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Research Article

Effect of Different Litter Size on The Rate of Postpartum Uterine Involution in Hu Sheep

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Abstract: In this study, we investigated the effects of different litter sizes on the rate of uterine involution in Hu sheep. Using B-mode ultrasonography, we recorded changes in the uterine horn diameter and the maximum uterine caruncle diameter in 60 primiparous Hu sheep from days 0 to 45 postpartum. The uterine horn diameter decreased gradually postpartum from 79±1.42 mm at day 0 to 10.87±0.5 mm at day 45 with singleton parturition, from 91±6.58 mm at day 0 to 10.63±0.32 mm at day 45 with twin parturition, and from 107±3.67 mm at day 0 to 11±0.87 mm at day 45 with triplet parturition. The time to complete uterine involution postpartum was 30, 35 and 40 days postpartum in singleton, twin and triplet parturitions, respectively. (P<0.05). The maximum uterine caruncle diameter also decreased gradually from 12.43±0.91 mm at day 3 to 8.82±0.27 mm at day 10 with singleton parturition, from 12.14±1.19 mm at day 3 to 8.31±0.94 mm at day 10 with twin parturition, and from 12.5±0.66 mm at day 3 to 7.94±0.95 mm at day 10 with triplet parturition (P>0.05). Thus, different litter sizes had a greater effect on postpartum uterine horn recovery than on the uterine caruncle. Furthermore, the rate of uterine involution in ewes with singleton parturition was significantly higher than that with triplet parturition. Our findings provide a reference for improving the reproductive performance of Hu sheep.

Keywords: *Hu sheep, Litter size, Uterine involution, Ultrasound*

Hu Koyunlarında Farklı Batın Genişliklerinin Doğum Sonrası Uterus İnvolüsyonu Oranına Etkisi

Öz: Bu çalışmada, Hu koyunlarında farklı batın genişliklerinin uterus involüsyon hızına etkilerini araştırdık. B-mod ultrasonografi kullanarak, doğum sonrası 0 ila 45. gün arasındaki 60 primipar Hu koyununun uterus kornu çapı ve maksimum uterus karunkül çapındaki değişiklikleri kaydettik. Doğum sonrası 0 ile 45. gün arasında uterus korn çapları tekil doğum yapanlarda 79±1.42 mm'den 10.87±0.5 mm'ye, ikiz doğuranlarda 91±6.58 mm'den 10.63±0.32 mm'ye ve üçüz doğuranlarda 107±3.67 mm'den 11±0.87 mm'ye düştü. Doğum sonrası uterus involüsyonunun tamamlama süresi, tekil, ikiz ve üçüz doğumlarda sırasıyla 30, 35 ve 40 gündü (P<0.05). 3. günden 10. güne maksimum uterus karunkül çapları tekil doğumlarda 12.43±0.91 mm'den 8.82±0.27 mm'ye, ikiz doğumlarda 12.14±1.19 mm'den 8.31±0.94 mm'ye ve üçüz doğumlarda 12.5±0.66 mm'den 7.94±0.95 mm'ye düştü (P>0.05). Bu nedenle, farklı batın genişliklerinin, doğum sonrası uterus kornularının iyileşmesi üzerine etkisi, rahim kıkırdağına göre daha büyüktü. Ayrıca, tekil doğum yapan koyunlarda uterus involüsyon oranı, üçüz doğum yapana göre önemli ölçüde daha yüksekti. Bulgularımız, Hu koyunlarının üreme performansının iyileştirilmesi için bir referans sağlamaktadır.

Anahtar sözcükler: *Hu koyunu, Batın genişliği, Uterus involüsyonu, Ultrason*

Introduction

The reproductive performance of small ruminants has important economic benefits, as it determines the annual number of offspring. Uterine involution refers to a physiological process in which the uterus of a dam returns to its non-pregnant state and function after parturition. Several techniques are used to study uterine involution, such as dissection, hormone content determination, and ultrasonography [1-3]; however, ultrasonography is a non-invasive technique that is not only less harmful to dams, but also allows intuitive and accurate monitoring

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of uterine changes during uterine involution. Uterine involution has also been extensively studied in other animals, including Holstein cows [4-6], horses [7], in pigs [8-10], in bitches [11] and cats [12].

Uterine involution in sheep is affected by many factors including nutrition level, season of parturition, breed and parity [13-17]. The time of uterine involution in subtropical sheep lambing in February was 29.4±1.2 day, lambing in June was 33.9±1.1 days [14]. Medan et al.[15] showed that the time to complete uterine involution in Libyan goats was 28-35 days, while Hauser et al.[16] reported that the process was completed in approximately 17 days in an improved German land breed of sheep. The uterine body and caruncle of Baladi goats was basically recovered by day 19 postpartum, and this time was not related to parity [17]. In this study, we investigated the effect of litter size on the rate of uterine involution in primiparous Chinese Hu sheep using B-mode ultrasonography. This breed can produce up to three litters of 1-7 lambs within two years and has excellent traits such as strong environmental adaptability, perennial oestrus, good lactation performance, and rapid growth and development. Adult rams and ewes weigh approximately 65 kg and 40 kg, respectively, and are ready for mating at 6 months of age [18].

This investigation of the effects of different litter sizes on the rate of uterine involution in Hu sheep will provide a reference for improving the reproductive performance of Hu sheep.

Material and Methods

Experimental Animals

A total of 300 Hu sheep aged between 8 and 18 months and weighing 40-60 kg, were included in this study from May to June 2021. All sheep were from the breeding base of Runkangyuan, Xinjiang, China, and housed under conditions of natural light. Ewes were fed a complete diet prepared according to their nutritional requirements and allowed free access to water with mineral salts provided in a licking block throughout the experiment. All lambs were suckled by the ewes.

Primiparous Hu sheep were artificially inseminated after estrus synchronization treatment. Then they were submitted to an ultrasonographic evaluation to determine the number and location (on the same side of the uterine horn) of fetuses was determined by B-mode ultrasonography on day 40 of the pregnancy.

Experimental Design

According to the number of lambs, 60 ewes with a mean body condition score (BCS) of 3.48/5 according to Russel et al.[19] in the northern hemisphere were selected for analysis of uterine involution and allocated to the following groups: Group A, singleton parturition (n=20; average weight = 41.2 kg); Group B, twin parturition (n=20; average weight = 40.3 kg); and Group C, triplet parturition (n=20; average weight = 40.8 kg). Postpartum uterine involution was monitored in all sheep by transrectal ultrasonography measurement of the uterine horn diameter and the maximum uterine caruncle diameter on days 0, 3, 7, 10, 14, 18, 22, 26, 30, 35, 40 and 45.

Ultrasonography and Image Analysis of the Uterine Horn and Caruncle

Uterine involution was monitored in all postpartum sheep in the standing position by transrectal ultrasonography (7.5-MHz linear-array; HS-1600, HONDA, Japan) performed by an operator wearing sterile gloves. After application of a layer of coupling agent, the probe was slowly inserted into the cleaned rectum with the scan detection window facing down. The bladder was used as a reference object to rotate the left and right sides for scanning to obtain the best field of view of the uterine horn and caruncle [20,21]. Transrectal ultrasonography was performed at the uterine angular curvature. Images of the section corresponding to the maximum uterine caruncle diameter were recorded, and the length of the external diameter was measured. The diameter of uterine caruncle was determined by measuring at least three uterine chambers [22]. The section diameter was calculated as (long axis length + short axis length)/2.

Statistical Analysis

Data were statistically analyzed using SPSS 20.0 (Statistical Product and Service Solutions 20.0) software. All data were expressed as mean ± standard error of mean (SEM). One-way ANOVA was used to evaluate differences between groups. Duncan's test was used to evaluate differences in the uterine horn diameter and maximum uterine caruncle diameter. $P<0.05$ was set as the threshold for statistical significance.

Results

Ultrasonography of the Uterine Horn of Hu Sheep in the Postpartum Period

Images of the bladder filled with urine *(Fig. 1-A2)* and when empty *(Fig. 1-B5)* were used as a reference for ultrasonography of the uterus. The uterine horns were identified by transrectal ultrasonography using the bladder as a reference. The size and volume of the uterine horns were gradually reduced with time postpartum in all groups *(Fig. 1)*.

Although the uterine horn was too large to be displayed by ultrasonography before day 3 postpartum, uterine residual fluid was clearly observed *(Fig. 1-A1 white circle)*. The structures of the uterine wall, including the mucosal layer,

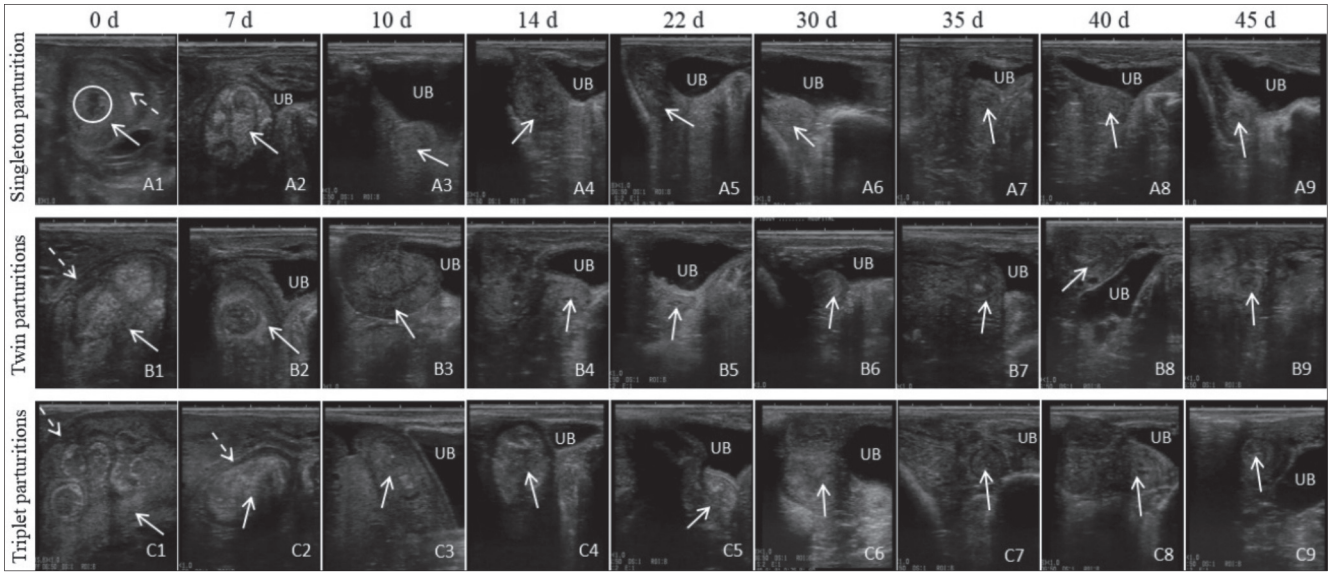

Fig 1. Ultrasonography of uterine horns in Hu sheep 0-45 days postpartum, as detected with a 7.5-MHz transducer. UB, bladder; uterine horn *(solid arrow);* uterine wall *(dashed arrow);* uterine residual fluid *(white circle).* The number of days postpartum are shown above the images

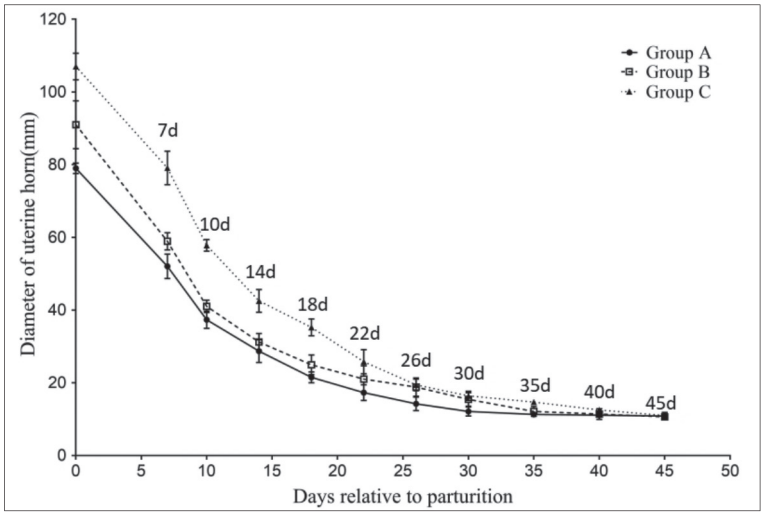

Fig 2. Changes of uterine horn diameter 0-45 days postpartum in Hu Sheep

were also clearly visible *(Fig. 1-B1, dotted arrow)*. Until day 7 postpartum, the whole uterine horn was visible in cross-section. The image of the uterine wall structure blurred gradually from day 10 postpartum and the uterine residual fluid disappeared *(Fig. 1-A3, solid arrow)*. The uterine horn contracted gradually from day 14 to day 26 postpartum. In Groups A, B and C, uterine involution was completed on days 30, 35 and 40 postpartum, respectively. On day 45 postpartum, the uterine horns were similar in size and shape (oval or round) in all groups *(Fig. 1-A9,B9,C9)*.

Changes in Uterine Horn Diameter of Hu Sheep in the Postpartum Period

The diameter of the uterine horn decreased rapidly from day 0 to day 14 postpartum, and was followed by a slow recovery. On day 7 postpartum, the diameters of the uterine horn in Groups A, B and C were 52±0.75 mm, 58.9±2.39 mm and 63.3±13.2 mm, respectively. However, by day 14 postpartum, the diameters of the uterine horn were 28.7±3.13 mm, 31.2±2.36 mm and 42.5±3.12 mm, representing reductions of 73.86%, 72, 68%, and 67.19% in Groups A, B and C, respectively. The diameters of the uterine horn in Groups A, B and C were stable by days 30, 35 and 40 postpartum, respectively, with uterine horn diameters ≤130 mm ($P<0.05$) *(Fig. 2)*.

Ultrasonography of the Uterine Caruncle of Hu Sheep in the Postpartum Period

The uterine caruncle of sheep is attached to the endometrium, although the shape varies due to differences in the detection positions. The diameter of the uterine caruncle decreases with proximity to the fallopian tube and cervix. Therefore, in this study, the maximum uterine caruncle diameter was measured by B-mode ultrasound detection of a discoid shape at the uterine angular

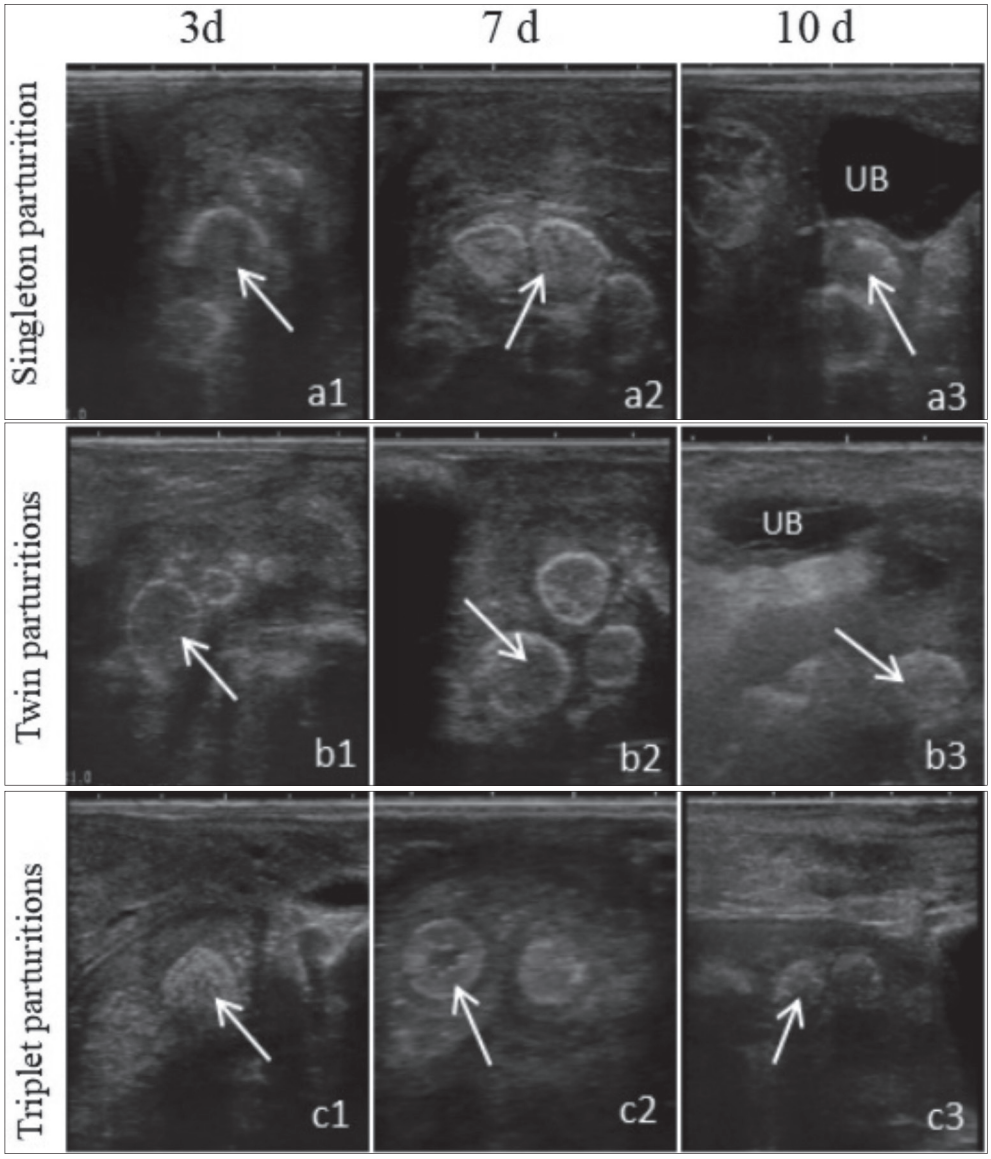

Fig 3. Ultrasonography of uterine caruncle in Hu sheep 3-10 days postpartum, detected with a 7.5-MHz transducer. UB, bladder; uterine caruncle *(solid arrow)*. The number of days postpartum are shown above the images

curvature [23,24]. The contour of the caruncle was clearly seen to be round or oval from days 3 to 10 postpartum and the diameter of the caruncle began to decrease with increasing time postpartum until it was essentially undetectable after day 10 *(Fig. 3)*.

Changes in the Maximum Uterine Caruncle Diameter of Hu Sheep in the Postpartum Period

On day 3 postpartum, the maximum diameters of the uterine caruncle of ewes in Groups A, B and C were 12.43±0.91mm, 12.14±1.19 mm and 12.5±0.66 mm, respectively. By day 7 postpartum, the maximum diameter of the uterine caruncles in Groups A, B and C were reduced to 10.86±0.86 mm, 10.0±0.12 mm and 9.97±1.48 mm, respectively, and 8.82±0.27 mm, 8.31±0.94 mm and 7.94±0.95 mm, respectively, by day 10 postpartum. After day 10, the uterine caruncles in all groups were almost undetectable *(Fig. 4)*.

Discussion

Ultrasonography is widely used in sheep production, not only for disease diagnosis, but also for determination of the fetus number in early pregnancy. B ultrasound has emerged as a non-invasive method for monitoring of uterine involution in sheep. Complete uterine involution is defined as a uterine horn ≤200 mm in transverse diameter and no fluid collection in the uterine cavity [25].

The time to complete uterine involution is affected by different factors. In our study, the process occurred most intensively during the first 14 days postpartum, corresponding to a total reduction of 50%. Thereafter, the

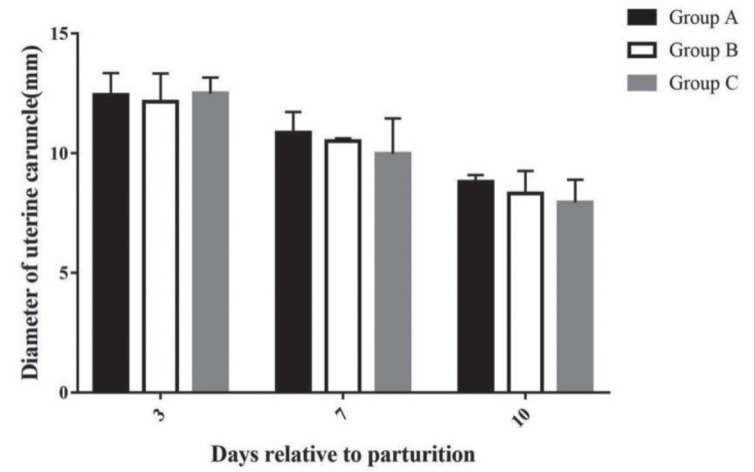

Fig 4. Changes in the maximum uterine caruncle diameter 3-10 days postpartum in Hu sheep

involution rate decreased and stabilization was reached by days 30, 35 and 40 for singleton, twin and triplet parturitions, respectively. Completion of uterine involution has been reported to occur by day 18 postpartum in sheep and by day 21 in goats [26]. Compared with the results of our study, this discrepancy in the time to complete uterine involution may be due to breed differences. Medan et al.[15] showed that uterine involution was completed on day 35 postpartum in ewes after lambing in January and February, and on day 28 postpartum after lambing in March. Hayder et al.[14] also found that the mean interval for complete uterine involution was shorter in ewes after lambing in February, compared to those lambing in June (29.4±1.2 days vs. 33.9±1.1 days). This was similar to the rate of uterine involution after singleton and twin parturitions observed in our study. Gomes et al.[27] found that uterine depth regression stabilized on average on days 35 and 49 postpartum for singleton and twin parturitions, respectively.

Necropsy samples showed revealed the appearance of edema in the degenerated uterine caruncle on day 14 postpartum. Following complete necrosis of the caruncle surface on day 16 postpartum, the surface regained cleanliness, luminescence, although some necrotic caruncle tissue remained attached to the uterine wall at day 21 [28]. Finally, epithelialization of the uterine caruncle completed the regeneration by 28 days postpartum [29]. Ahmed et al.[30] observed uterine caruncles within 10 days postpartum in ewes, which was consistent with the present study. The diameter of the uterine caruncle measured 2.02±0.16 cm on day 2 postpartum and regressed to 1.24±0.17 cm on day 8 [16]. Ioannidi et al.[13] reported that the caruncle was undetectable after day 20 postpartum.

The uterine caruncle and uterine fluid decreased gradually in the first week postpartum. Ababneh and Degefa [17] found the diameter of the uterine caruncle was 2.86±0.43 cm on day 2 postpartum and 1.33±0.22 cm on day 9. In the present study, the uterine caruncle tended to decrease significantly in size in all sheep during the first week postpartum. The diameter of uterine caruncle on day 10 postpartum was significantly smaller than that on day 3 postpartum (8.36±0.78 mm vs. 12.36±0.83 mm; $P<0.05$). Furthermore, there were no significant differences in the diameters of the uterine caruncle among the three groups at 3 days postpartum ($P>0.05$).

In this study, we observed a rapid reduction in the uterine horn diameter from days 7-22 postpartum, followed by a more gradual decreased from days 22-40 postpartum. The time to completion of uterine involution increased significantly postpartum with the number of parturitions ($P<0.05$). Uterine involution was completed by day 30 postpartum in ewes with singleton parturition, by day 35 postpartum with twin parturitions, and by day 40 postpartum with triplet parturitions. Thus, our findings provide evidence that different litter sizes have influence the rate of uterine involution and represent a reference for improving the reproductive performance of Hu sheep.

Availability of Data Materials

The datasets produced during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contribution

WB Zeng and YP Wang designed the study, conducted the experiments, analyzed the data, and drafted the manuscript. ZL Liu designed the study and drafted the manuscript.

WQ Zhang CH Zhu, X Chen and YK Zhao conducted parts of the experiments and collected samples.

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