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Wykorzystanie ultrasonografii w ocenie przebiegu ciąży i diagnostyce prenatalnej u owiec

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SPIS TREŚCI

1. WYKAZ PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

Rozprawa doktorska pod tytułem „Wykorzystanie ultrasonografii w ocenie przebiegu ciąży i diagnostyce prenatalnej u owiec" została udokumentowana publikacjami powiązanymi tematycznie:

1. Brzozowska A., Wojtasiak N., Błaszczyk B., Stankiewicz T., Wieczorek-Dąbrowska M., Udała J. 2020: The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs. Large Animal Review, 26, (3), 119-126, IF: 0,417 (Załącznik nr 1).
2. Brzozowska A., Stankiewicz T., Błaszczyk B., Chundekkad P., Udała J., Wojtasiak N. 2022: Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep. BMC Veterinary Research, 18, 326, IF: 2,792 (Załącznik nr 2).

2. STRESZCZENIE

Celem naukowym rozprawy doktorskiej było zbadanie parametrów morfometrycznych łożyska i ocena przepływów krwi w krążeniu łożyskowo-pępowinowym oraz określenie możliwości wczesnej diagnozy ciąży u owiec z wykorzystaniem techniki ultrasonograficznej.

Badania wykonano na owcach pomorskich i rasy Suffolk utrzymywanych na fermie owiec w Zakładzie Doświadczalnym Instytutu Zootechniki Państwowego Instytutu Badawczego w Kołbaczu. Owce były kryte w czasie ich naturalnego sezonu rozrodczego, nie stosowano metod synchronizacji rui. Okres trwania ciąży określono na podstawie dnia pokrycia i potwierdzano retrospektywnie po porodzie.

W badaniach przeprowadzono analizę morfometryczną łożysk pozyskanych po porodzie uwzględniając wiek maciorek, wielkość miotu, płeć i masy urodzeniowej jagniąt. Wykorzystując ultrasonografię dopplerowską przeprowadzono ocenę hemodynamiki w naczyniach łożyskowo-pępowinowych u ciężarnych owiec. Określono też przydatność różnych parametrów ultrasonograficznych we wczesnej diagnozie ciąży u owiec.

Uzyskane wyniki wykazały, że parametry morfometryczne owczych łożysk zależą od wieku maciorek, wielkości miotu, płci i masy urodzeniowej jagniąt. Dlatego też parametry te powinny być uwzględniane w ultrasonograficznej ocenie rozwoju łożyska u owiec.

Przeprowadzone badania wskazują na możliwość wykorzystania ultrasonografii dopplerowskiej w monitorowaniu hemodynamiki naczyń łożyskowo-pępowinowych podczas ciąży u owiec. Wykazano, że zarówno lokalizacja naczynia tętniczego w krążeniu łożyskowo-pępowinowym, jak i wiek ciążowy mają istotny wpływ na parametry hemodynamiczne tych naczyń. Uzyskane wyniki dostarczają też nowych informacji dotyczących przepływów krwi w naczyniach zlokalizowanych w łożyszczach, co przyczyni się do pełniejszego poznania zmian hemodynamicznych w łożysku owczym. Uzyskane wyniki potwierdzają przydatność transrektalnej ultrasonografii we wczesnej diagnozie ciąży u owiec.

Wskazują też, że identyfikacja i obrazowanie ciałka żółtego za pomocą ultrasonografii B-Mode może być bardzo wczesną i prostą metodą potwierdzającą skuteczne krycie u owiec.

3. ABSTRACT

The scientific objective of the doctoral dissertation was to analyse the morphometric parameters of the placenta and to assess blood flow in the placental and umbilical cord circulation as well as to determine the possibility of early pregnancy diagnosis in sheep using the ultrasound technique.

The research was carried out on Pomeranian and Suffolk sheep kept on a sheep farm at the Experimental Department of the National Research Institute of Animal Production in Kołbacz. Sheep were mated during their natural breeding season, no oestrus synchronization methods were used. The duration of gestation was determined based on the day of mating and was confirmed retrospectively after parturition.

In the research, a morphometric analysis of placentas obtained after parturition was carried out, taking into account the age of the ewes, litter size, sex and birth weight of lambs. Using Doppler ultrasonography, an assessment of haemodynamics in the placental and umbilical vessels in pregnant sheep was carried out. The usefulness of various ultrasound parameters in the early diagnosis of pregnancy in sheep was also determined.

The obtained results showed that the morphometric parameters of sheep placentas depend on the age of ewes, litter size, sex and birth weight of lambs. Therefore, these parameters should be taken into account in the ultrasonographic assessment of placental development in sheep.

4. CEL NAUKOWY

Celem naukowym rozprawy doktorskiej było badanie parametrów morfometrycznych łożyska i ocena przepływów krwi w krążeniu łożyskowo-pępowinowym oraz określenie możliwości wczesnej diagnozy ciąży u owiec z wykorzystaniem techniki ultrasonograficznej.

Dla realizacji nadrzędnego celu naukowego wyznaczono następujące cele szczegółowe:

1. Określenie parametrów morfometrycznych łożysk owczych w zależności od wieku maciorek, wielkości miotu, płci i masy urodzeniowej jagniąt.
2. Określenie i porównanie parametrów dopplerowskich w naczyniach tętniczych i żylnych zlokalizowanych w części matczynej i płodowej łożyszczy oraz w naczyniach pępowinowych podczas ciąży u owiec.
3. Określenie przydatności różnych parametrów ultrasonograficznych we wczesnej diagnozie ciąży u owiec.

5. WPROWADZENIE I UZASADNIENIE PODJĘCIA BADAŃ

Obecnie ultrasonografia w czasie rzeczywistym jest jedną z ważniejszych technik umożliwiającą wykrywanie i monitorowanie ciąży u owiec. Pozwala ona na wczesną diagnozę ciąży, a w późniejszych jej etapach na monitorowanie rozwoju płodu i szacowanie wieku ciążowego (Karen i in. 2009, Eerdoğan i.in. 2016).

Ważnym parametrem w ultrasonograficznej diagnozie ciąży u owiec jest obecność łożyszczy, które już w pierwszym trymestrze ciąży widoczne są jako echogeniczne struktury w kształcie miseczek (Kaşikçi i in. 2011, Stankiewicz i in. 2020). W miarę rozwoju ciąży łożyszcza powiększają swoje rozmiary, stąd badania nad możliwością wykorzystania pomiarów łożyszczy w określeniu wieku ciążowego (Metodiev i.in. 2014, Lawrence i in. 2016, Stankiewicz i in. 2020). Należy jednak uwzględnić, że największy wzrost średnicy łożyszczy obserwuje się do 90. dnia ciąży (Stankiewicz i in. 2020). Po tym czasie wielkość łożyszczy utrzymuje się na zbliżonym poziomie, natomiast wzrasta ich ukrwienie. Ponadto wielkość i liczba łożyszczy może zależeć nie tylko od wieku ciążowego, ale od wielu innych czynników, np. od wieku maciorek, wielkości miotu, płci i masy urodzeniowej jagniąt.

Duży wgląd w poznanie wpływu tych czynników mają badania przeprowadzone na łożyskach i błonach płodowych pozyskanych po porodzie. Wyniki tych badań umożliwiły bardziej precyzyjną ocenę ultrasonograficzną łożysk podczas ciąży. Ponadto analiza preparatów morfometrycznych owczych błon płodowych i łożysk pozyskanych po porodzie uwidacznia układ naczyń pępowinowo-łożyskowych. Jest to bardzo pomocne w ultrasonograficznej ocenie przepływu krwi w tych naczyniach podczas ciąży.

Wykształcenie funkcjonalnego krążenia maciczno-łożyskowego i płodowo-łożyskowego jest jednym z najwcześniejszych procesów, które decydują o dalszych losach ciąży. U owiec angiogeneza łożyskowa rozpoczyna się już w 18 dniu (Reynolds i Redmer 1995, Bairagi i in. 2016, Mourier i in. 2017). Rozwój łożyska trwa przez cały okres ciąży i jest ściśle związany z rozwojem naczyń krwionośnych w łożysku, ponieważ zwiększenie przepływu krwi jest niezbędne dla zaspokojenia potrzeb płodu i prawidłowej wymiany składników między organizmem matki i płodu (Stankiewicz i in. 2020).

U owiec łożysko jest typu liścieniowatego, składającego się z kilkudziesięciu łożyszczy. Każde łożyszcze jest funkcjonalną jednostką zbudowaną z części matczynej powstałej z endometrium pokrywającym brodawki maciczne i części płodowej, powstałej z połączenia beznaczyniowej kosmówki i unaczynionej omoczni (Reynolds i in. 2005, Sammin i in. 2009). Są one miejscem matczyno-płodowej wymiany składników pokarmowych, gazów oddechowych i produktów przemiany materii (Borowicz i in. 2006, Vonnahme i in. 2008, Sammin i in. 2009).

Badania architektury łożyszczy u owiec wykazały, że unaczynienie części matczynej tworzą rozgałęzienia macicznych tętnic promieniowych, które wnikają do brodawki macicznej od jej podstawy, a następnie rozciągają się wzdłuż jej wypukłej powierzchni. Naczynia te penetrują łożyszcze tworząc liczne rozgałęzienia i rozległą sieć naczyń włosowatych. Żyły biegną w podobny sposób, ale w przeciwnym kierunku niż tętnice (Hafez i in. 2010). Natomiast w części płodowej naczynia wchodzą i opuszczają liścień w strefie wnęki łożyszcza (Sammin i in. 2009, Hafez i in. 2010). Zwykle są to pojedyncze naczynia tętnicze, które w obszarze wnęki ulegają rozwidleniu na naczynia boczne, a następnie ulegają dalszym rozgałęzieniom tworząc sieć naczyń włosowatych (Hafez i in. 2010). Z kolei liczba naczyń żylnych odchodzących z liścieni jest zwykle większa, ponieważ istnieją anastomozy między naczyniami krwionośnymi z sąsiednich łożyszczy.

Jest coraz więcej dowodów, że zaburzenia w krążeniu łożyskowo-płodowym powodują nieprawidłowości w rozwoju płodu, wpływają negatywnie na wzrost płodu i prowadzą do niskiej masy urodzeniowej (Wu i in. 2006, Reynolds i in. 2013). Zwiększa to ryzyko zachorowalności i śmiertelności we wczesnych stadiach życia postnatalnego (Greenwood i in. 1998, Sawalha i in. 2007). Dlatego tak ważne są badania monitorujące przepływ krwi w krążeniu łożyskowo-płodowym.

Obecnie coraz częściej stosowaną techniką w tych badaniach jest ultrasonografia dopplerowska, która jest skutecznym, nieinwazyjnym narzędziem dającym informacje o charakterystyce unaczynienia i przepływach krwi. Przeprowadzone dotychcazas badania u owiec dotyczyły głównie przepływów w tętnicach pępowinowych, macicznych i płodowych (Elmetwally i Bollwein 2016, Santos i in. 2021). Niewiele jest jednak danych na temat wskaźników przepływów krwi w naczyniach krwionośnych tworzących bezpośrednie unaczynienie łożyszczy.

Ważne są też badania nad oceną wskaźników przepływu w naczyniach żylnych, tym bardziej, że analiza widma dopplerowskiego w żyle pępowinowej może być użytecznym wskaźnikiem w ocenie prawidłowego rozwoju płodu (Acharya i in. 2004). Badania te mają duże znaczenie poznawcze na temat życia wewnątrzmacicznego i mogą być użytecznym wskaźnikiem w ocenie prawidłowego rozwoju płodu.

Znaczenie ultrasonografii to również możliwość wczesnej diagnozy ciąży, co ma duże znaczenie w zarządzaniu hodowlą stada. Dlatego tak ważne są badania nad oceną ultrasonograficznych parametrów pozwalających wykryć ciążę i określić liczbę zarodków w możliwie jak najszybszym okresie.

6. METODYKA BADAŃ, UZYSKANE WYNIKI I ICH OMÓWIENIE

Badania wykonano na owcach pomorskich i rasy Suffolk utrzymywanych na ekologicznej fermie owiec w Zakładzie Doświadczalnym Instytutu Zootechniki Państwowego Instytutu Badawczego w Kołbaczu (Kołbacz, Polska: szerokość geograficzna 53'30"N). Owce utrzymywane były w systemie pastwiskowo-alkierzowym. Żywienie odbywało się zgodnie z normami przyjętymi dla tego gatunku, w oparciu o zielonkę pastwiskową i inne pasze objętościowe oraz treściwe, w zależności od pory roku. Zwierzęta miały stały dostęp do wody i lizawek solnych. Owce były kryte w czasie ich naturalnego sezonu rozrodczego (wrzesień) w sposób naturalny (krycie z ręki), nie stosowano metod synchronizacji rui. Okres trwania ciąży określono na podstawie dnia pokrycia i potwierdzano retrospektywnie przyjmując, że ciąża trwała 148 dni (Waziri i in. 2017, Stankiewicz i in. 2020).

6.1. Realizacja celu pierwszego (cel szczegółowy I)

Dla realizacji tego celu badania przeprowadzono na owcach pomorskich w różnym wieku (pierwiastki i wieloródki), od których pozyskiwano łożyska bezpośrednio po porodach. W badaniach przeanalizowano 99 łożysk z ciąż pojedynczych i 29 łożysk z ciąż mnogich (monochorialnych i dichorialnych). Po porodzie określono płeć i masę urodzeniową jagniąt. W przeprowadzonej analizie morfometrycznej uwzględniono masę, długość i szerokość łożyska, określono liczbę, masę i wielkość liścieni, a także średnicę pępowiny.

Analiza wyników wykazała istotne różnice w wielkości łożyska i liścieni w zależności od wieku maciorek. Całkowita masa łożyska i średnia średnica liścieni była istotnie większa u wieloródek niż u pierwiastek. Z kolei w łożyskach pozyskanych od owiec młodszych stwierdzono istotnie większą liczbę liścieni niż w łożyskach pozyskanych od wieloródek. Wyniki te wskazują, że wielkość i liczba liścieni zależy od wieku maciorek, co powinno być uwzględniane w ultrasonograficznej ocenie łożyszczy podczas ciąży.

W przeprowadzonej pracy wykazano też dodatnie korelacje między masą urodzeniową jagniąt a badanymi parametrami morfometrycznymi łożyska. Ponadto stwierdzono, że parametry morfometryczne łożyska zależą od płci płodu i wielkości miotu. Wykazano, że łożyska z ciąż z płodami męskimi były większe. Również masa liścieni i średnica liścieni w tych łożyskach była większa niż w łożyskach z płodami żeńskimi.

Masa i długość łożyska, liczba liścieni były istotnie większe w łożyskach z ciąż pojedynczych niż bliźniaczych. Natomiast średnica liścieni była istotnie większa w łożyskach z ciąż bliźniaczych. Nie stwierdzono natomiast zależnych od wieku maciorek, płci jagniąt i wielkości miotu istotnych różnic w średnicy pępowiny.

Przeprowadzone badania dostarczają też dokumentację fotograficzną obrazującą charakterystykę unaczynienia łożyska owczego, co może być pomocne w ultrasonograficznej ocenie przepływów krwi w naczyniach pępowinowo-łożyskowych.

Wyniki tej pracy opublikowano w artykule pt. „The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs".

6.2. Realizacja celu drugiego (cel szczegółowy II)

Dla realizacji tego celu badania przeprowadzono na 16 owcach rasy Suffolk. Aby wyeliminować wpływu wielkości miotu na badane parametry w badaniu analizowano tylko ciąże pojedyncze (uwzględniając wyniki uzyskane podczas realizacji celu pierwszego), które diagnozowano podczas ultrasonograficznego badania transrektalnego i potwierdzano podczas porodu. Ujednolicono też wiek maciorek - badania przeprowadzono u wieloródek w wieku od 3 do 4 lat.

Dla wykonania pomiarów przepływu krwi w tętnicach pępowinowych i łożyszczach badanie rozpoczęto od 35 dnia i kontynuowano przez cały okres ciąży w odstępach kilkudniowych. Pomiary przepływu krwi w żyłach przeprowadzono w okresie od 70 do 90 dnia ciąży.

Badanie ultrasonograficzne wykonywano za pomocą aparatu ultrasonograficznego (EDAN U50) wyposażonego w głowicę linearną o częstotliwości do 4 MHz (Model, V742UP) i sektorową o częstotliwości do 5 MHz (Model, C352UB). W początkowym okresie ciąży badanie ultrasonograficzne wykonywano transrektalnie, a w kolejnych okresach ciąży owce badano przezbrzusznie. Badanie ultrasonograficzne wykonano u ciężarnych owiec, które nie były wcześniej poddane działaniu środków uspokajających.

Każdorazowo u każdej owcy badano wolną część pępowiny w pobliżu wstawki brzusznej oraz 5 losowo wybranych łożyszczy, których skanowanie przeprowadzono przy użyciu ultrasonografii w trybie B. W celu identyfikacji naczyń tętniczych i żylnych w pępowinie, części płodowej i matczynej łożyszczy wykorzystano kolorowego Dopplera.

Po zlokalizowaniu naczyń krwionośnych dokonano pomiarów przepływu krwi w tętnicach i żyłach wykorzystując pulsacyjną ultrasonografię dopplerowską. Określono następujące parametry dopplerowskie: szczytową prędkość skurczową (PSV), końcową prędkość rozkurczową (EDV), stosunek PSV/EDV, indeks oporności (RI) i indeks pulsacji (PI).

Przeprowadzone badania wykazały, że większość parametrów dopplerowskich w tętnicach pępowinowych, liścieniowych i części matczynej łożyszczy była istotnie skorelowana z dniem ciąży. Zarówno w tętnicach pępowinowych, jak i w łożyszczach prędkość przepływu krwi wzrastała, a indeksy oporności i pulsacji malały wraz z zawansowaniem ciąży.

W pracy zanotowano też istotne różnice w wielkości badanych wskaźników dopplerowskich między tętnicami zlokalizowanymi w części matczynej i płodowej łożyszczy oraz tętnicami pępowinowymi.

Wartość PSV w tętnicach pępowinowych była istotnie wyższa niż w tętnicach zlokalizowanych w łożyszczach. W tętnicach pępowinowych i liścieniowych końcowa prędkość rozkurczowa w początkowym okresie ciąży była niewykrywalna, natomiast od 55 dnia do końca ciąży zanotowano stopniowy i postępujący wzrost tego parametru. Uważa się, że pojawienie się końcowej prędkości rozkurczowej związane jest z regularnością cykli i zmniejszeniem częstości akcji serca płodu (Elmetwally 2012). Z kolei w tętnicach części matczynej wartość EDV była istotnie wyższa niż w liścieniach i pępowinie.

Indeks oporności i wskaźnik pulsacji w początkowym okresie ciąży były istotnie wyższe niż w późniejszych okresach ciąży. W tętnicach części matczynej łożyszczy indeksy te były istotnie niższe niż w liścieniach i pępowinie. Można sądzić, iż jest to związane z ochroną łożyszczy (Saghian i in. 2019) wskazują bowiem, że zbyt duża prędkość i ciśnienie krwi może uszkodzić delikatne kosmki łożyska, zwłaszcza w początkowym okresie ciąży. Ponadto zbyt wysoki opór naczyniowy w łożysku może obniżać wymianę składników gazowych i odżywczych, co może być przyczyną niskiej masy urodzeniowej noworodków i śmiertelności okołoporodowej (Reynolds i in. 2006; Saw i in. 2018).

W żyłach łożyskowych wykazano niemal stałą prędkość przepływu. Natomiast w żyle pępowinowej początkowa i końcowa prędkość były wyższe niż w żyłach łożyskowych. Być może te wyższe wartości w żyłach pępowinowych związane są z ich lokalizacją w pobliżu płodu. Przepływ żylny jest bowiem bardziej podatny na zakłócenia związane z ruchem płodu niż przepływ tętniczy (Kiserud 2003).

Przeprowadzone badania wskazują na możliwość wykorzystania ultrasonografii dopplerowskiej w monitorowaniu hemodynamiki naczyń łożyskowo-pępowinowych podczas ciąży u owiec.

Wyniki tych badań zostały opublikowane w artykule pt. „Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep".

6.3. Realizacja celu trzeciego (cel szczegółowy III)

Dla realizacji tego celu przeprowadzono wczesną diagnozę ciąży u owiec rasy Suffolk z wykorzystaniem ultrasonografii transrektalnej (USG EDAN U50, głowica linearna o częstotliwości 4 MHz). W badaniu uwzględniano następujące parametry: wielkość i echogeniczność przekroju macicy, obecność ciałek żółtych na jajnikach oraz obecność zarodka i pęcherzyka zarodkowego w macicy. Notowano też czas, w którym po raz pierwszy widoczne były łożyszcza i pępowina. Parametry te oceniano od 17 dnia po pokryciu w odstępach kilkudniowych.

Opierając się na wyglądzie macicy, obecności zarodka i pęcherzyka zarodkowego u badanych owiec ciąża została zdiagnozowana między 20 a 28 dniem po pokryciu (22.21±2.35). W obrazie ultrasonograficznym macica w przekroju poprzecznym miała wygląd owalnych, dobrze widocznych hipoechogennych struktur.

W tym okresie widoczne były już zarodki, a od 25 dnia pęcherzyki zarodkowe. Obrazy powiększonych, wypełnionych płynem przekrojów macicy uważane są za jedne z wcześniejszych oznak ciąży (Braganca i in. 2016, Rickard i in. 2017). Natomiast bezpośrednim wskaźnikiem ciąży jest obecność zarodka i pęcherzyka zarodkowego.

W przeprowadzonej pracy stwierdzono, że pomocnym parametrem w diagnozie wczesnej ciąży może być obecność ciałek żółtych na jajnikach. W ocenie ultrasonograficznej jajników wykonywanej od 17. dnia stwierdzono obecność ciałek żółtych, które utrzymywały się przez kolejne tygodnie ciąży. Ciałka żółte widoczne były jako szare echogenne owalne struktury bez centralnej jamki. Do 56 dnia średnica ciałek żółtych była zbliżona, a w kolejnych dniach uległa istotnemu zmniejszeniu. Dlatego zidentyfikowanie ciałka/ciałek żółtych za pomocą ultrasonografii B-Mode może być bardzo wczesną i prostą metodą potwierdzającą skuteczne krycie u owiec.

Kolejnym wskaźnikiem ciąży była obecność łożyszczy. W przeprowadzonej pracy pierwsze łożyszcza zaobserwowano na przełomie pierwszego i drugiego miesiąca ciąży. Były one widoczne na powierzchni endometrium jako obszary o zwiększonej echogeniczności w stosunku do hipoechogennego światła macicy. Średnica pierwszych uwidocznionych łożyszczy wynosiła 7.73±0.69 mm. Pępowina po raz pierwszy uwidoczniona była około 33 dnia ciąży.

Wyniki tej pracy opublikowano w artykule pt. „Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep".

Ponadto stwierdzenie obecności łożyszczy w ultrasonograficznym badaniu transrektalnym zostało również wykorzystane podczas realizacji pierwszego celu pracy, której wyniki zostały opublikowane w artykule pt. „The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs".

7. PODSUMOWANIE I WNIOSKI

1. Uzyskane wyniki wykazały, że parametry morfometryczne owczych łożysk zależą od wieku maciorek, wielkości miotu, płci i masy urodzeniowej jagniąt. Dlatego też parametry te powinny być uwzględniane w ultrasonograficznej ocenie rozwoju łożyska u owiec.
2. Przeprowadzone badania wskazują na możliwość wykorzystania ultrasonografii dopplerowskiej w monitorowaniu hemodynamiki naczyń łożyskowo-pępowinowych podczas ciąży u owiec.
3. Uzyskane wyniki wskazują, że zarówno lokalizacja naczynia tętniczego w krążeniu łożyskowo-pępowinowym, jak i wiek ciążowy ma istotny wpływ na parametry hemodynamiczne tych naczyń. Wyniki te umożliwią pełniejsze poznanie zmian hemodynamicznych w łożysku owczym.
4. Przeprowadzone badania dotyczące parametrów hemodynamicznych w żylnych naczyniach pępowinowych i łożyskowych są badaniami wstępnymi u owiec, ale mogą stanowić inspirację do dalszych badań w tym zakresie.
5. Uzyskane wyniki potwierdzają przydatność transrektalnej ultrasonografii we wczesnej diagnozie ciąży u owiec. Wskazują też, że identyfikacja i obrazowanie ciałka żółtego za pomocą ultrasonografii B-Mode może być bardzo wczesną i prostą metodą potwierdzającą skuteczne krycie u owiec.

8. PIŚMIENNICTWO

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9. WYKAZ ZAŁĄCZNIKÓW

Załącznik 1: publikacja

Brzozowska A., Wojtasiak N., Błaszczyk B., Stankiewicz T., Wieczorek-Dąbrowska M., Udała J. 2020: The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs. Large Animal Review, 26, (3), 119-126.

Załącznik 2: publikacja

Brzozowska A., Stankiewicz T., Błaszczyk B., Chundekkad P., Udała J., Wojtasiak N. 2022: Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep. BMC Veterinary Research, 18, 326.

Załącznik 3: Oświadczenia współautorów o procentowym udziale w przygotowaniu publikacji.

Załącznik 1.

The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs

The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs

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SUMMARY

The objective of this study was to determine morphometric parameters of sheep placenta and the birth weight of lambs and their relationship with the type of pregnancy, litter sex and age of ewes. Placenta was obtained directly after delivery from Pomeranian sheep (n=128), including single (n=99) and multiple (n=29) pregnancies. For twin pregnancies, monochorionic and dichorionic placentas were taken into account. The following were determined: lamb birth weight (BWL), placental weight (PW), placental length (PL), placental width (WP), cotyledons number (CN), cotyledons weight (CW), mean diameter of the cotyledon (MDC), umbilical cord diameter (DUC). One-way ANOVA variance analysis was used for statistical comparisons and the Pearson and/or Spearman's correlation coefficient for correlation analysis. The primiparous were shown to have significantly lower PW and MDC ($p<0.05$) compared to older sheep. On the other hand, in the case of PL ($p<0.05$) and CN ($p<0.01$), which were significantly higher in the primiparous compared to multiparous. A significantly higher BWL was found in the male lambs than in the female lambs ($p<0.05$). Significantly higher PW, MDC ($p<0.01$) and CW ($p<0.05$) were found in the placenta from which male lambs were born. There was also a significantly higher BWL in male lambs ($p<0.05$) born from dichorial twin pregnancy. CW was significantly higher in the dichorial placenta from which the male lambs were born ($p<0.05$). Comparing the placenta from single and twin dichorial pregnancies, it was shown that type of pregnancy had a significant impact on the development of some placental indices and the birth weight of lambs. Significantly heavier lambs were born from single pregnancy ($p<0.01$). Also PW and PL ($p<0.01$) and CN ($p<0.01$) were significantly higher in single pregnancy placentas. In turn, MDC ($p<0.05$) was significantly higher in twin placental dichorial pregnancies. Monochorial placenta were characterized by significantly larger PW and CW ($p<0.05$) from which the male lambs were born. The obtained results showed that morphometric parameters of sheep placenta and birth weight of lambs depend on the type of pregnancy, litter sex and age of ewes. These results may be helpful in assessing postpartum placenta in this animal species. In addition, recorded differences in placenta parameters and birth weight of lambs may be useful in ultrasound assessment of placental and fetal development during pregnancy.

KEY WORDS

Cotyledon, litter size, placenta, lamb, sheep.

INTRODUCTION

The results regarding reproductive parameters are of key importance in sheep breeding. They particularly concern the assessment of the course of pregnancy, whose proper development may affect the health of newborn lambs. Among many factors, placenta plays the key role[1]. It starts when an embryo is implanted and from that moment, it play a significant role in the development of pregnancy[2]. This is where the morphological and functional analysis of the placenta originated. It has been shown that some morphometric parameters of the placenta can directly affect fetal growth and development, and thus the success of pregnancy[3]. On the example of sheep placenta, it was confirmed that its certain indicators can directly contribute to placental insufficiency, and thus constitute one of the factors that determine the mortality of newborn livestock[4]. Therefore, this type of work is important in the diagnosis and monitoring of pregnancy, especially *in vivo*[5]. Currently, ultrasound is increasingly used for this purpose, the use of which is associated with the ability to correctly interpret it [5]. Therefore, you should first learn about morphology of the placenta, including actual dimensions of its structures, which is best done by direct testing[6,7], e.g. non-invasive, using the placenta expelled immediately after delivery. This type of work can have practical significance, especially in controlling pregnancy and increasing productivity in large livestock farms[5]. They can also be helpful for breeders in preparing for deliveries from multiple pregnancies or the occurrence of heavy births and other complications during this period, which results in minimizing infant mortality[5]. However, previous studies have not tak-

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Table 1 - The number of male lambs and female lambs born and died up to 2 weeks of age from single and multiple pregnancies.

Type of pregnancy (n)		Lambs			
		alive[1]		dead[2]	
		male lambs	female lambs	male lambs	female lambs
Single (n= 99)		43	51	3	2
Twin (n=28)	dichorial (n=18)	16	16	2	2
	monochorial (n=10)	8	12	-	-
Triplet (n=1)		2	1	-	-

[1]live born lambs
[2]dead lambs born and those that did not survive up to 2 weeks of age

Table 2 - Mean (±SD) birth weight of lambs and morphometric parameters of sheep placenta from single pregnancies in ewes of different ages.

Parameters		Age of ewes			Statistically significant differences
		I (1 year; n=20)	II (2-5 years; n=41)	III (5> years; n=33)	
		1	2	3	
BWL (g)	Mean ± SD	5375.00±767.69	5226.83±712.05	5163.64±648.47	NS
	Range	3600.00-6500.00	3800.00-6900.00	3800.00-6500.00	
PW (g)	Mean ± SD	305.45±68.71	307.24±66.10	318.85±78.06	1<3; p<0.05
	Range	200.00-403.00	196.00-494.00	187.00-482.00	
PL (cm)	Mean ± SD	153.06±17.57	141.12±17.39	143.23±21.39	1>2; p<0.05
	Range	126.70-185.00	116.00-181.40	106.60-214.00	
WP (cm)	Mean ± SD	54.03±7.77	55.88±7.90	57.07±9.85	NS
	Range	37.50-69.00	40.00-76.70	40.70-74.90	
CN	Mean ± SD	86.20±14.64	69.24±17.91	70.27±18.06	1>2,3; p<0.01
	Range	55.00-113.00	37.00-98.00	40.00-107.00	
CW (g)	Mean ± SD	92.82±39.75	102.39±23.53	96.83±36.54	NS
	Range	43.00-166.00	53.00-135.00	33.50-170.00	
MDC (cm)	Mean ± SD	2.09±0.36	2.33±0.46	2.24±0.24	1<2; p<0.05
	Range	1.45-2.68	1.65-3.95	1.73-2.89	
DUC (cm)	Mean ± SD	0.64±0.11	0.64±0.11	0.62±0.13	NS
	Range	0.50-0.80	0.50-0.90	0.40-0.90	

Statistical significance of p < 0.01; p < 0.05; SD - standard deviation; NS - no significant; BWL - lamb birth weight; PW - placental weight; PL - placental length; WP - placental width; CN - cotyledons number; CW - cotyledons weight; MDC - mean diameter of cotyledons; DUC - umbilical cord diameter.

en into account all the morphometric parameters of the placenta that can be tested, as well as the relationships between them, taking into account fetal and maternal factors. One of the aspects not yet discussed in the available literature is the analysis of sheep's placenta in multiple pregnancy, especially twin occurring frequently in sheep[8, 9], taking into account the classification into a monochorial placenta, having one chorion and a common placenta for developing fetuses and dichorial, where they are two chorions and completely separate placenta for each fetus in twin pregnancy[10]. It is also worth examining the effect of fetal sex on the placenta[11] and mother's age, with which reproduction parameters are known to change[12,13]. Therefore, it is so important to include the foregoing aspects in the analysis of placenta, which are not fully explained in the available literature. The purpose of this study was to examine morphometric parameters of the sheep placenta depending on the type of pregnancy, litter sex and age of ewes, and to analyze the birth weight of lambs.

MATERIAL AND METHODS

The study was carried out on Pomeranian sheep kept on an organic sheep farm in the Experimental Plant of the National Research Institute of Animal Production in Kołbacz (Kołbacz, Poland: latitude 53°30' N). The Pomeranian sheep are the main breed in the population of meat and wool sheep raised in the north-western region of Poland. This breed is included in the livestock genetic resources conservation program. In the study, sheep were kept in a pasture-alcove system, under conditions of uniform feeding. Nutrition was carried out according to standards adapted for this species, based on fodder, and oth-

Table 3 - Mean (± SD) birth weight of lambs and morphometric parameters of sheep placenta from single female and male pregnancies.

Parameters		Sex of lambs: female (n=51) 1	Sex of lambs: male (n=43) 2	Statistically significant differences
BWL (g)	Mean ± SD Range	5082.35±613.42 3800.00-6500.00	5418.60±756.64 3600.00-6900.00	1<2; p<0.05
PW (g)	Mean ± SD Range	294.24±68.53 187.00-494.00	330.74±68.45 196.00-482.00	1<2; p<0.01
PL (cm)	Mean ± SD Range	146.23±19.99 117.00-214.00	142.22±18.33 106.60-185.00	NS
WP (cm)	Mean ± SD Range	54.49±7.87 37.50-68.00	57.58±9.17 41.00-76.70	NS
CN	Mean ± SD Range	73.10±18.04 37.00-107.00	73.35±19.13 37.00-113.00	NS
CW (g)	Mean ± SD Range	89.61±33.07 33.50-166.00	111.59±26.33 67.00-170.00	1<2; p<0.05
MDC (cm)	Mean ± SD Range	2.18±0.43 1.45-3.95	2.33±0.30 1.74-3.15	1<2; p<0.01
DUC (cm)	Mean ± SD Range	0.61±0.12 0.40-0.90	0.66±0.11 0.50-0.90	NS

Statistical significance of p < 0.01; p < 0.05; SD - standard deviation; NS - no significant; BWL - lamb birth weight; PW - placental weight; PL - placental length; WP - placental width; CN - cotyledons number; CW - cotyledons weight; MDC - mean diameter of cotyledons; DUC - umbilical cord diameter.

Table 4 - Mean (± SD) birth weight of lambs and morphometric parameters of sheep placenta from dichorial twin pregnancies including lambs sex.

Parameters		Sex of lambs: female (n=16) 1	Sex of lambs: male (n=16) 2	Statistically significant differences
BWL (g)	Mean ± SD Range	3800.00±307.06 3400.00-4300.00	4141.67±716.63 2400.00-4900.00	1<2; p<0.05
PW (g)	Mean ± SD Range	242.25±32.13 213.00-292.00	274.00±74.18 197.00-436.00	NS
PL (cm)	Mean ± SD Range	110.90±21.48 75.80-141.40	123.46±27.62 91.00-187.40	NS
WP (cm)	Mean ± SD Range	54.73±6.72 44.20-64.90	52.67±8.69 39.00-68.80	NS
CN	Mean ± SD Range	50.00±20.23 35.00-98.00	58.67±21.29 36.00-102.00	NS
CW (g)	Mean ± SD Range	64.50±2.12 63.00-66.00	87.86±9.96 69.00-96.00	1<2; p<0.05
MDC (cm)	Mean ± SD Range	2.50±0.39 2.00-2.96	2.42±0.29 2.01-2.89	NS
DUC (cm)	Mean ± SD Range	0.60±0.14 0.40-0.80	0.67±0.10 0.60-0.90	NS

Statistical significance of p < 0.01; p < 0.05; SD - standard deviation; NS - no significant; BWL - lamb birth weight; PW - placental weight; PL - placental length; WP - placental width; CN - cotyledons number; CW - cotyledons weight; MDC - mean diameter of cotyledons; DUC - umbilical cord diameter.

er forages and concentrate feeds, depending on the time of year (summer and winter nutrition). In the grazing season from May to October, sheep grazed in the meadow, and in the fold they received oats, hay and straw. In the winter, from November to April, the sheep stayed in the fold, where they were fed with oats, straw and hay. The animals had constant access to water and salt licks. Sheep were mated during their natural breeding season (September) in a natural way, no oestrus synchronization methods were used. Duration of pregnancy was determined based on the day of mating and the success of mating was confirmed by transrectal ultrasound (USG scanner 480, Pie Medical, linear probe with frequency of 7.5 MHz) and vaginal mucus resistance measurements (ohmmeter Dramiński, Poland)[14]. To distinguish single and multiple pregnancy, abdominal ultrasound was performed (USG scanner EDAN U50, sectoral probe with frequency of 5 MHz). A total of 128 sheep were included in the study.

Data collection

Placentas were obtained immediately after deliveries. The following types of placenta were obtained from single pregnancies and twin pregnancies. In twin pregnancies there were: monochorial and dichorial (Fig. 1). After delivery, the sex and birth weight of lambs (BWL) were determined (veterinary weight; MENSOR WE15P2-A, Poland). In the first stage, placentas obtained from single (n=94) and twin (n=16) pregnancies, from which live lambs were born, were used for comparative analyses. On the other hand, the parameters of placentas obtained from monochorial twin pregnancies (n=10) were subjected to a separate analysis due to the type of placenta, which prevents comparative analysis with other types of placenta. Due to the presence of one large placenta, the total birth weight of lambs (TBWL) was determined for a given monochorial placenta. According to the classification given by Steven[15], monochorial placentas include chorionic sacs: joined by avascular tips and completely fused with placental vascular asymmetry, and without asymmetry of the placental vessels. The study also took into account the survival of lambs, distinguishing a group of live-born lambs and a group of dead lambs up to 2 weeks of age.

Placenta characteristics were determined based on the following parameters: placenta weight (PW), placenta length (PL), placenta width (WP), number of cotyledons (CN), the weight of cotyledons (CW), mean cotyledon diameter (MDC), umbilical cord diameter (DUC). The length of the placenta was measured using a non-stretchy cord according to the method described by Vernunft et al.[16]. Each placenta was placed on the countertop with the fetal surface outside and the umbilical cord arranged centrally. Estimation of organ length was done along the main curvature that was opposite the umbilical cord base (Fig. 2). Necrotic placenta elements were not included in the measurements. In turn, determination of the placenta width was based on the method given by Winder et al.[17]. The measurement was taken at the widest point of the placenta after it was cut along the curvature opposite the umbilical cord (Fig. 2). Then the number of cotyledons was estimated and isolated from the placenta, then the diameters of randomly selected 10 cotyledons from the placenta were measured[8]. Cotyledon diameters were estimated by averaging the length and width (cm) measurements[18] using a caliper (Fig. 3). Then the obtained values were averaged and the mean diameter of the cotyledons per test placenta was obtained. All isolated cotyledons from the placenta were weighed using the same scale as placental and lamb weight measurements. The umbilical cord diameter was measured using a caliper.

Statistical analysis

Statistical analysis of the study results was performed using the Statistica program version 13.3 (StatSoft, Poland). Mean values and standard deviations were calculated. One-way analysis of variance (ANOVA) was used to determine the significance of differences between the normal distribution means. The Duncan test was used as the post-hoc test. For variables that did not meet the parameters of parametric tests, the non-parametric Kruskal-Wallis test was used to analyze the variance. A multiple comparison test was used as the post-hoc test. Correlation analysis was done by calculating Pearson and/or Spearman's correlation coefficients. The level of statistical significance was $p < 0.05$.

Figure 1
Type of placentas in twin pregnancies: A - monochorial placenta; B - dichorial placenta.

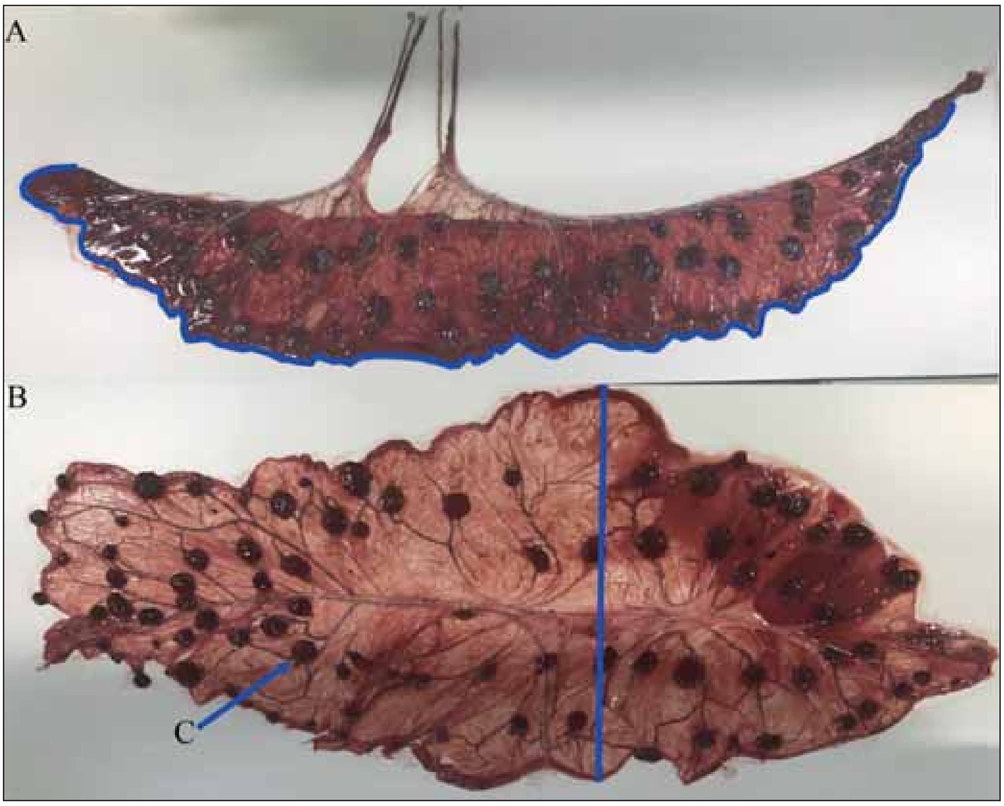

Figure 2
Representative picture of sized ovine placentas of one litter; the line demonstrates the measurement of placental length (A) and width (B); C-cotyledon.

RESULTS

Table 1 presents numbers of pregnant sheep, pregnancy types, live and dead lambs up to two weeks of age. The sex of lambs was also taken into account. The number of sheep with single pregnancy was more than three times higher than the number of sheep with multiple pregnancies. In the examined sheep were born more male lambs than female lambs. In twin pregnancies, there were more dichorial than monochorial ones. The mortality rate of lambs was similar in both sexes (Table 1).
Table 2 presents the average values of morphometric parameters of placenta and birth weight of lambs from single pregnancies depending on the age of the ewes. There were significant differences in placental weight, with the lowest weight for the youngest ewes, and the highest for sheep over 5 years of age. The length of nulliparous placenta was greater than that of older sheep, but significant differences were noted between nulliparous placenta and ewe placenta at the age of 2-5 years. Nulliparous placenta also had more cotyledons than the older sheep placenta. On the other hand, the mean diameter of the cotyledon was smaller than in older sheep's placentas, with significant differences between the cotyledons of nulliparous placenta and the cotyledons of sheep aged 2-5 years. In the remaining parameters, no significant differences were found between the examined groups of ewes (Table 2).
The average birth weight of lambs and morphometric parameters of the placenta from single pregnancies, taking into account the sex of lambs, is given in Table 3. Male lambs had a significantly higher birth weight than female lambs. Also, the placental weight from male pregnancies was significantly higher than from female pregnancies. Similarly, the differences in cotyledon weight and mean diameter of cotyledon were shaped. However, no statistical differences were found in other parameters (Table 3).
Table 4 shows the average values of analyzed parameters of the placenta and birth weight for female and male lambs born from dichorial twin pregnancies. The birth weight of male lambs was significantly higher than that of female lambs. The differences in cotyledon weight were similar. However, no significant differences were found in other parameters examined (Table 4).
Table 5 presents the results regarding the comparison of the birth weights of lambs and placental morphometric parameters between single and twin dichorial pregnancies. Lambs from single pregnancies had a significantly higher birth weight than lambs with twin dichorial pregnancy. The placenta weight was also significantly higher in single pregnancies than in lambs from dichorial twin pregnancies. The differences in placental length and the number of cotyledons were similar. In turn, the mean diameter of cotyledon was significantly larger in twin dichorial placentas than in single pregnancies. No significant dif-

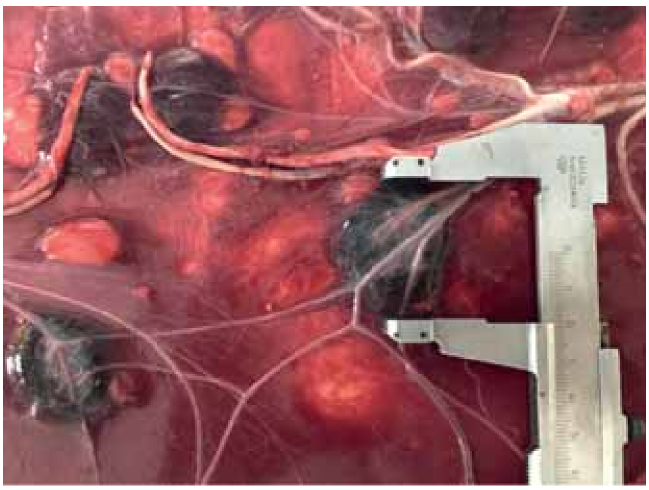

Figure 3 - Measurement of cotyledon using a caliper.

Table 5 - Mean (± SD) birth weight of lambs and morphometric parameters of sheep placenta from single and twin dichorial pregnancies.

Parameters		Type of pregnancy		Statistically significant differences
		single (n=94)	dichorial twin (n=32)	
		1	2	
BWL (g)	Mean ± SD Range	5236.17±699.44 3600.00-6900.00	4005.00± 601.29 2400.00-4900.00	1>2; p<0.01
PW (g)	Mean ± SD Range	310.94±70.53 187.00-494.00	261.30±61.81 197.00-436.00	1>2; p<0.01
PL (cm)	Mean ± SD Range	144.42±19.26 106.60-214.00	118.17±25.38 75.80-187.40	1>2; p<0.01
WP (cm)	Mean ± SD Range	55.85±8.61 37.50-76.70	53.54±7.79 39.00-68.80	NS
CN	Mean ± SD Range	73.21±18.45 37.00-113.00	55.20±20.79 35.00-102.00	1>2; p<0.01
CW (g)	Mean ± SD Range	98.10±32.19 33.50-170.00	82.67±13.45 63.00-96.00	NS
MDC (cm)	Mean ± SD Range	2.25±0.38 1.45-3.95	2.45±0.33 2.00-2.96	1<2; p<0.05
DUC (cm)	Mean ± SD Range	0.63±0.12 0.40-0.90	0.64±0.12 0.40-0.90	NS

Statistical significance of p < 0.01; p < 0.05; SD - standard deviation; NS - no significant; BWL - lamb birth weight; PW - placental weight; PL - placental length; WP - placental width; CN - cotyledons number; CW - cotyledons weight; MDC - mean diameter of cotyledons; DUC - umbilical cord diameter.

ferences were found in other parameters (Table 5).
Table 6 presents the average total birth weight of twin lambs and placental parameters from monochorial twin pregnancies. The average placental weight from female twin pregnancies was significantly lower than from male twin pregnancies. In contrast, the weight of cotyledons was significantly higher in placenta from male twin pregnancies than from female ones. No statistical differences were found in other parameters (Table 6).
Table 7 shows correlation coefficients between the tested morphometric parameters of the placenta, and the age of ewes and the birth weight of lambs. It was shown that the birth weight of lambs was positively correlated with placental weight, placental length, cotyledon number and weight. There were no significant correlations between the age of ewes and placental parameters (Table 7).

DISCUSSION

Morphometric parameters of the sheep placenta were examined depending on the type of pregnancy, litter sex and age of ewes, as well as the analysis of lambs birth weight. The obtained results confirmed that the foregoing factors had an impact on some morphometric parameters of the placenta and the birth weight of newborn lambs. Some results of research on given parameters were similar to those observed in the works of other authors. Additional results were also obtained that could expand knowledge in this area of sheep research.
In this study, no impact of sheep age on the weight of newborn offspring was found. Similar observations were noted in studies of Karakus and Atmaca[19]. On the other hand, other authors showed that age of ewes no affects the birth weight of lambs[20].
This study showed that the number of placental cotyledons decreased with increasing age, and the mean diameter of cotyledon increased. Results regarding the number of cotyledons were similar to those obtained in another study[21], however they differed from the results obtained by Kolosov et al.[3] and Pettigrew[22]. It can be presumed that in older sheep, the decrease in the number of cotyledons and the increase in the cotyledon diameter with age can lead to minimizing losses in the exchange of nutrients between the mother and the fetus. Parraguez et al.[4] showed similar conclusions regarding the number and diameter of cotyledons. It is known that cotyledons as fetal placenta (placentomes) are responsible for nutrition of the fetus, due to the penetration of metabolites from these structures from the mother[23]. It is possible that a smaller amount of cotyledons may affect the weight of lambs born to older sheep than by nulliparous. Confirmation of this hypothesis can be the results of this work.
As the pregnancy progresses, the size of cotyledons may change due to nutritional requirements of the fetus[23]. It can be assumed that the larger diameter of cotyledon is intended to increase the penetration of nutrients into the fetus. It can be assumed that this is not enough to offset the smaller number of cotyledons per placenta. Thus, it can affect the birth weight of lambs.
This study also noted significant differences in placental length between primiparous and older sheep. Placental length had highest values in youngest sheep. However, there is no analysis of this indicator in sheep available in the literature, which is why it is so important to continue research in this area.
Significant differences in some placenta parameters and birth weight were noted between female and male lambs in single pregnancies. It was shown that placental weight, cotyledon

Table 6 - Mean (± SD) total birth weight of lambs from monochorial twin pregnancies and morphometric parameters of placenta.

Parameters		Twin		Statistically significant differences
		female (n=6)	male (n=4)	
		1	2	
TBWL (g)	Mean ± SD Range	7966.67±801.66 6500.00-8600.00	7875.00±309.57 7600.00-8300.00	NS
PW (g)	Mean ± SD Range	435.33±77.75 332.00-540.00	523.75±37.62 485.00-573.00	1<2 ; p<0.05
PL (cm)	Mean ± SD Range	203.82±35.85 167.50-271.50	203.90±15.47 182.00-215.40	NS
WP (cm)	Mean ± SD Range	57.82±3.37 53.00-61.00	56.10±10.01 44.50-64.90	NS
CN	Mean ± SD Range	95.33±11.00 80.00-108.00	105.25±14.50 88.00-120.00	NS
CW (g)	Mean ± SD Range	153.75±61.45 112.00-244.00	223.00±31.11 201.00-245.00	1<2 ; p<0.05
MDC (cm)	Mean ± SD Range	2.34±0.34 1.96-2.94	2.34±0.24 2.04-2.61	NS
ADUC (cm)	Mean ± SD Range	0.63±0.08 0.50-0.70	0.68±0.10 0.60-0.80	NS

Statistical significance of $p < 0.01$; $p < 0.05$; SD - standard deviation; NO - no significant; TBWL - total lamb birth weight; PW - placental weight; PL - placental length; WP - placental width; CN - cotyledons number; CW - cotyledons weight; MDC - mean diameter of cotyledons; ADUC - average umbilical cord diameter.

weight and their diameters were affected by the sex of the lambs. Similar results were obtained in the studies of other authors[8,24]. It is possible that this is due to the different requirements of the male and female fetus[25,26]. The male lambs, due to their higher birth weight, compared to female lambs, need more nutrients delivered through the placenta. Therefore, it may increase placental weight, cotyledon weight, and mean cotyledon diameter in the placenta where the fetus was male.

The study also analyzed twin dichorial placentas, where cotyledons had a larger weight in the placenta from which the male lambs were born. As in the case of single pregnancy, the weight of cotyledons was greater in placenta from dichorial twin pregnancies from which male lambs were born. Similarly, the birth weight of lambs was higher for male twins.

Other authors' conclusions indicated that the size and weight of the litter affect the weight of the placenta and the size of cotyledons[27]. The smaller number and weight of cotyledons in twin pregnancy dichorial placentas compared to single pregnancy placentas confirm these assumptions. In contrast, the increase in cotyledon diameter in twin pregnancies compared to those with single pregnancies may be the result of the placenta trying to minimize losses in nutrient transport to twin fetuses, caused by a reduced amount of cotyledons in each part of the dichorial placenta per fetus. Previous studies showed that twin pregnancies caused changes in maternal and fetal physiology differently from single pregnancies[28].

In this study, differences were noted in the influence of litter size on individual morphometric parameters of the placenta. Some authors suggested that in cases where the placenta was burdened with multiple pregnancy, specific interactions occurred between developing offspring[11]. There may be uneven competition for already limited resources in the twin placenta, which may subsequently lead to differences in the birth weight of twin lambs.

This study also considers the second type of monochorial twin placenta in pregnancy. Monochorial placenta due to their structure carry a greater risk of anastomosis of placental vessels [29]. It is possible that the occurrence of anastomoses of these vessels limits the access to nutrients to one of the twin fetuses, and thus affects its development and birth weight[30]. The available literature has not yet included the classification of twin placenta into dichorial and monochorial in sheep, and thus the morphometric parameters of the placenta in this aspect have not been analyzed. That is why it is so important to continue re-

Table 7 - Correlation coefficients between placental morphometric parameters and the age of ewes and lamb weight.

	Age of ewes	Lamb birth weight
Age of ewes	-	
Lamb birth weight	0.04	-
Placental weight	0.13	0.75**
Placental length	0.03	0.72**
Placental width	0.07	0.15
Cotyledons number	-0.10	0.54**
Cotyledons weight	0.23	0.69**
Mean diameter of cotyledons	0.17	0.004
Umbilical cord diameter	-0.14	0.14 (*) p<0.05; (**) p<0.01

search and extend the analysis to include placental types and their possible impact on the morphometric parameters of the placenta and on fetal development.

CONCLUSIONS

The obtained results indicate that processes in the body of a ewe during pregnancy occur on many levels. These processes are directed in particular at creating favorable conditions for fetal development, which in turn affects the survival of newborns. It can be assumed that the proper course of pregnancy, including the proper development of the placenta and obtaining its best parameters can affect success in the delivery period. It is similar with birth weight in farm animals, which has a great impact on the survival and further development of the offspring[30]. The obtained results showed that the morphometric parameters of sheep placenta and the birth weight of lambs depend on the type of pregnancy, litter sex and age of ewes. These results should be helpful in assessing postpartum placenta in this animal species. In addition, recorded differences in placenta parameters and birth weight of lambs may be useful in ultrasound assessment of placental and fetal development during pregnancy.

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Załącznik 2.

Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep

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RESEARCH

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Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep

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Abstract

Background: Ultrasonography is one of the most important techniques that enable the detection and monitoring of pregnancy. One such study using this technique is the assessment of the hemodynamics of fetal and umbilical blood vessels.

However, there is little data on blood flow in the placentomes, which is the basic structural unit of the sheep's placenta. Therefore, the aim of this study was to determine the Doppler parameters in the arterial vessels of the caruncles, cotyledons and the umbilical cord as well as measuring venous flow rates during the entire gestation period of the sheep. Additionally, the usefulness of various other ultrasound parameters in the early diagnosis of pregnancy in sheep was analyzed.

Results: Most of the Doppler parameters in umbilical, cotyledonary and caruncular arteries were significantly correlated with the day of pregnancy ($p < 0.01$). In the early stages of pregnancy, the peak systolic velocity (PSV), regardless of the location of the artery, was significantly lower than that in the later stages of pregnancy ($p < 0.01$). PSV was also found to be significantly higher in the umbilical artery than in the cotyledonary and caruncular arteries ($p < 0.01$).

Until the 50th day of pregnancy, the end diastolic velocity (EDV) was not found in the umbilical and cotyledonary arteries. EDV was significantly higher in the caruncular arteries than in the cotyledonary and umbilical arteries ($p < 0.01$). The resistance index (RI) and pulsatility index (PI) in the early stages of pregnancy were found to be significantly higher than that in the later stages of pregnancy ($p < 0.01$). The RI and PI were significantly lower in the caruncular arteries than in the arteries of the cotyledons and umbilical cord ($p < 0.01$). In the umbilical vein, all Doppler parameters were observed to be significantly higher than those in the placentomal veins ($p < 0.01$ or $p < 0.05$). Using transrectal ultrasound, pregnancy was detected between 20 and 28 days after mating. The ovaries were observed to have corpora lutea, the diameter of which was fairly consistent from the 17th to the 56th day of pregnancy.

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Conclusions: It has been demonstrated that both the location of the arterial vessel in the placental-umbilical circulation and the gestational age have a significant impact on hemodynamic parameters. The results also provide new insights about the blood flow in caruncular and cotyledonary arteries, which could contribute to a more holistic understanding of hemodynamic changes in the placentas of sheep. Analyzing haemodynamic parameters in the umbilical and placental veins are preliminary studies in sheep, but it could inspire further research in this field. Furthermore, the research conducted confirms the practicality and convenience of transrectal ultrasonography in the early diagnosis of pregnancy in sheep and also indicates that the identification and imaging of the corpus luteum using B-mode ultrasonography can be a very early and simple method of confirming effective mating in sheep.

Keywords: Placenta, Umbilical cord, Arteries, Veins, Corpus luteum, Doppler ultrasound, Pregnancy, Sheep

Background

Real-time ultrasound is one of the most important techniques for detecting and monitoring pregnancy in sheep. These studies take into account the characteristic images of the uterus, the presence of the embryo and the embryonic vesicle, the embryo's heartbeat, and the presence of placentomes [1–4]. During the detection of pregnancy using ultrasound, attention is also paid to the presence of the corpora lutea on the ovaries. In pregnancy, the corpus luteum formed after ovulation does not undergo luteolysis [5]. The detection of early pregnancy is of great importance in the management of a herd, especially since about 30–50% of embryos do not survive this initial period [6]. Early detection of pregnancy also makes it possible to begin the study of intrauterine development as soon as possible, especially since the pregnant sheep is a valuable biomedical model [7–11]. One of the earliest processes that determine the fate of the pregnancy is the development of functional uteroplacental and fetoplacental circulation. In sheep, placental angiogenesis begins as early as day 18 [12, 13]. The development of the placenta continues throughout pregnancy and is closely related to the development of blood vessels in the placenta, since increased blood flow is necessary for meeting the needs of the growing fetus and for the proper exchange of materials between the mother and the fetus [3, 14]. In sheep, the placenta is of the cotyledonous type, consisting of several dozen placentomes [15]. Each placentome is a functional unit composed of the maternal part (caruncle), formed from the endometrium covering the uterine papillae, and the fetal part (cotyledon), formed by the union of the avascular chorion and the vascularized allantois [8, 16]. Studies on the architecture of the placenta in sheep have shown that maternal vascularization is formed by branches of uterine radial arteries that penetrate the caruncle from its base and then extend along the convex surface of the caruncle. These arteries penetrate the placenta creating numerous branches and an extensive network of capillaries. The vein pattern is similar, but in the opposite direction to that of the arteries [17]. On the other hand, cotyledonary vessels enter and leave the cotyledons from the region of the placentome hilum [16, 17]. They are usually single arterial vessels, which at the area of the hilum, divide into lateral vessels and then branch out to form a capillary network [17]. Conversely, the number of veins leaving the cotyledons is usually greater due to the presence of anastomoses between blood vessels from adjacent placentomes. This vascular system is clearly visible in the postpartum fetal membranes [15]. Oxygenated and nutrient-rich blood flows from the placenta to the fetus through the umbilical veins, and deoxygenated blood returns to the placenta through the arteries [16]. In sheep, the umbilical vascular system is made up of two arteries and two veins. Along the umbilical cord, these vessels do not fork, but in the chorioallantoic area, they separate into two umbilical trunks, each of which consists of one artery and one vein. This phenomenon is well documented in postpartum morphometric research of sheep membranes [15]. There is increasing evidence that disturbances in the placental-fetal circulation causes abnormalities in fetal development, negatively affect fetal growth and lead to low birth weight [18, 19] and this increases the risk of morbidity and mortality in the early postnatal stage [20, 21]. Therefore, it is crucial to monitor the placental-fetal blood circulation throughout pregnancy. Currently, an increasingly frequently used technique in these studies is Doppler ultrasonography, which is an effective, non-invasive tool that provides information on the characteristics of vascularization and blood flow. Studies conducted so far in sheep have focused mainly on blood flow in the umbilical, uterine and fetal arteries [3, 4, 22]. Doppler indices obtained from these studies have provided clinically useful information. However, there is little data available that helps characterize blood flow in the blood vessels that are responsible for forming direct vascularization of the placentomes. Research evaluating blood flow indices in venous vessels also appears to be interesting, especially since the analysis of the Doppler spectrum in the umbilical vein may be a useful parameter to consider in the assessment of the proper development of the fetus [23]. Therefore, the aim of this study was to determine and compare the Doppler parameters in the arterial vessels of the caruncles, cotyledons and umbilical cord during the entire gestation period in sheep. The flow rates in the placentomal and umbilical veins were also determined. Additionally, the relevance of various ultrasound parameters in the early diagnosis of pregnancy in sheep was also analyzed.

Results

Pregnancy was detected between the 20th and 28th day post mating (22.21 ± 2.35) based on the uterine appearance and the presence of the embryo and embryonic sac, in the examined sheep.

Ultrasound examinations carried out during the aforementioned interval showed that the uterus was oval in shape with clearly visible hypoechoic structures in its cross-section. On day 21, the embryos were visible (Fig. 1), and on day 25 - the gestational sac. Succeeding this observation, ultrasound evaluation of the ovaries performed from the 17th day onwards, indicated the presence of corpora lutea, which persisted throughout the following weeks of pregnancy. The corpora lutea were visible as gray echogenic oval structures without a round anechoic central cavity. A selected image of the corpus luteum is shown in Fig. 2. By day 56, the diameters of the corpora lutea were similar to that of the previous days, but in the days following, it was observed to have decreased significantly. The mean values of the examined biometric parameters are presented in Table 1. The placentomes and umbilical cord were first visible at 30 ± 2.15 and 33 ± 1.53 days of gestation, respectively. The ultrasound images of the placentomes and umbilical cord in this period are shown in Figs. 3 and 4. The umbilical cord diameter and length during this period were 5.28 ± 0.54 and 10.76 ± 0.62 mm, respectively, while the diameter of the first visualized placentomes was 7.73 ± 0.69 mm.

Tables 2, 3, 4, 5 and 6 show the mean values of Doppler parameters of blood flow in the placental and umbilical arteries in pregnant sheep. Table 2 shows the mean PSV values. In the umbilical artery, in all the examined periods of pregnancy, PSV values were significantly higher than that in the placental arteries. Table 3 shows the mean EDV values. During the period from 35 to 50 days of pregnancy, no EDV was found in the measurements made in the umbilical artery and in the cotyledonary arteries. A positive value of this parameter was noticed only in subsequent stages of pregnancy. In the caruncular arteries, EDV was significantly higher than that in the cotyledons and in the umbilical cord, in all stages of pregnancy. Also PSV/EDV differed significantly depending on the location of the arterial vessel (Table 4). In the umbilical artery and cotyledons, this ratio could only be determined after day 55 of pregnancy. Table 5 shows the mean RI values. In caruncular arteries, RI was significantly lower than that in the cotyledons and umbilical cord, in all examined periods of pregnancy.

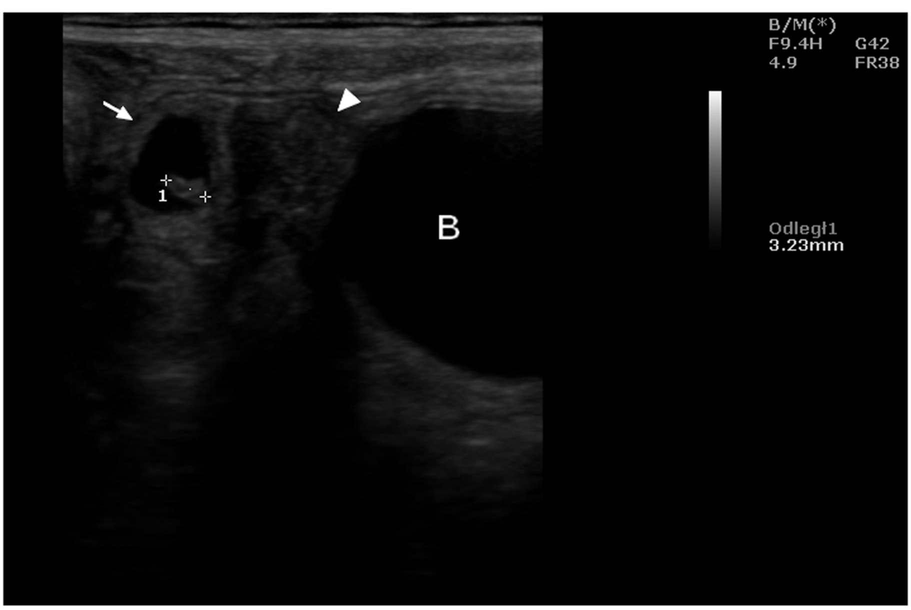

Fig. 1 Ultrasound image of the cross section of a sheep uterus in B-Mode on the 21st day of pregnancy. Arrow - cross-section of the uterus, arrowhead - corpus luteum, 1 - embryo, B - urinary bladder

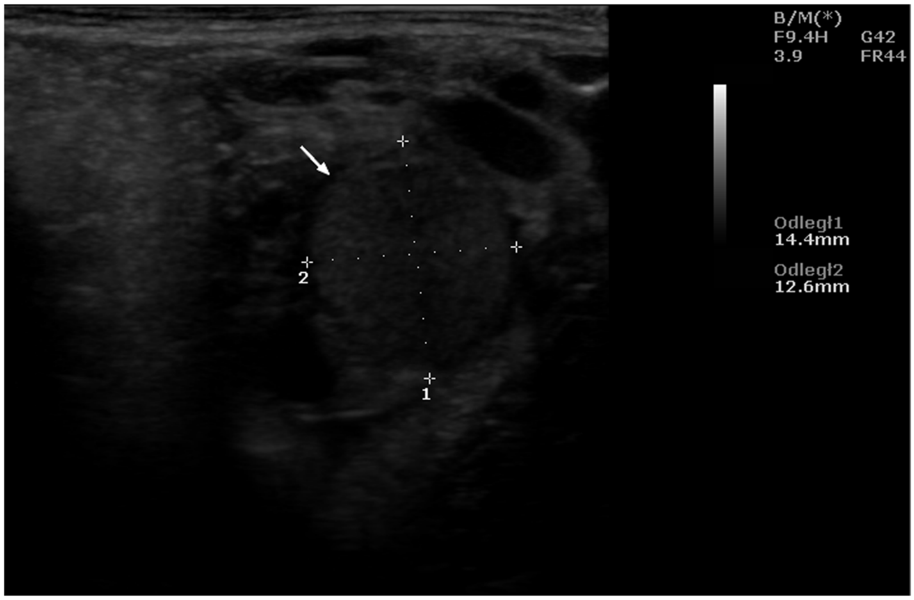

Fig. 2 Ultrasound image of the corpus luteum of a sheep in B-Mode on day 19 of pregnancy. Arrow – corpus luteum

Table 1 Mean (± SD) of the corpus luteum diameter, uterine cross-section, embryo and amniotic sac length at the beginning of pregnancy in sheep ($n = 16$)

Days of pregnancy	Parameters			
	Diameter of corpus luteum (mm)	Cross section of uterus (mm)	Length of embryo (mm)	Length of gestational sac (mm)
17–24	13.98 ± 1.83^{A}	11.29 ± 2.82^{A}	2.67 ± 0.67^{A}	–
25–32	13.21 ± 1.61^{A}	25.35 ± 3.00^{B}	7.16 ± 8.61^{B}	20.83 ± 0.47^{A}
33–40	12.99 ± 0.56^{A}	40.21 ± 7.94^{C}	26.50 ± 6.05^{C}	30.50 ± 6.42^{B}
41–48	13.02 ± 1.26^{A}	50.53 ± 3.58^{D}	37.35 ± 4.16^{D}	39.90 ± 4.61^{C}
49–56	13.67 ± 1.01^{A}	–	–	–
57–64	8.87 ± 1.00^{B}	–	–	–

A - indicates that the mean values in rows marked with different alphabets (A/ B/ C/ D) differ at $p < 0.01$

Also the PI values differed significantly depending on the location of the arterial vessel (Table 6). Table 7 shows the mean values of the Doppler parameters in the umbilical artery during consecutive stages of pregnancy. Whereas Tables 8 and 9 show the mean values of these parameters in the placental arteries. In the early stages of pregnancy, the values of PSV and EDV, regardless of the location of the vessel, were significantly lower than that in the later stages of pregnancy. On the other hand, PSV / EDV, RI and PI in the initial stages of pregnancy were significantly higher than that in the consecutive stages of pregnancy. Most of the Doppler parameters in the examined arterial vessels were significantly correlated with the day of pregnancy. The values of the correlation coefficients are presented in Table 10. Moreover, the analysis of variance showed that the values of Doppler parameters depended on both the period of pregnancy and the location of the artery. Example images of blood flow in umbilical arteries in different stage of pregnancy are presented in Figs. 5 and 6, and in placental arteries in Figs. 7, 8 and 9. The waveform of the Doppler spectrum in these vessels was of a pulsating nature, which in the umbilical artery and in the cotyledons, at the beginning of pregnancy, had a saw-tooth like appearance with only a systolic component but in the later stages of pregnancy, the diastolic breakdowns of the wave were also visible. Table 11 shows the mean values of the Doppler

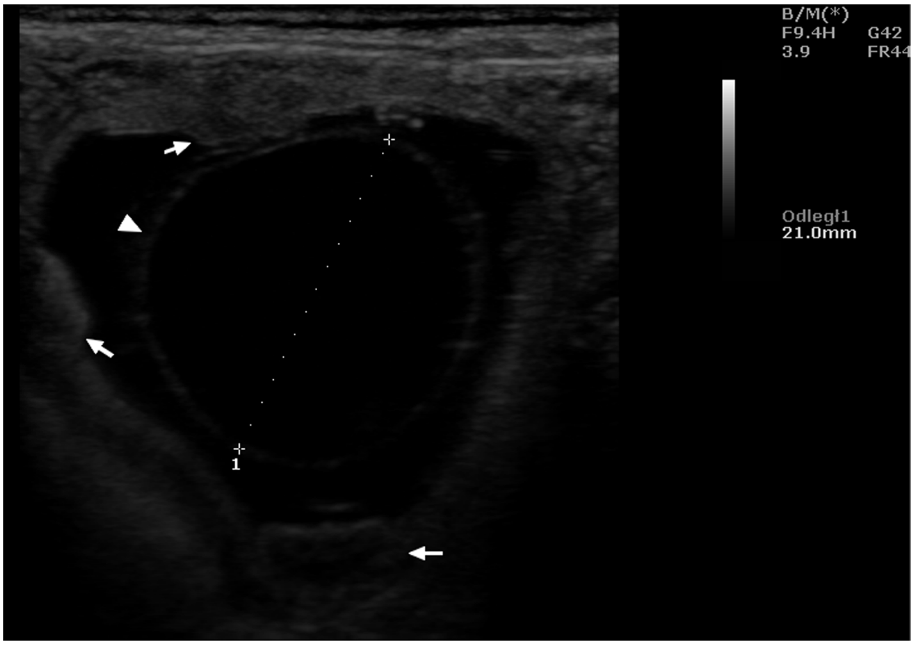

Fig. 3 Ultrasound image of the placenta of a sheep in B-Mode on day 21 of pregnancy. Arrows - placenta, arrowhead - wall of the embryonic sac.

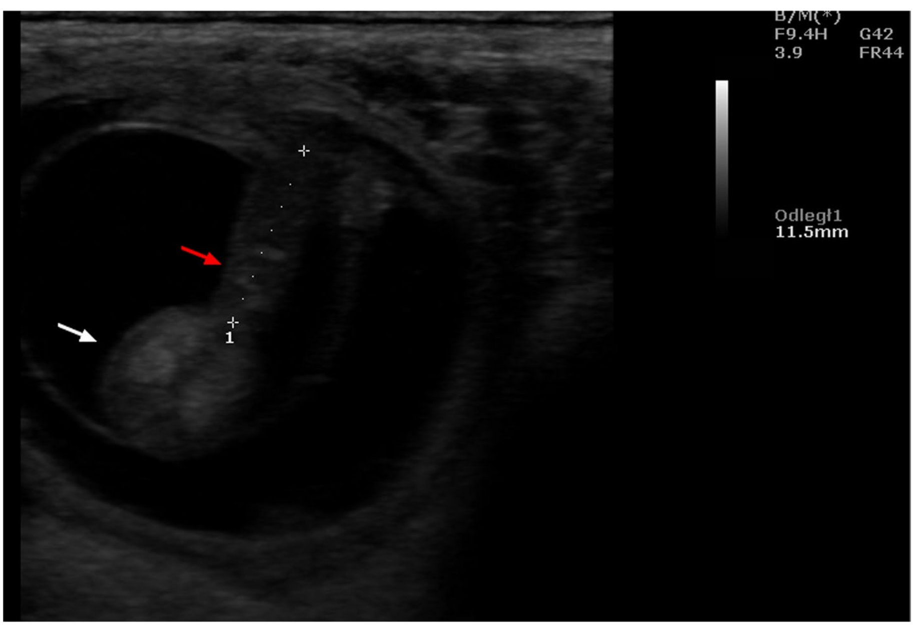

Fig. 4 Ultrasound image of the umbilical cord of a sheep in B-Mode on day 35 of pregnancy. Red arrow - umbilical cord, white arrow - embryo, arrowhead - embryonic vesicle sac.

parameters of blood flow in the placental and umbilical veins in the period between 70 and 90 days of pregnancy. In the umbilical vein, all parameters were significantly higher than those in veins located in the cotyledons and caruncles. The Doppler spectrum in the veins was observed to be flat and wavy (Figs. 10, 11 and 12). In some of the imaged data, two spectra were visible simultaneously: one characteristic for the artery and the other for the vein.

Table 2 Mean (± SEM) values of PSV (cm/s) from placental and umbilical arteries in pregnant sheep ($n = 16$)

Location of the artery	Period of pregnancy				
	(35–50 days)	(55–70 days)	(75–90 days)	(95–110 days)	(130–145 days)
Umbilical cord	22.69 ± 1.34^{A}	22.26 ± 0.74^{A}	26.15 ± 0.53^{Aa}	27.79 ± 1.23^{a}	31.24 ± 1.43^{a}
Cotyledon	10.33 ± 0.17^{B}	13.18 ± 0.61^{B}	14.49 ± 0.76^{Ba}	22.25 ± 0.79^{b}	22.87 ± 3.32^{b}
Caruncle	11.07 ± 1.34^{B}	14.34 ± 1.19^{B}	17.86 ± 0.51^{b}	26.2 ± 2,32^{a}	29.18 ± 3.25^{b}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

Table 3 Mean (± SEM) EDV values (cm/s) from placental and umbilical arteries in pregnant sheep ($n = 16$)

Location of the artery	Period of pregnancy				
	(35–50 days)	(55–70 days)	(75–90 days)	(95–110 days)	(130–145 days)
Umbilical cord	0.00	1.93 ± 0.23^{A}	4.25 ± 0.72^{Aa}	6.91 ± 0.66^{a}	9.07 ± 0.92^{a}
Cotyledon	0.00	0.04 ± 0.02^{B}	3.30 ± 0.34^{Ab}	6.10 ± 0.41^{a}	12.10 ± 1.13^{b}
Caruncle	2.10 ± 0.71	7.91 ± 0.92^{C}	9.13 ± 1.74^{B}	10.32 ± 1.85^{b}	12.21 ± 1.21^{b}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

Table 4 Mean (± SEM) values of PSV/EDV from placental and umbilical arteries in pregnant sheep ($n = 16$)

Location of the artery	Period of pregnancy				
	(35–50 days)	(55–70 days)	(75–90 days)	(95–110 days)	(130–145 days)
Umbilical cord	NO	8.67 ± 0.85^{A}	9.56 ± 1.57^{A}	4.60 ± 0.42^{a}	4.22 ± 0.58^{a}
Cotyledon	NO	12.03 ± 2.81^{B}	12.62 ± 4.42^{B}	3.78 ± 0.33^{b}	1.77 ± 0.48^{b}
Caruncle	0.95 ± 0.32	3.99 ± 1.10^{C}	4.84 ± 0.76^{C}	3.23 ± 0.23^{b}	2.42 ± 0.51^{b}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

NO – incalculable value

Table 5 Mean (± SEM) RI values from placental and umbilical arteries in pregnant sheep ($n = 16$)

Location of the artery	Period of pregnancy				
	(35–50 days)	(55–70 days)	(75–90 days)	(95–110 days)	(130–145 days)
Umbilical cord	1.00	0.95 ± 0.01^{A}	0.78 ± 0.08^{A}	0.76 ± 0.03^{a}	0.70 ± 0.04^{A}
Cotyledon	1.00	1.00	0.75 ± 0.03^{A}	0.67 ± 0.03^{b}	0.35 ± 0.12^{B}
Caruncle	0.87 ± 0.04	0.36 ± 0.08^{B}	0.42 ± 0.09^{B}	0.56 ± 0.12^{b}	0.52 ± 0.13

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

Table 6 Mean (± SEM) PI values from placental and umbilical arteries in pregnant sheep ($n = 16$)

Location of the artery	Period of pregnancy				
	(35–50 days)	(55–70 days)	(75–90 days)	(95–110 days)	(130–145 days)
Umbilical cord	3.01 ± 0.12^{A}	2.79 ± 0.12^{A}	1.65 ± 0.09^{A}	1.48 ± 0.13^{a}	1.17 ± 0.11^{a}
Cotyledon	2.26 ± 0.02^{Ba}	2.55 ± 0.14^{A}	1.37 ± 0.10^{A}	1.40 ± 0.06^{a}	0.64 ± 0.17^{b}
Caruncle	1.66 ± 0.17^{Bb}	0.73 ± 0.15^{B}	0.79 ± 0.11^{B}	0.91 ± 0.11^{b}	0.89 ± 0.09^{a}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

Table 7 Mean (± SEM) values of Doppler parameters in the umbilical artery during consecutive stages of pregnancy in sheep ($n = 16$)

Period of pregnancy	Parameters				
	PSV (cm/s)	EDV (cm/s)	PSV/EDV	RI	PI
35–50 days	22.69 ± 1.34^{Aa}	0.00^{A}	NO	1.00^{A}	3.01 ± 0.12^{Aa}
55–70 days	22.26 ± 0.74^{Aa}	1.93 ± 023^{Ba}	8.67 ± 0.85^{a}	0.95 ± 0.01^{A}	2.79 ± 0.12^{Aa}
75–90 days	26.15 ± 0.53^{b}	4.25 ± 0.72^{Bb}	9.56 ± 1.57^{a}	0.78 ± 0.08^{B}	1.65 ± 0.09^{b}
95–110 days	27.79 ± 1.23^{b}	6.91 ± 0.66^{BC}	4.60 ± 0.42^{b}	0.76 ± 0.03^{B}	1.48 ± 0.13^{B}
130–145 days	31.24 ± 1.43^{B}	9.07 ± 0.92^{BC}	4.22 ± 0.58^{b}	0.70 ± 0.04^{B}	1.17 ± 0.11^{B}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

NO – incalculable value

Table 8 Mean (± SEM) values of Doppler parameters in the cotyledonary artery during consecutive stages of pregnancy in sheep ($n = 16$)

Period of pregnancy	Parameters				
	PSV (cm/s)	EDV (cm/s)	PSV/EDV	RI	PI
35–50 days	10.33 ± 0.17^{Aa}	0.00^{A}	NO	1.00^{A}	2.26 ± 0.02^{A}
55–70 days	13.18 ± 0.61^{A}	0.04 ± 0.02^{A}	12.03 ± 2.81^{a}	1.00^{A}	2.55 ± 1.14^{A}
75–90 days	14.49 ± 0.76^{Ab}	3.30 ± 0.34^{B}	12.62 ± 4.42^{a}	0.75 ± 0.03^{B}	1.37 ± 0.10^{Ba}
95–110 days	22.25 ± 0.79^{B}	6.10 ± 0.41^{C}	3.78 ± 0.33^{b}	0.67 ± 0.03^{B}	1.40 ± 0.06^{Ba}
130–145 days	22.87 ± 3.32^{B}	12.10 ± 1.13^{D}	1.77 ± 0.48^{b}	0.35 ± 0.12^{C}	0.64 ± 0.17^{Bb}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

NO – incalculable value

Table 9 Mean (± SEM) values of Doppler parameters in the caruncular artery during consecutive stages of pregnancy in sheep ($n = 16$)

Period of pregnancy	Parameters				
	PSV (cm/s)	EDV (cm/s)	PSV/EDV	RI	PI
35–50 days	11.07 ± 1.34^{Aa}	2.10 ± 0.71^{A}	0.95 ± 0.32^{a}	0.87 ± 0.04^{Aa}	1.66 ± 0.17^{Aa}
55–70 days	14.34 ± 1.19^{Aa}	7.91 ± 0.92^{B}	3.99 ± 1.10^{b}	0.36 ± 0.08^{B}	0.73 ± 0.15^{B}
75–90 days	17.86 ± 0.51^{Ab}	9.13 ± 1.74^{D}	4.84 ± 0.76^{b}	0.42 ± 0.09^{B}	0.79 ± 0.11^{b}
95–110 days	26.20 ± 2.32^{B}	10.32 ± 1.85^{C}	3.23 ± 0.23^{b}	0.56 ± 0.12^{B}	0.91 ± 0.11^{b}
130–145 days	29.18 ± 3.25^{B}	12.21 ± 1.21^{C}	2.42 ± 0.51^{c}	0.52 ± 0.13^{b}	0.89 ± 0.09^{b}

A, a - indicate that mean values in the rows marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

Discussion

In this study, an early detection of pregnancy was performed using select ultrasound parameters and the Doppler indices of blood flow in the placental and umbilical vessels were determined. Pregnancy was detected at a time similar to that reported by other authors who used transrectal ultrasound for the early detection of pregnancy [1, 4, 24]. In these studies, the most frequently assessed parameters was the size of the uterus from a cross-sectional view. Images of enlarged, fluid-filled sections of the uterus are considered to be one of the earlier signs of pregnancy [25, 26]. On the other hand, a direct indicator of pregnancy is the presence of an embryo and an embryonic sac. In this study, embryos and embryonic sacs post mating were visible at times similar to those reported by other authors [26]. Additionally, it was found that the presence of corpora lutea on the ovaries may also be a helpful parameter.

Typically, during the sheep breeding season, the oestrus cycle lasts 17–19 days, with a short, 3–4 day follicular phase followed by a longer luteal phase [27]. Luteolysis begins around day 12, initiated by the uterine

Table 10 Pearson correlation coefficients (r) between the day of pregnancy and the Doppler parameters in the arterial vessels of the placentomes and umbilical cord in sheep ($n = 16$)

Location of the vessel	Parameters	Correlation coefficients	Significance level
Umbilical cord	PSV	0.64	$P < 0.01$
	EDV	0.74	$P < 0.01$
	PSV/EDV	−0.04	NS
	RI	−0.72	$P < 0.01$
	PI	−0.82	$P < 0.01$
Cotyledon	PSV	0.66	$P < 0.01$
	EDV	0.90	$P < 0.01$
	PSV/EDV	−0.04	NS
	RI	−0.90	$P < 0.01$
	PI	−0.68	$P < 0.01$
Caruncle	PSV	0.56	$P < 0.01$
	EDV	0.60	$P < 0.01$
	PSV/EDV	0.21	NS
	RI	−0.57	$P < 0.01$
	PI	−0.48	$P < 0.01$

NS correlation is statistically insignificant

secretion prostaglandin F2α (PGF2α) [28]. On the other hand, the gestational corpus luteum is resistant to the luteolytic effects of this prostaglandin [5]. Therefore, the presence of the corpus luteum 17 days after mating may indicate that luteolysis has not occurred. Corpus luteum regression causes the extinction of activity and a reduction in the size of the corpus luteum [29]. On the other hand, in the study, the diameter of the corpus luteum between 17 and 24 days after mating was similar in size to that recorded by Rickard et al. [26] in sheep between the ovulation and the pre-implantation period. Hence, the identification of the corpus luteum/corpora lutea using B-Mode ultrasonography can be a very early and simple method to confirm successful mating in sheep. It seems that an important observation is also the appearance of the corpus luteum, which were identified as gray, echogenic, oval structures without a round anechoic central cavity. Indirectly, this may indicate that these are later stages of the development of the corpus luteum. Earlier stages of the corpus luteum are characterized by the presence of a central cavity in sheep and goats [26, 30]. In terms of the use of corpora lutea assessment for the early detection of pregnancy in sheep, interesting results were presented by Braganca et al. [25], Arashiro et al. [31] and Dall et al. [32]. The authors indicate the possibility of using Doppler ultrasound to assess the vascularization of the corpora lutea in the detection of pregnancy. The corpus luteum in pregnant sheep is the main source of progesterone from days 13 to 55 of pregnancy. After this time, this function is taken over by the placenta. Placental progesterone production is sufficient to maintain pregnancy in ovariectomized sheep from day 55 of gestation onwards [16]. This may explain the differences in the corpus luteum diameter noted in this study, which

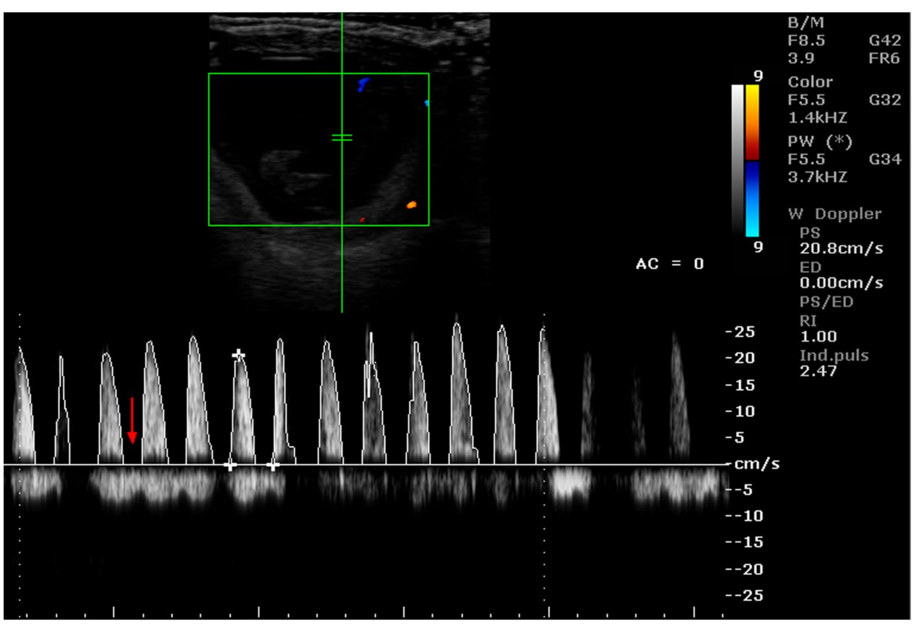

Fig. 5 Color Doppler ultrasound image showing hemodynamic measurements from the umbilical artery of a sheep on day 35 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index). Red arrow – no ED

Fig. 6 Color Doppler ultrasound image showing hemodynamic measurements from the umbilical artery of a sheep on day 130 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index). Red arrow – the presence of ED

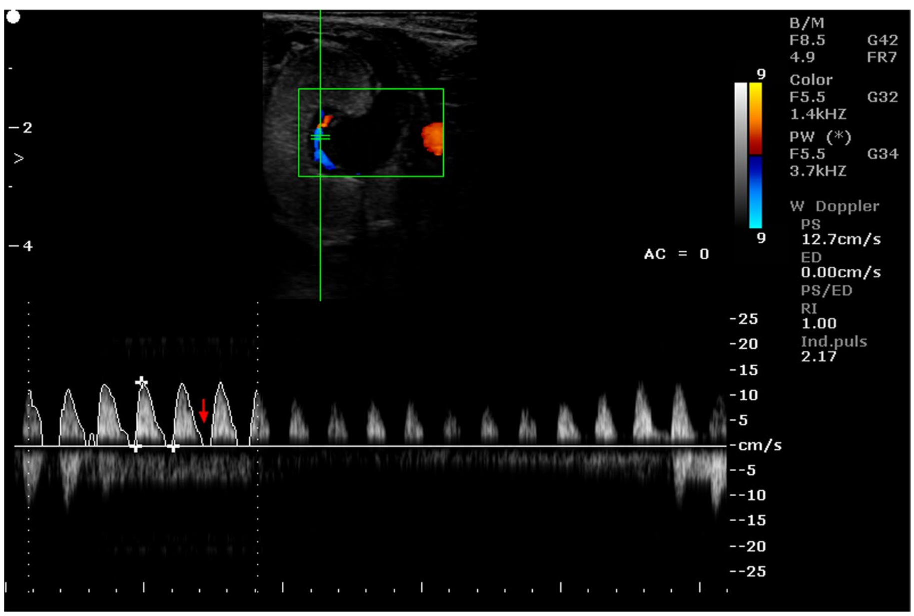

Fig. 7 Color Doppler ultrasound image showing hemodynamic measurements from the cotyledonary artery of a sheep on day 60 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index). Red arrow – no ED

significantly decreased at the end of the first trimester of pregnancy. Another indicator of pregnancy is the presence of the placentomes [3, 33]. In this study, the first placentomes were observed between the first and second months of pregnancy. They were visible on the endometrial surface as areas of increased echogenicity in contrast to the hypoechoic uterine lumen. Kaşikçi et al. [33] observed the first placentomes on day 25, and Rickard et al. [26] on day 29 after insemination.

In this study, the umbilical cord was visualized for the first time around the 33rd day of pregnancy, similar to that reported by other authors [4]. Rickard et al. [26]

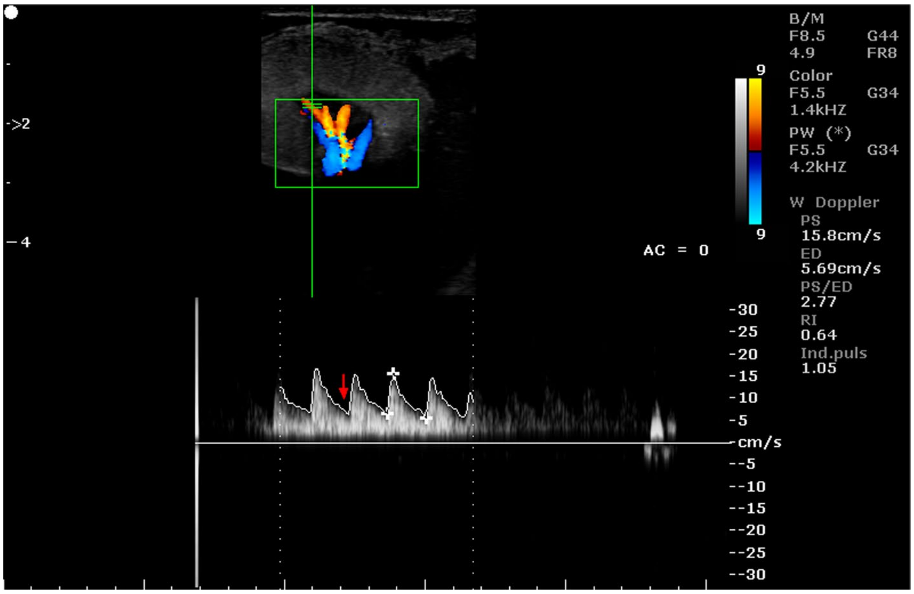

Fig. 8 Color Doppler ultrasound image showing hemodynamic measurements from the cotyledonary artery of a sheep on day 95 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index). Red arrow – the presence of ED

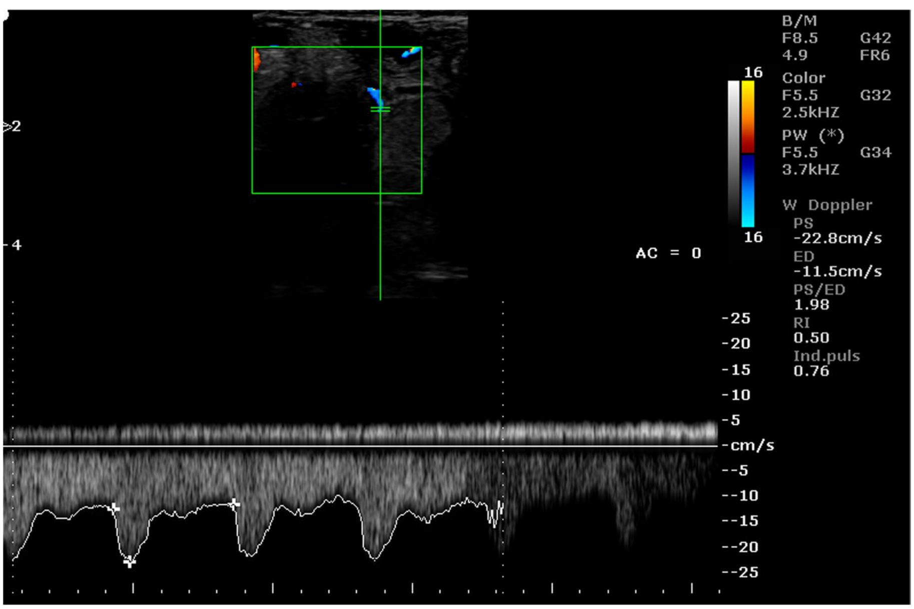

Fig. 9 Color Doppler ultrasound image showing hemodynamic measurements from the caruncular artery of a sheep on day 95 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index)

visualized the umbilical cord already on the 23rd day of pregnancy, while Kumar et al. [34] identified free movement of the umbilical cord on the 39th day of pregnancy. At this stage, the cross-section of the umbilical cord was still small, but large enough to obtain a good measurement of its diameter. Measurements of the umbilical cord diameter at the beginning of pregnancy were also made by other authors [3, 35]. From the 35th day of pregnancy, it was also possible to estimate the blood flow velocity and other Doppler indices in the umbilical vessels. It should be emphasized that stress-free conditions were maintained in the study and no pharmacological agents

Table 11 Mean (± SEM) values of Doppler parameters in the umbilical cord and placentomal veins in the period between 70 and 90 days of pregnancy in sheep ($n = 16$)

Parameters	Location of vessel		
	umbilical cord	**cotyledon**	**caruncle**
PSV (cm/s)	18.51 ± 1.18^{A}	12.97 ± 1.74^{B}	10.77 ± 0.94^{B}
EDV (cm/s)	13.44 ± 1.06^{a}	$11.50 \pm 1{,}91^{a}$	8.95 ± 0.84^{b}
PSV/EDV	1.44 ± 0.11^{a}	1.16 ± 0.06^{b}	1.22 ± 0.03
RI	0.32 ± 0.05^{A}	0.12 ± 0.01^{B}	0.18 ± 0.02^{B}
PI	0.38 ± 0.06^{Aa}	0.14 ± 0.02^{B}	0.21 ± 0.03^{b}

A, a - indicates that mean values in columns marked with different alphabets differ at $p < 0.01$ and $p < 0.05$, respectively

were used. During the examination, the sheep were in standing position, which although poses difficulty for the examiner, did not cause anxiety in the animals, which is extremely important in this type of examination. The possibility of carrying out hemodynamic measurements of the umbilical artery in sheep without the use of anesthesia has been demonstrated in previous studies as well [3]. In the umbilical artery, peak systolic velocity increased as pregnancy progressed and this pattern was also found in other studies in sheep [3, 35–37]. In this study, as pregnancy progressed, the resistance and pulsatility indices decreased, which was also observed in other studies in sheep [3, 4, 36–38]. The end diastolic velocity was undetectable during the initial stages of pregnancy, while a gradual and progressive increase in this parameter was recorded from day 55 to the end of pregnancy. It is believed that the emergence of end diastolic velocity is related to the regularity of the fetal heart cycle and a decrease in the fetal heart rate [39].

As pointed out by Lemley [40], end diastolic velocities are clearly visible on day 90 and only poorly visible on day 60 of gestation in sheep. Elmetwally and Meinecke-Tillmann [41] did not observe umbilical artery end-diastolic velocity in goats and sheep until 12 weeks of gestation. Perhaps these differences are due to the location from where the measurements were done. It cannot be ruled out that the end diastolic velocity in the umbilical cord, at the beginning of pregnancy, is less pronounced the farther away from the fetus and closer to the placenta it is. This would explain the results from the cotyledonary arteries noted in this study, which together with the umbilical vessels form the placental-fetal system [16, 17]. In these vessels, EDV was undetectable or very poorly visible until mid-pregnancy. In this study, the blood flow in the umbilical vessels was examined near the abdominal insert, and as reported by Acharya et al. [42], the location of the umbilical cord being closer to the fetus may play an important role in regulating blood flow. It has also been suggested that the fetuses can regulate blood flow themselves by changing the diameter of the umbilical veins in the umbilical ring [42]. This may explain the differences between the flow parameters in the umbilical and cotyledonary vessels noted in this study. Moreover, in this study, the pulsatility and resistance indices in the caruncular vessels were significantly

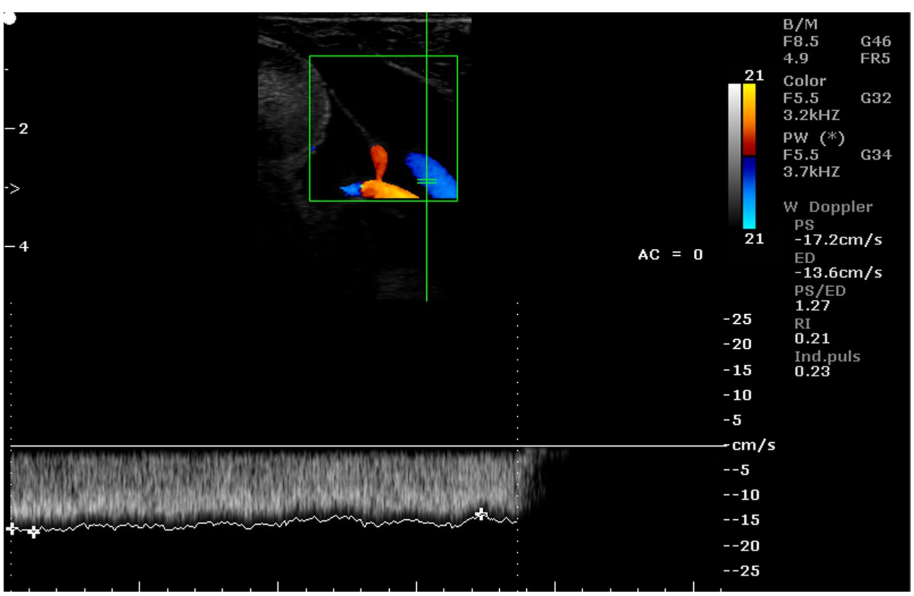

Fig. 10 Color Doppler ultrasound image showing hemodynamic measurements from the umbilical vein of a sheep on day 90 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index)

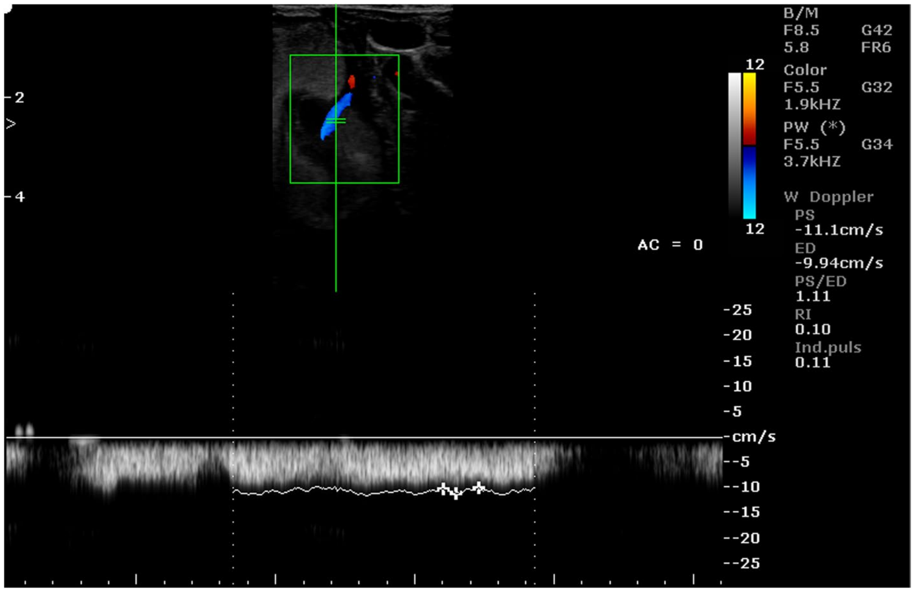

Fig. 11 Color Doppler ultrasound image showing hemodynamic measurements from the umbilical vein of a sheep on day 86 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index)

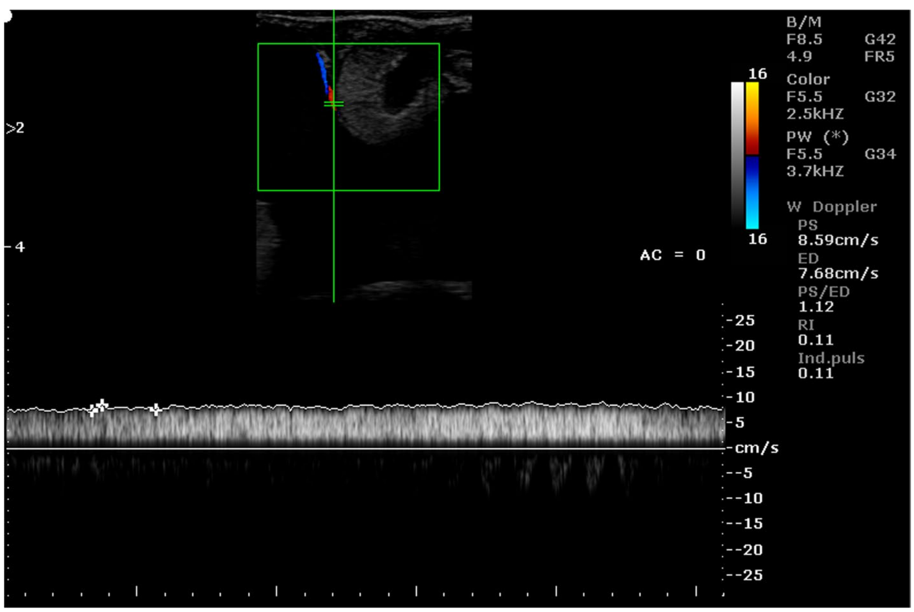

Fig. 12 Color Doppler ultrasound image showing hemodynamic measurements from the caruncular vein of a sheep on day 70 of pregnancy (PS - peak systolic velocity; ED - end-diastolic velocity; RI - resistance index; Ind. puls - pulsatility index)

lower and therefore perhaps it can be assumed that this is required for the protection of the placenta. Saghian et al. [43] indicate that very high blood velocity and pressure can damage the delicate villi of the placenta, especially in the early stages of pregnancy. Placentomes are an integral part of the exchange between the maternal-placental and placental-fetal circulation and are supplied with blood from both the uterine and fetal sides [16, 17]. In addition, very high vascular resistance in the placenta may reduce gas exchange and nutrient delivery, which causes low birth weight and perinatal mortality [44, 45]. In the presented study, both in umbilical and placental arteries, the velocity of blood flow increased, and the indices of resistance and pulsatility decreased with the advancement of pregnancy.

The relationship between the gestational age and the hemodynamics of the umbilical cord and placental vessels noted in this study, is also confirmed by the significant correlation between the Doppler parameters and the day of pregnancy. Such changes in vascular hemodynamics are most likely offset by the significant increase in volumetric blood flow in umbilical and placental vessels with the progression of pregnancy [8, 46]. However, the study noted significant differences in the size of the examined Doppler indices between the caruncular arteries and the umbilical and cotyledonary arteries. The end diastolic velocity in the arteries of the caruncle was shown to be higher and the pulsatility and resistance indices lower than that in the umbilical and cotyledonary arteries. As reported by Riesen et al. [47], an increase in end diastolic velocity causes an increase in blood flow and a decrease in the resistance index. The differences noted in the study indicate that the hemodynamics in the placental vessels differs in the fetal and maternal parts of the placenta. Undoubtedly, it is related to the basic function of the placenta, which is the exchange of material between the maternal and fetal circulation [16, 17, 46]. Moreover, the placenta is also a metabolically active organ [9] and it therefore seems likely that a reduction in blood flow would first negatively affect the placenta, as this organ is the first to experience a reduced supply of nutrients and important substrates [7]. Doppler ultrasonography can distinguish between arterial and venous blood flow in umbilical vessels [48]. The blood flow in umbilical arteries is always pulsating, and the diameter of these vessels is larger than the diameter of the veins and increases with the progress of pregnancy [3].

In contrast, in the umbilical veins, the flow is continuous and not pulsating. The occurrence of pulsations may be associated with abnormal fetal development and perinatal complications, as observed in humans [49]. In this study, the Doppler spectrum showing the blood flow in the placental and umbilical veins was flat and wavy. In some of the imaged data, especially in the umbilical vessels, the two spectra were visible simultaneously: one characteristic for the artery and the other for the vein. Similar images were also presented in the umbilical veins of goats [34]. An almost constant flow velocity was demonstrated in the cotyledonary and caruncular veins. In contrast, in the umbilical vein, the peak systolic velocity and the end diastolic velocity were higher than that in the placental veins. Perhaps these higher values in the umbilical veins are due to their proximity to the fetus. As reported by Pennati et al. [50], umbilical vein flow velocity profiles vary along the umbilical cord. Venous flow is more susceptible to disturbances related to fetal movement than arterial flow [51]. In this study, a high value of diastolic velocity, close to the peak systolic velocity, was recorded in the examined venous vessels. The lack of the end diastolic velocity is believed to reflect an increased resistance in placental-fetal circulation, which may have adverse effects [52].

Conclusion

The obtained results indicate that both the location of the arterial vessel in the placental-umbilical circulation and the gestational age have a significant impact on hemodynamic parameters. The results also provide new insight into blood flow in caruncular and cotyledonary arteries, which will contribute to a more holistic understanding of hemodynamic changes in sheep's placenta. The studies conducted on haemodynamic parameters in venous umbilical vessels and placental vessels are preliminary studies in sheep, but may inspire further research in this field. Moreover, this research confirms the usefulness of transrectal ultrasonography in the early detection of pregnancy in sheep. We also indicate that the identification and imaging of the corpus luteum using B-mode ultrasound can be a very early and simple method of confirming effective mating in sheep.

Material and methods

Animals and the layout of the experiment

The study was carried out on 16 Suffolk sheep kept on an organic sheep farm in the Experimental Station of the National Research Institute of Animal Production in Kołbacz (Poland: latitude 53′30″ N). The sheep were kept in the pastures and indoor systems. The feeding was carried out in accordance with the standards adopted for this species, which is based on pasture green, other roughage and concentrated fodder, depending on the season. The animals had constant access to water and salt licks. The examined sheep are healthy multiparous females, aged 3 to 4 years, with an even body weight (55–60 kg). The sheep were mated during their natural breeding season (September). The estrus was detected using a teaser ram, and mating was hand-service. The duration of pregnancy was determined on the basis of the date of mating. The effectiveness of mating was examined with the transrectal ultrasound (USG scanner EDAN U50, linear probe with 4 MHz frequency). After delivery, the date of conception was confirmed retrospectively by assuming that the pregnancy lasted 148 days [3]. The following parameters were taken into account in the early detection of pregnancy: the size and echogenicity of the uterine cross-section, the presence of corpora lutea on the ovaries, and the presence of the embryo and the gestational sac in the uterus. The time when the placentomes and umbilical cord were first visible was also noted. These parameters were assessed from the 17th day after mating at intervals of several days. In order to measure the blood flow in the umbilical arteries and the placentomes, the study was started on day 35 and continued throughout the pregnancy at intervals of several days. These analyses included 5 periods: 1st period (35–50 days), 2nd period (55–70 days), 3rd period (75–90 days), 4th period (95–110 days) and 5th period (130–145 days). The blood flow in the veins was measured from day 70 to day 90 of pregnancy. In order to eliminate the possible influence of the size of the litter on the examined parameters, only single pregnancies were analyzed, which were diagnosed during transrectal examination and confirmed during delivery.

Ultrasound examination

Ultrasound examinations were performed on pregnant sheep that had not been previously sedated. The examination was always performed by the same experienced and trained operator, in a quiet and dimly lit room. The ultrasound examination was performed using an ultrasound scanner (USG scanner EDAN U50) equipped with a linear probe with a frequency of up to 4 MHz (Model, V742UB) and a sector probe with a frequency of up to 5 MHz (Model, C352UB).

In the initial stages of pregnancy, ultrasound was performed transrectally, and in subsequent stages of pregnancy, sheep were examined transabdominally. Sheep were in standing position during the ultrasound examination. Prior to insertion of the probe, fecal pellets were removed from the rectum. About 20 ml of ultrasound gel was introduced rectally for better visualization of the organs [3, 14, 32]. Prior to transabdominal examination, the inguinal and caudal abdomen were shaved and the skin was cleansed with soap and water. A sufficient amount of transmission gel was applied before the ultrasound examination. Each sheep was examined for the free part of the umbilical cord near the abdominal insertion and for 5 randomly selected placentomes, which were scanned using ultrasound in the B mode. Color Doppler was used to identify arterial and venous vessels in the umbilical cord, cotyledons and caruncles (Fig. 13). Each time, after locating the blood vessels, the blood flow in the arteries and veins was measured using the Doppler pulse wave ultrasound technique. The following Doppler parameters were determined: peak systolic velocity (PSV), end diastolic velocity (EDV), PSV/EDV ratio, resistance index [RI = (PSV-EDV)/PSV] and pulsatility index [PI = (PSV-EDV)/mean velocity]. The flow angle during the examination was kept as close to 0 degrees as possible, with appropriate angle adjustments being made when necessary. Doppler imaging for each sheep lasted from 20 to 30 minutes. Doppler measurements of blood flow in the arteries were made on 5–7 continuous, regular waves of the Doppler spectrum, while venous flows took into account the entire visible spectrum. Measurements were not recorded during maternal and fetal movements. In the event of any signs of distress or tachypnea, the examination was discontinued and repeated at a later time.

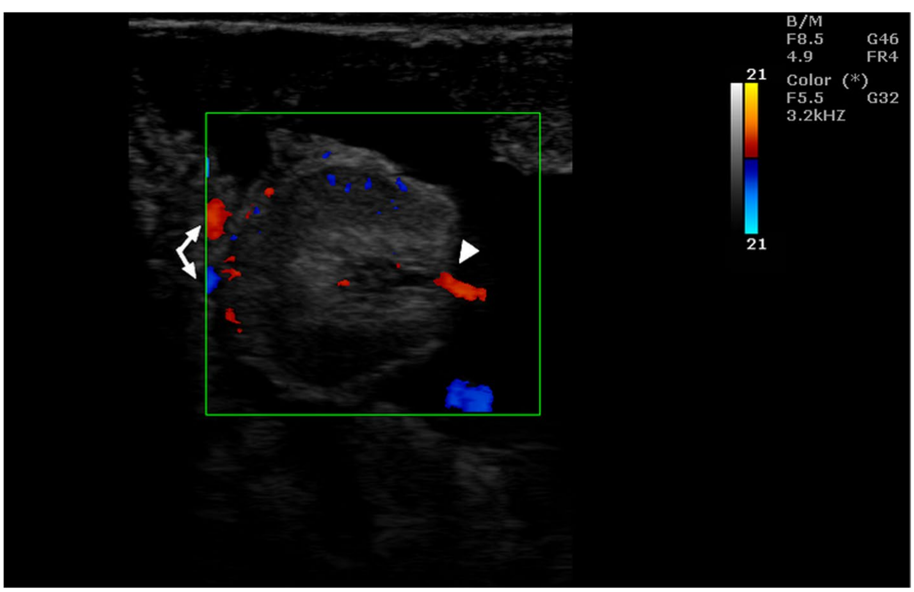

Fig. 13 Color Doppler ultrasound of a sheep placenta on day 70 of pregnancy. Arrows - vessels of caruncle, arrowhead - vessels of cotyledon

Statistical analysis

The obtained results were further submitted for statistical analysis. The results concerning the ultrasound biometric parameters assessed in the early period of pregnancy are represented as mean ± SD, while the results of the Doppler parameters are represented as mean ± SEM. Differences between the means of individual groups were analyzed using the post hoc test. Duncan's multiple range test was used to verify the significance of differences at $P<0.01$ and $P<0.05$. In order to determine the influence of the gestation period and the location of the blood vessel on the blood flow parameters, the multivariate analysis of variance (ANOVA) was performed, where the grouping variable was the gestational age and the location of the arterial vessel, and the dependent variable was the Doppler parameter. The F-test was used to determine the significance level. The correlations between the examined parameters and the day of gestation were calculated using the Pearson rank correlation coefficient (r). Statistical analyses were conducted using the STATISTICA version 13.3, Stat Soft, Poland.

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Not Applicable.

Authors' contributions
AB - research concept and carrying it out, analysis of the results, preparation of the manuscript for printing;TS - research concept and carrying it out, analysis of the results, preparation of the manuscript for printing; BB - research concept and conducting, analysis of the results, preparation of the manuscript for printing;PC - analysis of results, correction of the English language; JU - participation in conducting the experiment; NW - participation in conducting the experiment. All authors read the manuscript and approved it.

Authors' information
Not Applicable.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Animal procedure (ultrasound examination) conducted for this study is in accordance with the "Act on the protection of animals used for scientific purposes" of the Republic of Poland. This act is fully compliant with the EU directive no. 2010/63/EU [53] for the protection of animals used for scientific purposes. This experiment was approved by the Local Ethical Committee for Experiments on Animals in Poznań, Poznań University of Life Sciences (Resolution No. 20/2018). All reported methods are in accordance with the ARRIVE guidelines.

Consent for publication
Not Applicable.

Competing interests
Authors declare no competing interests that could have appeared to influence the findings reported in this paper.

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Publisher's Note

Załącznik 3.

Oświadczenia współautorów o procentowym udziale w przygotowaniu publikacji

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ZUT w Szczecinie
dr hab. inż. Arkadiusz Pietruszka, prof. ZUT
w/m

Oświadczenia współautorów pracy zespołowej wskazujące ich wkład do pracy doktorskiej mgr inż. Angeliki Brzozowskiej.

Niniejszym oświadczam, że udział **mgr inż. Angeliki Brzozowskiej** w przygotowaniu publikacji: Brzozowska A., Stankiewicz T., Błaszczyk B., Chundekkad P., Udała J., Wojtasiak N. 2022: *Ultrasound parameters of early pregnancy and Doppler indices of blood vessels in the placenta and umbilical cord throughout the pregnancy period in sheep*. BMC Veterinary Research, 18, 326, **wynosi 65%**.

Brzozowska Angelika
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Jednocześnie udział pozostałych współautorów w przygotowanie powyższej publikacji jest szacowany na:

dr hab. inż. Tomasz Stankiewicz, prof. ZUT: 10% ....
dr hab. inż. Barbara Błaszczyk, prof. ZUT: 10% Błaszczyk Barbara
BSc Pavitra Chundekkad: 5% ....
prof. dr hab. inż. Jan Udała: 5% ....
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w/m

Oświadczenia współautorów pracy zespołowej wskazujące ich wkład do pracy doktorskiej mgr inż. Angeliki Brzozowskiej.

Niniejszym oświadczam, że udział **mgr inż. Angeliki Brzozowskiej** w przygotowaniu publikacji: Brzozowska A., Wojtasiak N., Błaszczyk B., Stankiewicz T., Wieczorek-Dąbrowska M., Udała J. 2020: *The effects of non-genetic factors on the morphometric parameters of sheep placenta and the birth weight of lambs.* Large Animal Review, 26, (3), 119-126, **wynosi 65%**.

Brzozowska Angelika
Podpis czytelny Doktoranta

Jednocześnie udział pozostałych współautorów w przygotowanie powyższej publikacji jest szacowany na:

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