 (NS)

VLDL (mg/dL)

VLDL levels are much higher in single-born ewes, indicating enhanced transport or metabolic activity

- **Single-born:** 11.200 ± 0.37
- **Twin-born:** 5.140 ± 0.42
- P-value ≈ 0.0015 (Very Highly Significant at $p < 0.01$)

Table 3 and 4: Effect of type of birth on reproductive hormones parameters of Awassi ewes

Blood Parameter	Type of Birth		P-Value
	Single	Twin	
LH NS.	0.370 ± 0.03	0.316 ± 0.01	0.06
FSH NS	0.408 ± 0.02	0.416 ± 0.01	0.17
Progesterone NS.	0.192 ± 0.05	0.136 ± 0.01	0.22
Estrogen NS.	62.822 ± 4.84	69.642 ± 7.67	0.28
No of sample	5	5	

NS Non-significant differences among groups.

*Significant differences among groups p-value < 0.05

**highly significant differences among groups p-value < 0.01

LH (ng/mL)

Although there is a tendency for single-born ewes to possess extreme values of LH, but statistically significant difference is not evident.

- **Single-born:** 0.370 ± 0.03
- **Twin-born:** 0.316 ± 0.01
- P-Value ≈ 0.06 (NS)

FSH (ng/mL)

No significant differences in serum FSH levels were recorded in the two groups

- **Single-born:** 0.408 ± 0.02
- **Twin-born:** 0.416 ± 0.01
- P-value ≈ 0.17 (NS)

Progesterone (ng/mL)

Levels of progesterone are comparable in the two groups

- **Single-born:** 0.192 ± 0.05
- **Twin-born:** 0.156 ± 0.01
- P-value ≈ 0.22 (NS)

Estrogen (pg/mL)

No difference is apparent between the groups of the estrogen values

- **Single-born:** 62.822 ± 4.84
- **Twin-born:** 69.642 ± 7.67
- P-value ≈ 0.28 (NS)

Discussion

The body condition of a ewe is the result of the overall dynamics of many systems responding to internal and external signals. Litter has profound internal influence, especially in the pregnant and lactating stages. Study A offers a single image of these effects in Awassi ewes that this paragraph shall break down in the context of the rest of literature.

Hematological system changes dramatically during pregnancy in order to facilitate the growth of the fetus, the development of the placenta, and the expansion of maternal tissues. These adaptations include regulation of blood volume, erythrocyte production, and leukocyte trafficking (Piccione *et al.*, 2009) [12]. In study A, Red Blood Cell count (RBC),

White Blood Cell count (WBC), Hemoglobin (Hb) and Platelet count (PLT) did not differ significantly between single and twin born Awassi ewes. This finding indicated that under the given conditions and time of measurement in Study A, the basal haematological status in Awassi ewes were not significantly disturbed by the no of fetuses carried. Nevertheless, this result is not consistent in literature, based on the breed of dogs, physiological stage and specific hematological parameter.

For example, studies of Santarosa *et al.* (2022) ^[15] about Dorper ewes painted a different picture. Their investigation showed that ewes bearing more than one lamb had significantly greater erythrogram values (haemoglobin and haematocrit), and N: L ratio in perhaps the ultimate period of physiologic stress: peripartum (day 140 gestation; 48 h post-parturition) compared to ewes with only a singleton lamb. The authors suggest that these differences in liver function may be a more energetic adaptation to the heavier oxygen and substrate requirements of two fetuses. A higher N: L ratio could also indicate that the animal is under greater stress when it gives birth; as noted by the citation in Santarosa *et al.* 2022 ^[15] twins have higher endogenous cortisol concentrations than non-twins (13). To make things even more complicated, it was reported by Gökçe *et al.* (2021) that anemia was a significant factor for twin-bearing ewes during later pregnancies. In addition, the RBC, PCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in twin-bearing ewes during parturition on Akkaraman Blackhead sheep were all higher than those observed in single-bearing ewes at that time. These results suggest that twin-bearing ewes have an increased erythropoietic stimulus, perhaps in response to the greater O₂ demands on their circulator system or else greater hemodilution effects brought about by the fact that there are more fetuses than normal to ply with nutrients. The marginally non-significant elevated WBC numbers in twin-born ewes (Study A) could possibly have reached significance with a larger sample size, or if specific leukocyte differentials (for example N: L ratio) had been the primary outcome, as stress-induced neutrophilia is a common feature in periparturient animals (Mohri *et al.*, 2007).

Differences in Study A versus Santarosa *et al.* (2022) ^[15] and Gökçe *et al.* (2021) may be the result of a synergy of sources. Breed specific responses in physiological parameters have been reported in sheep, Awassi, Dorper and Akkaraman ewes could have variation in their inherent ability to adapt to the environment or in the baseline hematological values. Properly timing of blood collection is critical. The haematological indices vary during gestation and after delivery significantly. Sampling in study A may have been too early or past the crucial period in which hemodynamic effects of twin pregnancy are most pronounced on blood parameters, compared with the peripartum emphasis of Santarosa *et al.* (2022) ^[15] or the late-gestation period in Gökçe *et al.* (2021). Nutrition management with a potential significant impact on hematopoiesis may also vary across studies. Finally, the sample size of Study A (N=5 per cohort) is too small to guarantee sufficient statistical power to detect minor but potentially biologically relevant differences. There are extensive changes in lipid metabolism in pregnancy and lactation to support the increased energy requirement for fetal growth and for milk production, afterward. These changes include mobilization of the maternal fat store, modifications in lipoprotein synthesis and clearance, and changes in the concentrations of different lipid fractions in the blood pool (Relling and Reynolds, 2007) ^[13].

Some of the most statistically significant findings of study A were recorded on the lipid profile of Awassi ewes. Single-born ewes were distinguished by significantly higher blood concentrations of Triglycerides (TG) and Very Low-Density Lipoprotein (VLDL), the main carrier of endogenous TG. Twin-born ewes, however, had significantly higher level of High-Density Lipoprotein (HDL) also known as “good cholesterol” because they transport cholesterol in the reverse direction. Total Cholesterol and Low-Density Lipoprotein (LDL) did not differ significantly between groups. These findings provide very strong evidence that birth type in Awassi ewes is characterised by different metabolic tactics or adaptive responses to lipid responses. The high HDL in twin-born ewes, as implied by the authors of Study A, might imply more beneficial profile of lipid or improved cardiovascular adaptation and, thus, better turnover of lipid or antioxidant status. On the other hand, greater TG and VLDL levels observed in single-born ewes could be indicative of variations in energy mobilization, partitioning or even an increased tendency for fat deposition in response to energy intake greater than is demanded by a single fetus.

Interpretation of these nuanced results in the context of the whole literature is not straightforward, as studies vary in the influencing lipid forms, breeds studied, and physiological status. The extracts presented by Al-Thuwaini *et al.* (2021) who also studied Iraqi Awassi ewes, offered an interesting hint that twin-bearing ewes were the heavier hyperlipidemia. This result is in opposition to the non-significant direction of total cholesterol levels between Studies a (See Figure 2) and indicates potential intra-breed variation or effects of subtle differences in experimental conditions or animal care. However, the full text of Al-Thuwaini *et al.* (2021) that have prevented more thorough comparison of other important lipid components like TG, HDL, LDL and VHDL that would be indispensable for direct breed specific comparison with study A.

Lipid metabolism in ovine pregnancy is a complex process that is greatly affected by the increased energy requirements of fetal development and growth, placental growth, and preparation for lactation, as well as hormonal fluctuations (e.g., concentrations of insulin, glucagon and growth hormone) and prevailing nutritional status. Pregnancy with twins is itself metabolically more demanding for the ewe and frequently leads to a state of negative energy balance, especially at the end of the gestation period. It may promote further NEFA mobilization from adipose tissue. Indeed, there are reports from some studies of higher maternal NEFA in twin-bearers vs singular-bearers, particularly in the face of nutritional challenge (snippets: More NEFA in twins, not significant). These mobilized NEFAs are then delivered to the liver for oxidation, re-esterification to TG for export as VLDL or, if liver capacity is exceeded, production of ketone bodies or the induction of hepatic lipidosis. Study A's results TG/VLDL way on higher levels in the singles and HDL level in twins, although that many people would hypothesize twin load the greater are quite surprising Single-born ewes may have been using more energy to maintain their maternal reserves. At the same time they could even have chosen a different route of fat-depositing or a different pattern of lipid synthesis. And surely those ewes that had both twin lambs for Study A should actually have been set up with greater ability to adapt metabolically (with increased peripheral tissue uptake of VLDL-derived fatty acids, or better HDL-aided cholesterol efflux). Such intricate relations suggest that in future studies the amount of information on dietary intake (EE balance in detail) and data from a greater variety of metabolic

hormones enzymes needs to be included in order to completely comprehend differences between these lipid profiles of organization underlying physiological details.

The endocrine system, especially reproductive, is also important for pregnancy, fetal development, and the initiation of parturition. WELL FUNCTIONS Hormones of interest are Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), progesterone, and various forms of estrogen.

Study A did not observe any significant difference in plasma LH, FSH, progesterone or estrogen concentrations between single and twin-born Awassi ewes. This implies that the systemic levels of these important reproductive hormones in the animals at the time of sampling were not obviously affected by birth type, for the hormones analysed. But this discovery that is not wholly compatible with other studies. For instance, according to Kamil (2024) (in a study that include levels that likely included Awassi type of ewes), type of birth does contain a significant impact on estrogen around partum time in particular multiparous ewes, twin-bearing that higher EWES associated with estrogen concentrations. The examination of estrogen and progesterone also is important since estrogen values are elevated markedly in late gestation of equine gestation and are placenta-driven in origin; they are essential in preparing the uterus for parturition and in stimulating mammary development. Greater estrogen in twin-bearing ewes may be indicative of larger placental mass or stronger fetal cue.

Endocrine patterns may vary greatly in primiparous (single bearing) and multiparous (subsequent bearing) ewes as a result of variation in endocrine maturity and prior reproductive experience. On the basis of the brief summary for study A, it did not seem to stratify according to parity and, in turn, may obscure differences that would be expected to occur in the nulliparous and/or the multiparous groups. Indeed, the time when samples are collected (in relation to the exact stage of the oestrous cycle (if non-pregnant), stage of gestation or postpartum) is crucial as concentrations of the reproductive hormones change considerably and rapidly in response to these physiological conditions. Differences in timing of sampling points, even by a few days hours) around important dates such as parturition, may result in vastly different results. For example, progesterone secretion, secreted mainly by the corpus luteum with additional production from the placenta, is indispensable for maintaining pregnancy and automatically decreases just before delivery in sheep. LH and FSH are normally inhibited in pregnancy by negative feedback of progesterone and estrogen. The absence of an effect in Study A might suggest that sampling coincided with a time when these hormones were basally low (LH/FSH during mid-late pregnancy) or had not yet diverged as a functional consequence of litter size (e.g., progesterone prior to the preparturient decline, or estrogen prior to the final surge).

The comparison as a whole demonstrates that the physiological response to carrying singles vs. twins is complicated and probably depends on multiple factors such as breed, parity, physiological status (gestation, peri-partum, lactation), time of measurement, and environmental condition. Previous researchers have reported altered lipid metabolism in response to lambing type when comparing twin (or more) and single 685 bearing ewes ^[5, 36, 46] and between upgraded Awassi and Merino ewes (sired by Awassi rams) ^[21] which were the focus of Study A. Here, we have significant differences for lipid metabolism (TG, VLDL, HDL), which differed over lambing type, but not for haematology or reproductive hormones, in our particular sheep system. The

disagreement with previous findings, namely Santarosa *et al.* (2022) ^[15] on peripartum hematology, on oestrogen and Al-Thuwaini *et al.* (2021) on cholesterol and sex hormones highlight this complexity. Study A is limited by the low statistical power and small sample sizes (N=5 for each group) interfere with the ability to detect subtle differences or generalize the findings widely. Future studies with larger populations and more precise recording of parity and standardized sampling across physiological conditions are necessary to understand the subtle effects of birth type on sheep physiology.

Conclusion

Study A indicates that birth type influence lipid metabolism in Awassi ewes, twin-born ewes have higher HDL, while single-born ewes have higher TG and VLDL. In contrast, no differences were observed in the basal hematological parameters or the main reproductive hormones in the conditions of the present study.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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How to Cite This Article

Al-Charak AGH. Study the effect of type of birth on some physiological parameters in sheep. *International Journal of Veterinary Sciences and Animal Husbandry.* 2025;10(9):362-367.

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