Original Paper

Evaluation of pregnancy rate and length of pregnancy after intracervical insemination in zwartbles sheep

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The artificial insemination and the oestrus cycle synchronization are some of the most frequently used biotechnical methods of reproduction not only for sheep, but also for the other spieces of livestock. They are used mainly to achieve the shortening of the lambing period and to achieve easier breeding. An exact estimation of the lambing period is essential for the breeding process optimization, as it allows better preparation for the lambing period. This experiment monitors 48 Zwartbles sheep. Before the artificial insemination itself, the oestrus cycle of the sheep was synchronized using intravaginal sponges Ovigest and the subsequent intracervical insemination was performed using fresh, diluted and chilled semen extracted from rams of the Zbyšek and Zachari lineages. The pregnancy rates after insemination and after the subsequent breeding of the barren ewes in a harem were determined according to the pregnancy detection and the subsequent lambing times. Marking the exact date and time of insemination and lambing allowed to determine the overall pregnancy lengths in the inseminated animals. The overall pregnancy rate after insemination was 41.7% and the overall pregnancy rate after the subsequent breeding in a harem was 93.8%. The overall pregnancy period in animals after insemination varied between 141.6 and 147.8 days and the peak of lambing took place between 144.1 and 146.8 days.

Keywords: insemination, pregnancy, reproduction, synchronization, oestrus

1   Introduction

Similarly to other livestock, sheep breeding is usually performed through natural breeding or artificial insemination (Louda and Hegedüšová, 2009). A range of biotechnical methods are used, from ejaculate extraction and oestrus cycle synchronization, to different methods of synchronization (Louda and Hegedüšová, 2009; Malá et al., 2011). These biotechnical methods can assure decrease of the work load and time restraints of the breeding process, they can assure a better data recording and health monitoring of the animals. It can lead to shortening of the lambing period, which subsequently leads to better balance in the flock, as well as giving the possibility of situating the lambing into a suitable time period (Čunát et al., 2013; Sándor et al., 2011).

The natural mating is basic and the most common method used in the Czech Republic, which is used mainly in a smaller breeding programes (Malá et al., 2011). Contrarily, the artificial insemination leads to much lower pregnancy rates and it allows us to speed up the genetic gain. Similarly, it leads to better record keeping and health monitoring of the animals. Moreover, it allows for lower numbers of rams to be kept at the breeding programmes and it makes it easier to situate the lambing into a more suitable time period of the year (Kadlečík and Kasarda, 2006; Louda and Hegedüšová, 2009).

The methods used for oestrus cycle synchronization can be divided into the natural (controlled lighting regime, flushing, the ram effect) and the artificial (feed additives, intravaginal sponges, subcutaneous implants), and for better

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efficiency, these individual methods can be combined (for example the ram effect + flushing/intravaginal sponges); (Čunát et al., 2013; Horák et al., 2012; Louda and Ježková, 2002; Říha, 1999).

The artificial insemination is performed using fresh, chilled and diluted semen. Previously extracted doses stored in liquid nitrogen for a longer period of time are used only rarely, since this method of conservation leads to a rapid decrease of fertility of the insemination dose after its defrosting (Louda et al., 2001; Ntemka et al., 2018). A similar situation is reported with horses (Rečková and Filipčík, 2020).

Insemination methods are further divided according to the place of the semen insertion. Generally, the deeper into the sexual apparatus of the ewe it is inserted, the higher probability of pregnancy, but also the higher price and difficulty of the method. These methods are thus divided into intravaginal, intracervical, intrauterine, and laparoscopic (Čunát et al., 2013; Sándor et al., 2011).

The insemination doses are created from sperm extracted at the day of insemination into an artificial vagina after a jump on an ewe in heat. The extracted semen is then macroscopically and microscopically evaluated and its subsequent dilution is determined. For the dilution, commercial thinners, as well as milk-based thinners, can be used (Čunát et al., 2013). The milk used for dilution was pasteurized (min. 95 °C for 10 minutes) to inactivate lactein, an antibacterial agent that would act as toxic towards the semen in its active state (Salamon and Maxwell, 2000).

Due to the precise estimation of lambing times, and thanks to the estimated number of expected lambs, the care for the new-borns can be optimized and complications, which usually arise due to the bad lambing times management, can be eliminated.

Vaněk and Štolc (2002) state that the pregnancy periods vary. Kuchtík (2007) states the pregnancy period to be between 143 and 157 days and the peak of lambing takes place between the days 147 and 150. A similar time span (from 143 to 156 days) is presented by Gajdošík and Polách (1984). An average pregnancy period of 147 days is stated also by Tzanidakis et al. (2014) and Gootwine (2016).

The correct choice and application of biotechnical methods is crucial for optimization of reproduction outcomes. The aim of this research is to evaluate the pregnancy lengths of individual sheep.

2 Material and methods

This experiment focused on 48 Zwartbles breed sheep. All of the sheep were of age ranging between 2 and 8 years, with an average body condition score (BCS) of 3. The experiment were conducted from September 2019, when the synchronization of the oestrus cycle, insemination doses extraction and the insemination itself took place, to April 2020, when the lambing took place.

For the oestrus cycle synchronization, intravaginal sponges Ovigest (60 mg medroxyprogesterone acetate/sponge, LaboratoriosHipra, Spain) were used. These sponges were inserted inside the ewes' vaginas for fourteen days. After removal of the sponges, each ewe was injected with a lyophilized serum gonadotropin PMSG (0,2 ml/ewe, Sergon 200 IU, Biovetalvanovice, CZ). 56-60 hours after this injection, the ewes were inseminated.

Two rams, one from the Zbyšek (ZBS 0001 – 7 years) and one from the Zachari (ZAC0001 – 8 months) lineages were used for the ID (insemination dose) creation. The semen was extracted into an artificial vagina (Minitübe, Germany) at the day of insemination

The semen was then macroscopically and microscopically evaluated and the degree of dilution was determined due to the observed sperm concentration and motility. The diluted semen was then cooled down to 3°C in a cooling box, where it stayed until the insemination.

The insemination itself took place 56 to 60 hours after the PMSG application. The ewes were divided into two groups and the insemination took place on 11. 10. 2019, 13:00–14:30 and on 13. 10. 2019, 12:20–14:40.

Each ewe was fixed to a fixing pad and the oestrus symptoms (the colour and amount of mucus, blood perfusion, stiffness and openness of cervix, overall activity, etc.) were evaluated.

Before the insemination, the outer genitalia were cleaned and disinfected. The vagina and cervix were inspected using sheep vaginal speculum and the ID was then inserted through the speculum 1 to 2 cm deep into the cervix via a plastic applicator.

Three days after the insemination, the ewes were put into groups (harems) with the rams whose semen was used for the insemination. This was done to assure an increase in the pregnancy rate in the ewes in which the artificial insemination was not successful. The subsequent scanning showed in which ewes the artificial insemination was successful and which ewes became pregnant after the natural mating in a harem (found due to the size of the foetus). The overall number of ewes in which the artificial insemination was successful was estimated by subtracting the length of pregnancy period from the date of lambing.

The pregnancy scanning took place on the 43rd and 83rd day after insemination using OVI-SCAN (BCF technology, Scotland) and, along with the data on pregnant/barren ewes, the number of foetuses in the uterus was noted. Due to marking the exact day and time of insemination and lambing in individual ewes, the overall pregnancy durations were subsequently calculated.

STATISTICA 12.0 software and MS Excel 2016 were used for statistical assessment of the data.

3   Results and discussion

Figure 1 shows the overall pregnancy rates after the artificial insemination. For the ram of the Zbyšek lineage (ZBS0001), the pregnancy rate after insemination was 63.9% (14 pregnant ewes out of the 22 inseminated). This is considered above an average result, compared to the average 60% stated by Kuchtík et al. (2007). On the contrary, for the ram of the Zachari lineage (ZAC0001), the pregnancy rate was way below average with only 23.1% (6 pregnant ewes out of the 26 inseminated). The overall pregnancy rate after insemination was thus 41.7%.

After the artificial insemination, the barren sheep were let to breed naturally in the harems during the following oestrus cycle, which led to an increase in the pregnancy rate to 95.5% for the ZBS0001 ram and to 92.3% for the ZAC0001 ram. The overall pregnancy rate was thus 93.8% (45 pregnant ewes out of the 48 ewes monitored in the experiment). This outcome is slightly below average, since Louda and Hegedüšová (2009) state the average pregnancy rate in ewes after natural breeding to be 95%.

The lower pregnancy rate in the ZAC0001 ram was caused by a lower quality of the IDs, which could be caused by the lower age of the ram (8 months) or reproductive season time. The subsequent pregnancy rate after natural breeding was only slightly below average.
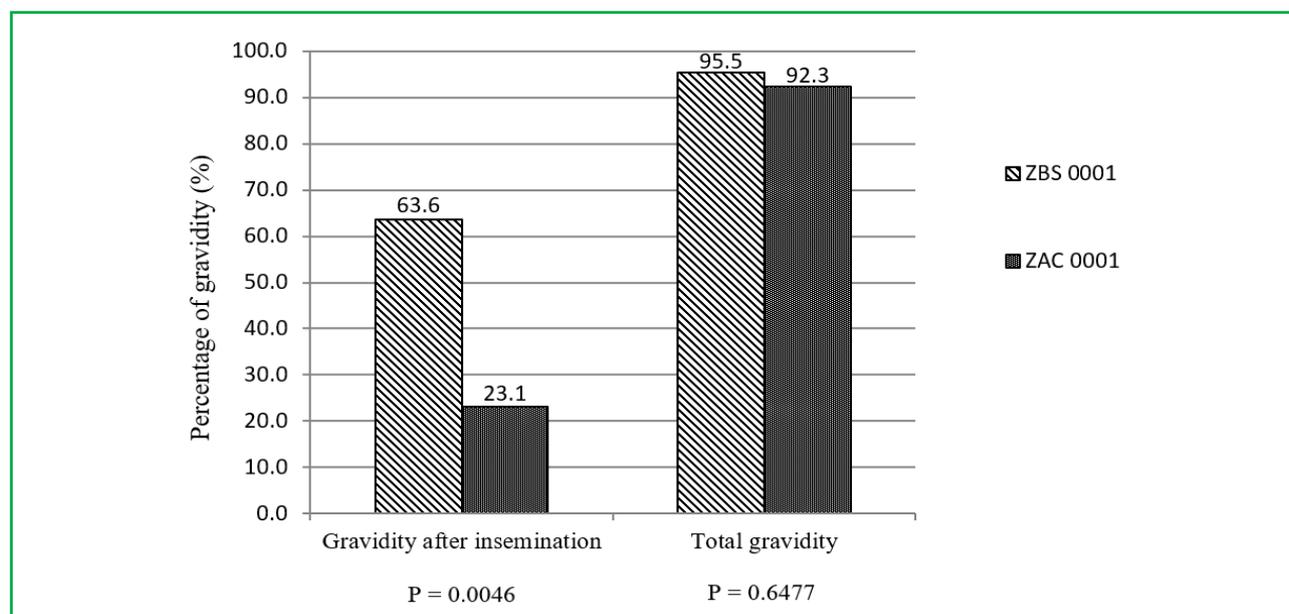
Figure 1   Comparison of the pregnancy rates after artificial insemination and the overall pregnancy rates in each ram
P <0.01 = high statistically significant differences, *P* >0.05 = non-significant differences

A highly statistically significant difference was noted between the ZBS0001 and ZAC0001 rams during the artificial insemination pregnancy rates. On the other hand, no statistically significant difference was noted within the overall pregnancy rates.

According to Vallejo et al. (2019) the pregnancy rate in ewes after oestrus cycle synchronization through hormonal prepapartions and a subsequent intracervical insemination was between 45% and 65%. He also states that after the subsequent ten-day harem breeding of the barren ewes the pregnancy rate increased above 80%.

Figure 2 and Table 1 show the lengths of pregnancy in individual sheep inseminated 11. 10. 2019 and 13. 10. 2019. During these phases a total of 48 ewes were inseminated. A total of 20 sheep got pregnant after the artificial insemination, and after the lambing itself the exact pregnancy lengths were calculated. These pregnancy lengths are stated in Figure 2.

The lambing itself took place in a span of 150 hours (6 days and 6 hours). The shortest pregnancy period (from insemination to lambing) was 141 days and 13.5 hours. The longest pregnancy period was 147 days and 19.5 hours. The main lambing period (see Figure 2 and Table 2) took place between the days 144.1 and 146.8, during which 70% of the animals went into lambing.

Table 1   Length of pregnancy in individual sheep (2019-2020)

Length of pregnancy (hours)										Average	SD	CV
3,397.5	3,441.5	3,447.7	3,457.3	3,469.2	3,474.1	3,477.6	3,480.2	3,488.5	3,490.0	3,488.32	34.7065	
3,491.1	3,496.0	3,497.5	3,498.3	3,505.5	3,515.0	3,522.7	3,530.0	3,539.4	**3,547.5**			
Length of pregnancy (days)												0.9949
141.56	143.40	143.65	144.05	144.55	144.75	144.90	145.01	145.35	145.42	145.34	1.4460	
145.46	145.67	145.73	145.76	146.06	146.46	146.78	147.08	147.48	147.81			

SD – standard deviation, CV – coefficient of variation, minimum and maximum in bold

The pregnancy lengths fluctuated between 141 and 147 days. However, only 20% of the sheep went into lambing during the threshold days. As opposed to the pregnancy lengths mentioned in the introduction (143 to 157 days), the results are way lower, and the average pregnancy length (Table 1) was 145.3 days, which is almost two days less than stated by Ingoldby and Jackson (2016).
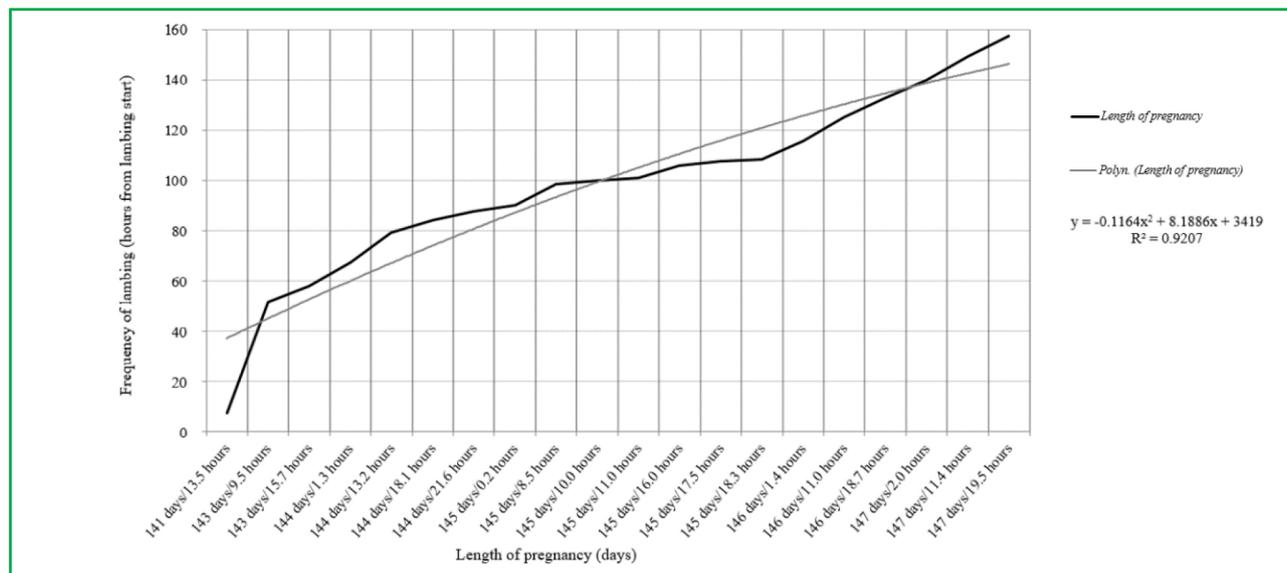
Figure 2   Length of pregnancy in sheep lambing in 2020

The pregnancy length can be influenced by a number of factors, such as the breed, the feed composition, microclimatic and macroclimatic conditions, sex of the lamb, number of foetuses or the lambs' weight (Iyiola-Tunji et al., 2010; Mavrogenis, 1992). For this reason, to gain more exact data, it is necessary to replicate this research and carry it out again on a larger number of animals,to evaluate the influence of these factors on the pregnancy length itself.

4   Conclusions

The pregnancy length in sheep that got pregnant during the artificial insemination was between 141.6 and 147.8 days. Seventy percent of the sheep went into lambing between 144.1 and 146.8 days, while the average pregnancy length was around 145 days.

As opposed to the average pregnancy lengths stated for sheep of any breed, the data collected in this research were lower.

The overall pregnancy rate after artificial insemination was 41.7%, after the subsequent natural mating in harems the pregnancy rate increased to 93.8%.

The results collected from this research will be used for replication of the experiment in a larger scale with a larger number of animals. The aim of this subsequent research will be optimisation of the insemination process and of the lambing preparations.

References

Čunát, L. et al. (2013). *Využití inseminace ovcí v chovatelské praxi*. Praha: Česká zemědělská univerzita.

Gajdošík, M. and Polách, A. (1984). *Chov oviec*. Bratislava: Príroda.

Gootwine, W. (2016). Sheep: Reproductive Management. *Husbandry of Dairy Animals*, 1(1), 887–892. https://doi.org/10.1016/B978-0-08-100596-5.21239-0

Horák, F. et al. (2012). *Chováme ovce*. Praha: Brázda.

Ingoldby, L. and Jackson, P. (2001). Induction od parturition in sheep. *Practice*, 23(4), 228–231. http://doi.org/10.1136/inpract.23.4.228

Iyiola-Tunji, T. et al. (2010). Relationship Between Gestation Length and Birth Weight in Nigerian Sheep and Their Crosses. *Animal Production*, 12(3), 135–138. http://doi.org/10.20884/1.anprod.2010.12.3.282

Kadlečík, O. and Kasarda, R. (2006). *Všeobecná zootechnika*. Nitra: Slovenská poľnohospodárska univerzita v Nitre.

Kuchtík, J. et al. (2007). *Chov ovcí*. Brno: Mendelova univerzita v Brně.

Louda, F. et al. (2001). *Inseminace hospodářských zvířat*. Praha: Česká zemědělská univerzita v Praze.

Louda, F. and Ježková, A. (2002). Biotechnické metody v reprodukci ovcí a koz. *Náš chov*. Retrieved August 17, 2020 from https://naschov.cz/biotechnicke-metody-v-reprodukci-ovci-a-koz/

Louda, F. and Hegedüšová, Z. (2009). *Inseminace ovcí – intenzifikační faktor šlechtitelské práce*. Certifikovaná metodika. Rapotín: Agrovýzkum Rapotín.

Malá, G. et al. (2011). *Chov dojných ovcí*. Zásady správné chovatelské praxe, Praha: Výzkumný ústav živočišné výroby.

Mavrogenis, A. P. (1992). Breed group and parity effects on gestation duration and litter size at birth of sheep. *Technical Bulletin Cyprus Agricurtural Research Institut*, 42(1992), 3–6.

Ntemka, A. et al. (2018). Current status and advances in ram semen cryopreservation. *Journal of the Hellenic Veterinary Medical Society*, 69(2), 911–924. https://doi.org/10.12681/jhvms.18014

Rečková, Z. and Filipčík, R. (2020). An analysis of Selected Aspects of Sperm Quality in Fresh and Cooled-Storage Stallion Semen. *Iranian Journal of Applied Animal Science*, 10(3), 405–408. Retrieved October 23, 2020 from http://ijas.iaurasht.ac.ir/article_675315_c1a9e1ab03a577c5ca5580877afaaa06.pdf

Říha, J. (1999). *Biotechnologie v chovu a šlechtění hospodářských zvířat*. Rapotín: Asociace chovatelů masných plemen.

Sándor, K. et al. (2011). Artificial Insemination of Sheep – Possibilities, Realities and Technique at the Farm Level In Artificial Insemination in Farm Animals, Iran: IntechOpen, pp. 27–50.

Salamon. S. and Maxwell, W. M. C. (2000). Storage of ram semen. *Animal Reproduction Science*, 62(1–3), 77–111. https://doi.org/10.1016/S0378-4320(00)00155-X

Tzanidakis, N. et al. (2014). Dairy sheep breeding. *LowInputBreeds technical note*, 1(1), 1–6.

Vallejo, D. A. et al. (2019). Pregnancy rates in hair sheep after Ovsynch synchronization and a combined intracervical fixed-time artificial insemination and 10-day mating period. *Veterinary World*, 12(11), 1779–1783. www.doi.org/10.14202/vetworld.2019.1779-1783

Vaněk, D. and Štolc, L. (2002). *Chov skotu a ovcí:* (přednášky pro Bc). Praha: Česká zemědělská univerzita.