BRIEF COMMUNICATION: Effect of dam size and nutrition during pregnancy on fetal testicular development in sheep

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Introduction

In male sheep, testicular development is almost complete at birth. Testicular cells start to differentiate as early as Day (D) 27 of gestation followed by steroidogenesis and the activation of associated enzyme systems (Rhind 2004). Sertoli cells are present at around D34 and replication continues postnatally (Hochereau-de Reviers et al. 1987). Seminiferous cords that will develop into tubules appear between D35 and D40 (Sweeney et al. 1997; Rhind et al. 2001). By D70 the rete testis is organised in the centre of testis (Sweeney et al. 1997) and between D35 and D85 the GnRH neuronal system develops in the hypothalamus (Caldani et al. 1995). Testicular gonocytes are typically observed between birth and 25 days after birth, and then progressively differentiate into spermatogonia up to 70 days after birth (Sharpe et al. 2003).

Previous studies have demonstrated that maternal undernutrition during pregnancy can impact on testicular development of the fetus. Maternal undernutrition decreased seminiferous tubule diameter at D99 of gestation (Bielli et al. 2001) and at 10 months of age (Kotsampasi et al. 2009), increased the number of Sertoli cells at two days of age (Bielli et al. 2002), decreased the number of Sertoli cells at 10 months of age (Kotsampasi et al. 2009) and increased the number of spermatocytes at 14 days of age (Rodríguez-González et al. 2012) in male offspring.

Effects such as these have the potential to affect reproductive capability of the male sheep in later life as testicular cell development is highly correlated with sperm productivity and quality (Martin et al. 2010). The present study investigated the effects of dam size and nutrition during pregnancy on the development of fetal testicular cells at D140 of gestation in sheep.

Materials and methods

Dams

Romney ewes of either heavy (H) (60.8 kg ± 0.18 (Standard error of mean), n = 450) or light (L) (42.5 kg ± 0.17, n = 450) live weight at the time of mating were oestrus-synchronised and artificially inseminated as previously described by Kenyon et al. (2009). Ewes were allocated to either *ad libitum* (A) or maintenance (M) (Total live weight gain equivalent to the expected increase in conceptus mass) nutrition from D21 to D140 of gestation, resulting in four treatment groups. The average pre- and post-grazing pasture covers during the period P21 to P140 were 2,304 ± 157 and 1,723 ± 150 kg DM/ha for the *ad libitum* regimen and 1,330 ± 140 and 804 ± 133 kg DM/ha for the maintenance regimen (Kenyon et al. 2009).

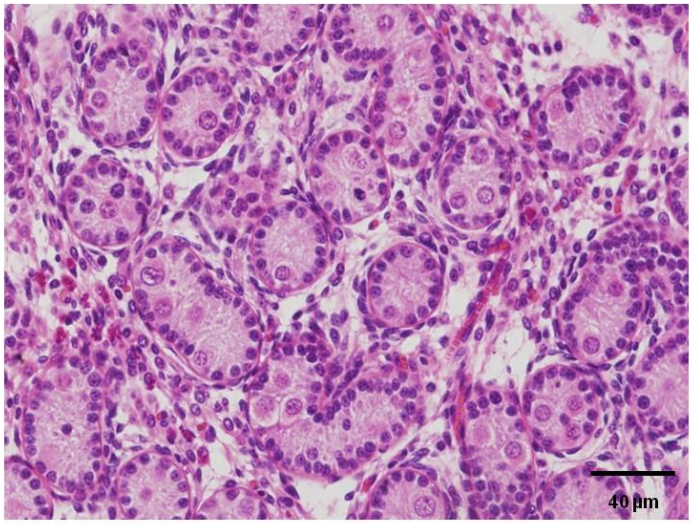
Fetal testicular collection

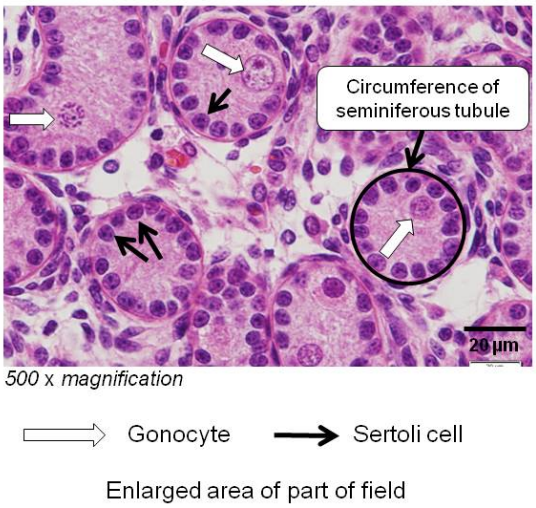
Seventy nine ewes were euthanised on D140 of gestation and fetuses collected (HA n = 21; HM n = 20; LA n = 18; LM n= 20 as previously described by Blair et al. 2011). Blair et al. (2011) reported fetal weight at D140 (HA-fetus 5.3 ± 0.2 kg; HM-fetus 4.7 ± 0.2 kg; LA-fetus 4.9 ± 0.2 kg; LM-fetus 4.5 ± 0.2 kg). Fetal weight of HM, LA, LM, LM fetuses did not differ from each other, but HA-fetuses were heavier than all other groups (Blair et al. 2011). Fetal testes from each group were dissected, weighed and place in Bouin's fixative for 20 hours. After this time, excess fixative was washed out in two changes of 70% ethanol and the testes stored in 70% ethanol before processing into paraffin wax (Leica Histoembedder, Leica Instruments GmbH, Nussloch, Germany).

Fetal testicular cells measurements

A total of 30 male testes were randomly selected (one testis per fetus) from 14 single and 16 twin fetuses from across the four treatment groups (HA = 8, HM = 8, LA = 7 and LM = 7). Paraffin embedded testes were weighed and 5 µm sections cut, 5 µm apart, to obtain five sections per fetus. The sections were stained with haematoxylin and eosin for morphological assessment. Total area of seminiferous tubules (250x magnification with a field area of 90,977 μm^2) was measured from two fields per section (10 fields per animal) (Figure 1). The circumference of 10 seminiferous tubules (round in shape with apparent gonocytes inside the tubule) was measured on each section, equal to 50 tubules per fetus, under 500x magnification. The total number of Sertoli cells and gonocytes were counted from the same seminiferous tubules used to measure the circumference. Measurement of the total area and number of Sertoli cells and gonocytes were conducted by using ImageJ software (Rasband 1997).

Figure 1 Histological slide to visualize the measured variables of number of seminiferous tubules per field of 90,977 µm^2 in area, circumference of seminiferous tubule, number of Sertoli cells per seminiferous tubule and number of gonocytes per seminiferous tubule in a sample of fetal testis at Day 140 of gestation.





Statistical analysis

Statistical analyses were carried out using the Statistical Analysis System (SAS 2008. SAS 9.2, SAS Institute, North Carolina, USA). Values for testes weight and mean values for total area and circumference of seminiferous tubules, total number of Sertoli cells and gonocytes from each animal were analysed using the MIXED procedure including fixed effects of fetal rank (singleton versus twin), dam size treatment (heavy versus light), dam feeding treatment (*ad-libitum* versus maintenance). All two-way and three-way interactions were included in the initial model. Testis weight was included as a covariate, except for testis weight analysis where fetal rank was included as a covariate. Dam size and nutrition, and the interaction between dam size and nutrition remained in all models to allow for testing of the study design.

Results and discussion

Testis weight

Results from the present study suggest that maternal size and nutrition did not affect fetal testis weight at D140 (Table 1). Similarly Kotsampasi et al. (2009) showed that maternal nutrition during

Table 1 Least square mean ± standard error of the mean for testis weight, total area of seminiferous tubules per field in a field area of 90,977 µm^2, circumference of seminiferous tubules (µm), total number of Sertoli cells and number of gonocytes per tubule at Day 140 of gestation for each dam size, dam nutrition and dam size by nutrition group.

Main effect	Treatment	Number of fetuses	Testis weight (g)	Total area of seminferous tubules (x10^4) (µm^2)	Circumference of seminiferous tubules (µm)	Number of Sertoli cells per seminiferous tubule	Number of gonocytes per seminiferous tubule
Dam size	Heavy (H)	16	3.4 ± 0.1	4.1 ± 0.7	124 ± 2	12.2 ± 0.2	1.26 ± 0.02^b
	Light (L)	14	3.4 ± 0.1	4.1 ± 0.8	127 ± 2	12.6 ± 0.2	1.19 ± 0.02^a
Dam nutrition	*Ad libitum* (A)	15	3.6 ± 0.1	4.0 ± 0.8	125 ± 2	12.5 ± 0.2	1.22 ± 0.02
	Maintenance (M)	15	3.3 ± 0.1	4.2 ± 0.8	126 ± 2	12.4 ± 0.2	1.23 ± 0.02
Size x Nutrition	HA	8	3.5 ± 0.2	3.9 ± 1.0	124 ± 2	12.5 ± 0.3	1.25 ± 0.03
	HM	8	3.4 ± 0.2	4.3 ± 1.0	125 ± 2	11.9 ± 0.3	1.27 ± 0.03
	LA	7	3.6 ± 0.2	4.0 ± 1.1	126 ± 2	12.4 ± 0.3	1.19 ± 0.03
	LM	7	3.2 ± 0.2	4.1 ± 1.1	128 ± 2	12.8 ± 0.3	1.20 ± 0.03

^{ab}Means between rows within a column with differing superscripts are significantly different (P<0.05).

pregnancy did not alter male lambs testis weight. However, the study by Bielli et al. (2001) showed testis weight was reduced when dams were exposed to restricted nutrition during pregnancy. The absence of an effect in the present study is likely to be due to a lack of a maternal undernutrition treatment.

Total area and circumference of seminiferous tubules

There was no effect of either dam size, dam nutrition or an interaction between size and nutrition for the total area and circumference of seminiferous tubules per field (Table 1). However, there was an effect of fetal rank, whereby singletons had a greater total area compared to twins (4.2 ± 0.8 x10^4 μm^2 versus 3.9 ± 0.9 x10^4 μm^2; $P = 0.02$). No effect of maternal nutrition on seminiferous tubule diameter was reported (Bielli et al. 2002). Other studies have reported reduced diameter (Kotsampasi et al. 2009) and reduced total area of seminiferous tubules (Sullivan et al. 2010) in offspring born to undernourished mothers. As testicular cell development is strongly associated with testicular weight (Jafariahangari et al. 2012), the absence of an effect on total area and circumferance of seminiferous tubules is likely to be due to the fact that there were no differences in testes weight.

Sertoli cell count

Studies typically focus on Sertoli cell development due to its important role in spermatogenesis (Sharpe et al. 2003). There was no effect of either dam size, dam nutrition or an interaction between size and nutrition treatments, or fetal rank on the number Sertoli cells per seminiferous tubule in the present study (Table 1). This has been previously reported (Bielli et al. 2001) with both a decrease (Kotsampasi et al. 2009) and an increase (Bielli et al. 2002) in the number of Sertoli cells having been found. The absence of an effect on Sertoli cell numbers in the present study is not surprising due to the lack of a difference in seminiferous tubule circumference. The number of Sertoli cells is highly correlated with the size of seminiferous tubules (Hochereau-de Reviers et al. 1987).

Gonocyte cell count

Male fetuses from H-ewes had a higher number ($P = 0.04$) of gonocyte cells in seminiferous tubules at D140 compared to males from L-ewes (Table 1). This result is interesting but somewhat unexpected given the lack of differences in the other parameters measured. Maternal nutrition and fetal rank had no effect on the number of gonocyte cells. To date no other studies have examined the effect of maternal size and maternal nutrition on gonocyte development.

Conclusion

Previous studies have investigated the impact of maternal undernutrition on fetal testes development, whilst the present study examined *ad libitum* and maintenance maternal feeding levels. The results reported here demonstrate that the feeding ewes above maintenance during pregnancy does not appear to affect testis weight, seminiferous tubule development, Sertoli cells number or gonocyte cell number in the fetal testis relative to feeding the ewes at maintenance. However, given the influence of maternal size on gonocyte cell number, the effect of maternal size on gonocyte functionality should be investigated as this is an early indicator of fertility potential in males.

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