Some Diagnostic And Treatment Considerations On Aborted Ewes

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Citation

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Abstract

This study aimed to investigate the serum, vaginal discharge of aborted ewes and stomach contents of aborted feti to throw the light on some factors causing abortion in ewes, beside to the effect of abortion on postpartum uterine rebound. This study conducted on 10 aborted ewes at 3rd to 4th month of pregnancy [aborted group] from a sheep flock [n=50] at the farm of Faculty of Veterinary Medicine, Zagazig University, and on another 10 healthy pregnant ewes at the same stage of pregnancy [control group]. The levels of some plasma elements [vitamin E, selenium, copper, phosphorus, zinc, magnesium and total protein] in the blood of aborted and control ewes were estimated. Swabs from the vaginal discharge and stomach contents of the aborted ovine feti were cultured for bacterial identification and antibiotic sensitivity. The completion of uterine involution [days] and uterine regression [cm] were assessed by B-mode Ultrasound Scanner [Pie-Medical Scanner-240] with a linear rectal transducer of 6-8 MHz as indicated by the transversal diameter and the presence of uterine lumen. This study showed that, the concentration of vitamin E, selenium, copper, phosphorus and magnesium were significantly lower ($P<0.05$) in aborted ewes than in the control. However, there was no significant difference in zinc and total protein levels between the two groups. The most prevalent bacterial isolates from the vaginal discharge were corynebacterium spp. [87.5%], staph. spp. [75%] and strept. spp. [62.5%], while the isolates from stomach contents of the aborted feti
were corynebacterium spp. [100%], followed by staph. spp., strept. spp., and anthracoid [50%/each]. The bacterial isolates were highly sensitive to procaine penicillin-G [100%] followed by dihydro-streptomycin sulphate [90%], gentamycin [80%] and ciprofloxacin [50%]. After normal delivery [control group], 7 swabs [70%] from the vaginal discharge showed E. coli and 5 swabs [50%] showed anthracoid isolates. The uterine involution in ewes completed on day 24 postpartum in spontaneously delivered ewes [control] without more measurable decrease, but it delayed in the aborted ewes to day 36 postpartum ($P<0.05$). Spontaneously delivered ewes showed a physiological regression of the uterus with a transversal diameter of 5.55(+/-0.02) cm on day 1, decreased to 1.87(+/-0.02) cm on day 24 postpartum [complete involution], while aborted ewes showed a uterine regression with transversal diameter of 5.89(+/-0.04) cm on day 1, decreased to 3.87(+/-0.01) cm on day 24, and delayed to 1.23(+/-0.01) cm on day 36 postpartum [complete involution].
This study concluded that the deficiency of some vitamins and minerals in the pregnant ewes may reduce the defense mechanism and increase the susceptibility of animal to infection during pregnancy which may lead to abortion, as well as delay the uterine rebound [involution or regression] after abortion.

INTRODUCTION

Abortion is one of great concern to the farmers due to loss of newborn, loss of fertility or even sterility resulting from prolonged uterine diseases and secondary bacterial infection of the genital tract. Single factor or combination of many factors may cause abortion in sheep. Trace elements may act as coenzymes, or stabilizers of secondary molecular structure. Their function has evolved from recognition of their essential function in cell metabolism. There has been special interest in effects of dietary trace element deficiencies on physiological function, particularly reproduction. Severe dietary deficiencies of trace elements including copper, selenium and zinc are commonly seen in ruminants. Hidiroglou (1979 and Minson (1990) suggested the presence of a close link between plasma levels of some vitamins and elements in aborted ewes and the reproductive performance. The deficiency of vit. E and selenium [Se] may cause nutritional muscular dystrophy or white muscle disease in young ruminants (Combs and Combs, 1986) and congenital death if it occurs before birth (McDowel, 1989; Combs and Combs, 1986; Norton and Campbell, 1990). Cu joins in formation of many enzyme systems and its deficiencies may result in those metabolic and clinical symptoms related to these enzymes. Phosphorus has been long recognized as an essential mineral for bone development, reproduction and energy transfer (Hidiroglou, 1979; Minson, 1990). In addition, Zn, Cu and Se transfer at high rates from pregnant ewes to fetus (Minson, 1990) and therefore, deficiencies of these elements may be a cause of ewe abortion.

The uterine infections, which are usually nonspecific, can reduce reproductive efficiency of ruminant livestock. The incidence and consequences of uterine infections are documented far more extensively for dairy cattle than for beef cattle, sheep, or goats (Lewis, 1997; Leontides et al., 2000). However, circumstances associated with increased risk of uterine infections in dairy cattle, such as dystocia, assisted births, retained fetal membranes, and unsanitary conditions at parturition, are common in sheep and predispose them to uterine infections (Fthenaki et al., 2000; Leontides et al., 2000).

During the postpartum period, the sheep should reestablish the functionality of the reproductive system and prepare for a new pregnancy. This includes uterine involution and resumption of cyclic ovarian activity. The finishing of uterine involution is a prerequisite to the maintenance of pregnancy. The time estimated for the completion of uterine involution in sheep varies between 17 and 40 days (Call et al., 1976, Kucharski et al., 1989, Rubianes and Ungerfeld, 1993, Rubianes et al., 1996). Many factors, such as dystocia, retention of fetal membranes, suckling, breed and season are implicated for the course of uterine involution (Bostedt, 1988; Rubianes and Ungerfeld, 1993; Rubianes et al., 1996). The evaluation of uterine involution based only on clinical examination is insufficient, because the uterus in sheep can not be palpated per rectum. Recently, the use of ultrasonography for the control of the uterine involution in sheep has been described (Hauser and Bostedt, 2002). This study aimed to recognize on some causes of abortion in sheep flock by investigation the level of some plasma elements [vit. E, Se, Zn, Cu, P, Mg and total protein], bacteriological identification of the vaginal discharge of aborted ewes and stomach contents of aborted feti, as well as the course of uterine rebound [time of uterine involution/days and uterine regression/cm in aborted ewes] after abortion and spontaneous delivery.

MATERIALS AND METHODS

ANIMALS

This study was conducted on 10 aborted ewes (between 3rd to 4th months of pregnancy) from a sheep flock [n=50] in the farm of the Faculty of Veterinary Medicine, Zagazig University. Another 10 healthy pregnant ewes at the same stage of pregnancy were used as a control group [selected from a number of replaced ewes in the flock]. The age of animals ranged between 2 to 4 years and weighing between 35 to 45 kg at the sampling time. All the animals were serologically negative to brucellosis when screened by Rose Bengal Plate Test, and toxoplasmosis when screened by Latex Agglutination Test [Quimica Clinica Aplica DA-SA. CN 340 km/1081-Pa Box 20 E 438-70 Amposta/Spain], and free from internal and external parasites through the periodical external inspection and fecal examination.

SAMPLING

Jugular blood samples from the aborted ewes (within 2-4 hours after abortion) and control ewes were taken using vacutainer tubes, and the plasma was extracted and stored -20°C. The control ewes were randomly selected from the flock and kept until ultrasonographical examination after spontaneous delivery. Vaginal swabs [8 samples from the aborted ewes and 10 samples from the control ewes], as well as stomach contents [2 samples] from the aborted feti; were collected on nutrient broth for the bacteriological identification.

SEROLOGICAL INVESTIGATIONS

Plasma level of vit. E (Kayden et al., 1973) was determinated spectrophotometricaly. Selenium level was determined with the method of Whetter and Ullrey (1978) using a spectroflouremeter. Zinc and Copper were determined according to Hudrik et al. (1983) by using atomic absorption spectrophotometer. Magnesium was determinated with titan yellow method described by Aras and Ersen (1975). Plasma phosphorus and total protein concentrations were measured with autoanalyser (Technicon RA-XT).

BACTERIOLOGICAL INVESTIGATION

1-Isolation and identification: Vaginal discharge and stomach contents of the aborted ovine feti were incubated at 37ºC for 6 hours in nutrient broth for activation of microorganisms. Lobes from each broth culture were streaked on nutrient agar, blood agar and Maconkey's agar. The plates were incubated at 37ºC for 24-48 hours. Different colonies were picked and purified. Identification of the isolates was performed according to Merchant and Packer (1969).

2-Sensitivity test: Antibiotic sensitivity test was carried out according to Ababneh and Degefa (2006) to select the suitable antibiotics for trials of treatment of the infected animals.

TREATMENT DESIGN

The aborted and control ewes were administered i.m. 5 ml daily for 5 consecutive days of Pen-Strep aqueous suspension [200,000 iu Procaine Penicillin-G and 20 mg/ml Dihydro-streptomycin Sulphate, GIBCO Co.]; according to the sensitivity test; in addition to Adevit-C [AD3E, Vit. A 50,000 iu – Vit. D3 25,000 iu – Vit. E acetate 20 mg/ml] 3 cm i.m. weekly; Copper sulphate 1/10000 [1gm/10 lb]; Minerals blocks for free licking. After treatment, the animals were rebred and noticed throughout the gestational period.

ULTRASONOGRAPHIC MONITORING OF THE UTERINE REBOUND

The ultrasonographic examination was carried out on day-1 and up to day-36 after abortion or spontaneous delivery to examine the time of uterine involution [days] and uterine regression [cm].

Ultrasonographic investigations were performed by using a Real Time B-mode Ultrasound Scanner (Pie-Medical Scanner, Genius-240) with a linear-array [transrectal probe] of 6-8 MHz. The animals were examined in standing position after the removal of the fecal matter. The probe fixed to an extension rod was inserted into the rectum. For scanning of the uterus, the probe was moved approximately 60° to each side around its longitudinal axis. Parameters for the evaluation of the time required for uterine involution and uterine regression were estimated including the transversal diameter of uterine horns of 2 cm and the lack of contents in the uterine cavity (Anderson et al., 1997). Ultrasonographic images of the uterus were documented by a video graphic printer (Mitsubishi P67E).

STATISTICAL ANALYSIS

Analysis of the data was performed using SAS analysis system package (Littel et al., 1991). Significant differences between the means were evaluated utilizing Duncan's Multiple Rang Test (DMRT) (Duncan, 1955).

RESULTS

The results showed that, the level of vit. E, Se, Cu and Mg in the plasma of aborted ewes was significantly lower ($P<0.05$) than the control group [Table 1]. However there is a less significant differences ($P<0.05$) between P-levels of aborted ewes and control group. Status of Zn and total protein were the same in both groups with no significant differences.

All the samples of vaginal discharge and stomach contents of the aborted ovine feti revealed bacterial isolates [Table 2]. The most prevalent bacterial isolates from vaginal discharge were corynebacterium spp. [87.5%], staph. spp. [75%] and strept. spp. [62.5%], while the isolates from stomach contents of the aborted feti are corynebacterium spp. [100%], followed by staph. spp., strept. spp., and anthracoid [50%/each]. The bacterial isolates were highly sensitive to procaine penicillin-G [100%] followed by dihydro-streptomycin sulphate [90%], gentamycin [80%] and ciprofloxacin [50%]. Regarding the bacteriological examination of the spontaneously delivered ewes, 7 swabs [70%] from the vaginal discharge showed E. coli and 5 swabs [50%] showed anthracoid isolates.

The postpartum uterus was well identified by its typical ultrasonographic pattern [Table 3 and figure 1]. The end of uterine involution was characterized by a small cross-sectional diameter of uterine horns and absence of lochia in uterus. The postpartum discharges were present in the vagina of aborted ewes and not more in control ones. If uterine involution was not completed, the transversal diameter of uterine horn was above 2 cm and lochia were accumulated within the lumen (aborted ewes). The uterine involution in ewes completed on day 24 postpartum in spontaneously delivered [control] ewes but delayed in the aborted ewes to day 36 postpartum ($P<0.05$). Ewes that spontaneously delivered showed a physiological regression of the uterus with a transversal diameter of 5.55 (+/-0.02) cm on day 1 postpartum, decreased to 1.87 (+/-0.02) cm on day 24 [complete involution], while the aborted ewes showed a uterine regression with a transversal diameter of 5.89 (+/-0.04) cm on day 1 postpartum, decreased to 3.87 (+/-0.01) cm on day 24 postpartum, and delayed to 1.23 (+/-0.01) cm on day 36 postpartum [complete involution]. Subsequently, there was a significant ($P<0.05$) delayed regression of the uterus after abortion in comparison to the spontaneously delivered ones.

The treatment protocol was associated with reduction in the incidence of abortion among the pregnant ewes. Moreover, the aborted ewes that were rebred after treatment became pregnant with no gestational problems and complete the pregnancy period till gave normal birth in the next season.

Figure 1

Table 1: Mean values of some elements in the plasma of aborted and healthy pregnant ewes [±S.E.].

Parameter	Estimated groups [Means±S.E.]	
	Aborted Group n=10	Control Group n=10
Vitamin E (μg/100 ml	111.00±0.53 [b]	152.10±0.80 [a]
Selenium (μg/100 ml)	8.30±0.30 [b]	11.00±0.36 [a]
Copper (μg/100 ml)	90.00±0.36 [b]	120.60±0.47 [a]
Phosphorus (mg/100 ml)	3.80±0.24 [b]	5.00±0.36 [a]
Zinc (μg/100ml)	134.50±0.58 [a]	136.30±0.49 [a]
Magnesium (mg/100 ml)	0.83±0.003 [b]	2.24±0.005 [a]
Total Protein (g/100 ml)	6.78±0.07 [a]	6.73±0.11 [a]

Means with different superscripts [a&b] in each row are significant [P<0.05].

Figure 2

Table 2: The isolated bacteria from the vaginal discharge of aborted ewes and stomach contents of aborted ovine feti and their sensitivity to different antibiotics.

Bacterial identification	Aborted ewes			
	Vaginal discharge (n=8)		Stomach contents (n=2)	
	No. of isolates	Percentage	No. of isolates	Percentage
Corynebacterium	7/8	87.5%	2/2	100%
Staph. spp.	6/8	75.0%	1/2	50.0%
Strept. spp.	5/8	62.5%	1/2	50.0%
Anthracoid	4/8	50.0%	1/2	50.0%
E. coli	2/8	25.0%	---	---
	Control ewes (n=10)			
E. coli	7/10	70.0%	---	---
Anthracoid	5/10	50.0%	---	---
Antibiotic sensitivity (n=10)				
Type of antibiotic		Sensitivity	Number	Percentage
Procaine Penicillin-G		++++	10/10	100%
Dihydro-Streptomycin sulphate		+++	9/10	90.0%
Gentamycin		+++	8/10	80.0%
Ceprofloxacin		++	5/10	50.0%

Figure 3

Figure 1: Ultrasonic images representing the uterine regression in aborted ewes, at day-1 [], day-12 [], delayed involution to day-36 [] after abortion.

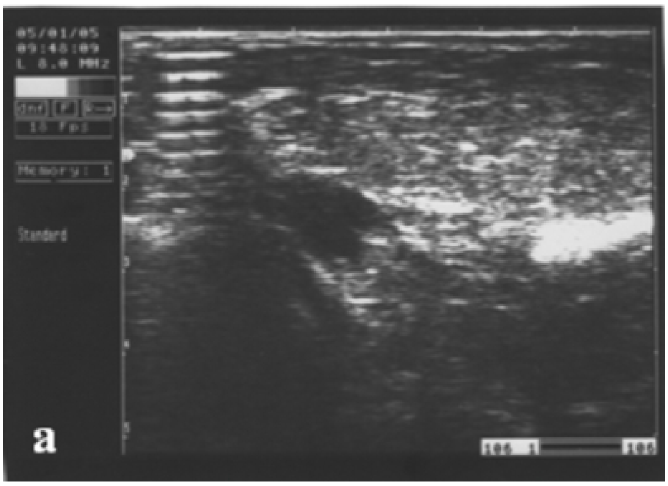

Figure 4

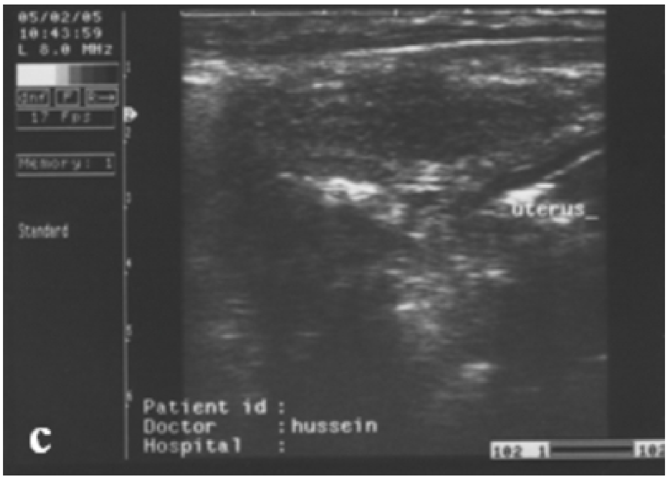

Figure 5

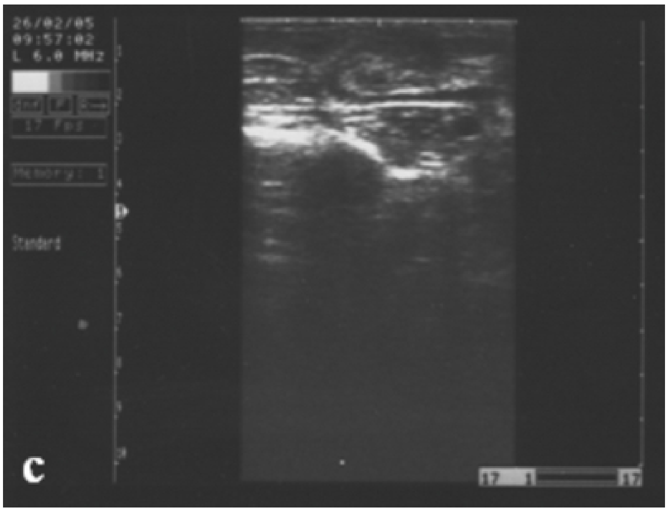

Figure 6

Table 3: Mean values [±S.E.] of the uterine involution (days) and uterine regression (cm) in aborted and spontaneously delivered ewes by ultrasonography.

Parameter	Aborted ewes N=10	Control ewes N=10
Uterine involution [days]	36.00±0.47 [a]	24.20±0.41 [b]
Uterine regression [cm]:		
-Day 1	5.89±0.04	5.55±0.02
-Day 6	5.44±0.02	4.99±0.03
-Day 12	4.83±0.01	3.54±0.01
-Day 18	4.12±0.07	2.45±0.02
-Day 24	3.87±0.01	1.87±0.02
-Day 30	2.59±0.02	1.36±0.01
-Day 36	1.23±0.01	1.12±0.05

Means with different superscripts [a&b] in each row are significant [P<0.05].

DISCUSSION

Status of vitamin E, Se, Cu, P and Mg levels in plasma of aborted ewes were significantly lower than the control group, but Zn and total protein levels were almost the same in both groups with no significant differences. The lower levels of Se and vit. E in the aborted ewes than in the control ewes is supported by results of those (Scales, 1974; Taylor et al., 1979; Stuart and Oehme, 1982; Kott et al., 1983). The deficiency of vit. E and Se may cause nutritional muscular dystrophy or white muscle disease in young ruminants (Combs and Combs, 1986) and congenital death if it occurs before birth (McDowel, 1989; Norton and Campbell, 1990). Also, there was a relationship between Se deficiency and bovine abortions (Taylor et al., 1979). However, Stuart and Oehme (1982) noted that a cause of abortion in cows and sows in North America was due to Se deficiency. New Zealand study showed that in areas where nutritional muscular dystrophy in lambs is severe, a high proportion of the ewes are barren. But feeding Se reduced embryonic mortality from 26 to 3% (Hartley, 1963). In the South Island of New Zealand, Se deficiency decreased the proportion of ewes that conceived by 9 and 15% when they were fed with a diet rich in Se (Scales, 1974). Kott et al. (1983) reported that pre weaning survival of lambs was increased by ewe treating with either Se and/or vit. E.

The low Copper content in diet of the ewes either prevented implantation or induced embryonic loss and fetal death (McChowell, 1968). In a study (Hidiroglou, 1979), nine ewes were fed a severely Cu deficient diet; five of which did not become pregnant and died between 23 and 34 weeks of the experiment and; of the remaining four ewes, two aborted and two produced stillborn lambs. However, copper is essential element for ruminants and its deficiencies occur in grazing animals in many parts of the world. Cu joins in formation of many enzyme systems and thus its deficiencies may reflect some metabolic and clinical symptoms related to these enzymes. Unanian and Feliciano-Silva (1984) informed that Cu status in aborted goats was lower and high incidence of early abortion could be associated with deficiency in copper. In addition, Anke et al. (1977) reported that Cu deficiency in ruminants caused abortion. In this study, the low level of Cu in aborted ewes than in control ewes come in agreement with the reports of McChowell et al. (1968), Anke et al. (1977), Hidiroglou (1979), and Unanian and Feliciano-Silva (1984).

The high incidence of early abortion in goats could be associated with deficiencies in P, Mg and total protein as reported by Unanian and Feliciano-Silva (1984). In the present study, status of Mg and P were significantly lower in aborted ewes than in non aborted ones which coincide with Unanian and Feliciano-Silva (1984) and Lylod et al. (1993). However, in contrast to the result of Unanian and Feliciano-Silva (1984), total protein levels in this study wasn't differ among two groups. Naturally occurring Zn deficiency is rare in livestock. In general, Zn may play a role in the reproductive processes has been obtained primarily from studies on experimentally induced Zn deficiencies (Hidiroglou, 1979). Pond and Wallece (1986) informed that there was no effect of dietary Zn supplementation on survival lambs. In this study, Zn status of ewes didn't statistically differ between two groups. This observation was confirmed by the results of Hidiroglou (1979) and Pond and Wallece (1986). Subsequently, the aborted ewes in this flock characterized by reduction in the levels of vit. E, selenium, copper, phosphorus, magnesium and it may play a role causing abortion in ewes.

The most prevalent bacterial isolates from the vaginal discharge of aborted ewes were corynebacterium spp. followed by staph. spp. and strept. spp., while the isolates from stomach contents of the aborted feti are corynebacterium spp., followed by staph. spp., strep. spp., and anthracoid. The bacterial isolates were highly sensitive to procaine penicillin-G followed by dihydro-streptomycin sulphate, gentamycin and ciprofloxacin. The investigation of bacterial flora of the genital tract and hormonal profiles during the postpartum period was performed by (Ababneh and Degefa, 2006). Staph. aureus and E. coli were isolated in pure or mixed culture from the uterus. Arcanobacterium pyogenes was isolated from swabs obtained from the vagina and cervix of one primiparous goat. Uteri and cervixes but not vagina were free of bacterial contamination by day 10 PP except for one pluriparous goat with scanty E. coli contamination on day 25 PP. Olson et al. (1984) were unable to isolate bacteria from uterine swabs obtained from 10 ewes, 1-14 days postpartum, at surgical hysterectomy. This come in agreement with this study, where the vaginal discharge of control ewes showed 7 isolates [70%] from E. coli and 5 isolates [50%] from anthracoid which may be due to contamination after delivery. These findings interpreted that corynebacterium as well as staph. and strept. spp may play a role in the occurrence of abortion in the ewes of this study.

The clinical investigation of uterine involution in sheep is difficult because the uterus can not be examined by rectal or abdominal palpation and uterine discharge ceases shortly after parturition due to the closure of the cervix. Uterine involution in sheep has been assessed histologically (Doboszynska et al., 1988; Krajnicakova et al., 1996), macroscopically at slaughter (Call et al., 1976; Doboszynska et al., 1988) and by means of radio-opaque markers and radiography (Tian and Noakes, 1991). In contrast to these methods B-mode real time ultrasound allows the noninvasive examination of uterus in sheep. The transrectal scanning is more reliable method than the transabdominal. The pilot study showed that, it was not possible to observe the uterus by transcutaneous scanning because to great distance of uterine horns to abdominal wall. The filled rumen limited also the assessment of the uterus using transabdominal scanning (Hauser and Bostedt, 2002). Consequently, our study confirmed that transrectal sonography is a useful and reliable technique to observe the uterine involution in sheep.

In the present study, the end of uterine involution was characterized by a small cross-sectional diameter of uterine horns and absence of lochia in uterus. The postpartum discharges were present in the vagina of aborted ewes and not more in control ones. If uterine involution was not completed, the transversal diameter of uterine horn was above 2 cm and lochia were accumulated within the lumen (aborted ewes), and there was a relationship between the course of uterine involution and puerperal discharges. Similar observations were made by Kucharski et al. (1989). The time of uterine involution varies in livestock species. On this way, Greyling and Van Niekerk (1991) reported that the diameter of the uterine horns of goats returned to the normal, non pregnant size by 27.9 days postpartum. Kiracofe (1980) reported that uterine involution was complete by 3 weeks postpartum in ewes, and by day 25 the uterus was similar in size to the non pregnant uterus. This is in agreement with the data being presented; uterine involution was completed by day 24 postpartum. After day 24, there was no measurable decrease in cross-sectional area of the uterine horns in spontaneously delivered ewes but delayed to day 36 in aborted ewes.

The spontaneously delivered ewes showed a physiological regression of the uterus with a transversal diameter of 5.55(+/-0.02) cm on day 1 postpartum, decreased to 1.87(+/-0.02) cm on day 24 [complete involution], while the aborted ewes showed a uterine regression with a transversal diameter of 5.89(+/-0.04) cm on day 1 postpartum, decreased to 3.87(+/-0.01) cm on day 24 postpartum, and delayed to 1.23(+/-0.01) cm on day 36 postpartum [complete involution]. Subsequently, there is a delayed regression in the uterus of ewes after abortion in comparison to the spontaneously delivered ones. This corresponded to studies of some authors that determined the time of uterine involution near to day 30 postpartum (O'Shea and Wright, 1984; Kucharski et al., 1989) or day 35 postpartum (Rubianes and Ungerfeld, 1993). In contrary, other authors (Rubianes et al., 1996; Hauser and Bostedt, 2002) observed that the end of uterine involution approximately at day 20 postpartum. The variability regarding the time required for a complete uterine involution may result from differences in breed, period of lambing, type of lambing and suckling. Ewes that spontaneously delivered showed a physiological regression of the uterus with a transversal diameter of 4.9±0.86 cm on day 1 postpartum which decreased to 1.84±0.14 cm until day 30 (Hauser and Bostedt, 2002). The uterine involution was delayed in ewes after manual obstetrics and cesarean section; in addition the incidence of fetal membranes retention with bacterial infections was increased leading to delayed uterine involution in aborted ewes. Subsequently, the uterine involution or regression in sheep finishes until day 24 postpartum in spontaneously delivered ewes [control] and delayed up to day 36 postpartum in aborted ewes [<2cm].

In conclusion, the deficiency of some vitamins and minerals in the pregnant ewes may reduce the defense mechanism and increase the susceptibility of animal to infection during pregnancy resulting in abortion, as well as delay the uterine rebound [involution or regression] after the occurrence of abortion.

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