Comparison of Gravid Uterine Parameters in Naturally Bred Ewes and Ewes after Transfer of In Vitro Produced Embryos, and in Single, Twin and Triplet Pregnancies.

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Abstract

Large offspring syndrome (LOS) may be initiated in ovine embryos after exposure to in vitro conditions. The objective of this study was to compare various parameters of the gravid uterus on day 140 after fertilization: (i) in ewes (n=4) bred naturally, and in ewes (n=18) after transfer of embryos produced in vitro, and (ii) in single, twin and triplet pregnancies after transfer of in vitro produced embryos. On day 5 after in vitro fertilization, two to three embryos with 16 or more cells were transferred to recipient ewes that were at day 5 after estrus. Pregnancy was verified by real-time ultrasonography on day 45 or later after breeding or embryo transfer (ET). On day 140 of pregnancy, the reproductive tract was collected from all ewes and the following parameters were determined: the number, sex and weight of fetuses, weight of uterus and fetal membranes, weight and numbers of placentomes. Weight of fetuses was similar for naturally bred ewes and ewes after ET, but the weight of individual placentomes was greater ($P<0.05$) for ewes after ET (8.5±0.5 g) than for naturally bred ewes (5.4±1.9 g) and the total weight of placentomes/ewe tended to be greater ($P<0.1$) for ewes after ET (0.66±0.05 kg) than for the naturally bred ewes (0.48±0.03 kg). The number of placentomes/ewe was less ($P<0.01$) for single pregnancies (67.0±4.2) than for twin (87.0±4.1) or triplet (85.7±3.7) pregnancies. The number of placentomes/fetus was greater ($P<0.01$) for single (67.0±4.2), less for twin (43.5±2.0) and least for triplet (28.5±1.2) pregnancies. However, the weight of each individual placentome was greater ($P<0.06$) for triplet (11.2±1.2 g) than for twin (8.3±0.8 g) or single (7.7±0.5 g) pregnancies. For single, twin and triplet pregnancies, the number of fetuses per ewe was negatively correlated with the weight of placentomes/fetus ($r^2= -0.65$; $P<0.003$), the number of placentomes/fetus ($r^2 = -0.86$; $P<0.001$) and the weight of the fetus ($r^2 = -0.80$; $P<0.001$) and positively correlated with the weight of individual placentome ($r^2= +0.50$; $P<0.03$). These data demonstrate the lack of large offspring syndrome, indicating that culture conditions were optimal for production of ovine embryos. The low number of placentomes/fetus seen in multiple pregnancies appears to be compensated for by the increase in the total number of placentomes and the weight of each placentome.

Introduction

In vitro production of embryos from domestic animals is used to augment conventional genetic improvement programs in agriculture and for research purposes. However, the adoption of advanced reproductive technologies for embryo production and transfer in ruminants may result in the occurrence of large offspring syndrome (LOS) also termed as fetal oversize syndrome (Young et al., 1998; Walker et al., 1996; Thompson et al., 1997; Farin et al., 2001). This syndrome has been associated with

increased dystocia and abnormal growth and development at fetal, neonatal and later stages in life. A number of treatments have been shown to cause this perturbation including in vitro culture of embryos and transfer of embryos to recipients (Young et al., 1998; Walker et al., 1996; Thompson et al., 1997; Farin et al., 2001). This emphasizes the need to optimize culture conditions to simulate natural uterine environment.

Several studies have demonstrated the effects of single, twin and triple pregnancies on fetal weight (Brown et al., 1998; Reynolds and Redmer, 1995), but parameters of the gravid uterus have not been evaluated in detail. The objective of this study was to compare various parameters of the gravid uterus on day 140 after fertilization: (i) in ewes bred naturally, and in ewes after transfer of embryos produced in vitro, and (ii) in single, twin and triplet pregnancies after transfer of in vitro produced embryos.

Material and Methods

Non-pregnant crossbred range ewes were divided into two groups, one group was allowed to breed naturally (n=4) and the other (n=18) was subjected to embryo transfer (ET). Ewes of mixed breeds (n = 15) were injected twice daily (morning and evening) with FSH-P (Sioux Biochemical, Sioux Center, IA; Jablonka-Shariff et al., 1994; Stenbak et al., 2001) on days 13 (5 units/injection, day 0 = estrus) and 14 (4 units/injection) of the estrous cycle. On the morning of day 15, ovariectomy was performed to collect ovaries (Reynolds et al., 1998). Oocytes were collected, matured and fertilized in vitro by frozen-thawed semen (Stenbak et al, 2001; Grazul-Bilska et al., 2003). Embryos were cultured for 5 days and then transferred at a stage of 16 or more cells to recipient ewes on day 5 of the estrous cycle. The estrous cycles of the recipient ewes had been synchronized so that their expected day of ovulation coincided with the day of IVF. Synchronization consisted of i.m. injection of $PGF_2\alpha$ (Estrumate, Schering-Plough Animal Health Corp., Union, NJ., 125 mg/ml/injection) in the morning and evening on day 8-12 of the estrous cycle. Two to three embryos with 16 or more cells were transferred to recipient ewes surgically by placing the embryos in the tip of the uterine horn ipsilateral to the ovary containing a corpus luteum. Four ewes were naturally bred with fertile Hampshire rams. The recipient ewes were placed with vasectomized rams beginning on day 6 after embryo transfer to check estrus. In addition, the presence of fetuses was determined by ultrasonography (Classic Ultrasound Equipment Ltd, Tequesta, FL) on day 45 or later after embryo transfer. On day 140 of pregnancy, recipient ewes and the ewes bred naturally were slaughtered and the gravid reproductive tract was collected. Then the number and weight of fetuses, weight of uterus and fetal membranes, weight and numbers of placentomes were determined.

Data were analyzed by using the general linear models (GLM) procedure of the Statistical Analysis System (SAS, 1985). When the F-test was significant, differences between specific means were evaluated using the Duncan test (Kirk, 1982). All data are reported as means ± standard error (SEM).

Results

Several parameters of the gravid uteri evaluated for the naturally bred ewes and for ET recipient ewes are shown in Table 1.

Table 1: Parameters of gravid uteri on day 140 after fertilization for ewes bred naturally and for ewes after transfer of embryos produced in vitro.

Parameter	Pregnancy after natural breeding	Pregnancy after transfer of in vitro produced embryos
Number of pregnant ewes	4	18
Weight of gravid uterus/ewe (kg)	10.48±0.26	13.76±1.13
Weight of fluid /ewe (kg)	1.96±0.26	3.13±0.48
Weight of uterus plus fetal membranes/ewe (kg)	3.11±1.0	2.13±0.13
Weight of fetal membranes/ewe (kg)	Not available	0.45±0.05
Total weight of placentomes/ewe (kg)	0.48 ± 0.03^{A}	0.66 ± 0.05^{B}
Total weight of placentomes/fetus (kg)	0.43±0.08	0.42±0.03
Total number of placentomes/ewe	67.5.0±4.3	77.8±3.4
Total number of placentomes/fetus	57.8±6.8	51.5±4.1
Mean weight of placentome/ewe (g)	5.43 ± 1.96^{a}	8.51 ± 0.53^{b}
Mean weight of fetus (kg)	5.19±0.78	4.92±0.17
Total fetal weight/ewe (kg)	6.78± 0.25 (5 fetuses)	8.49±0.63 (31 fetuses)
Range of fetal weight (kg)	3.1-7.1	2.95-6.4

^{a,b} $P<0.05$, ^{A,B} $P<0.10$; Values differ within a row.

The weight of the gravid uterus, weight of fetal membranes, weight of placentomes/ewe, total number of placentomes, mean weight of fetus and total fetal weight/ewe were similar for the naturally bred ewes and for ewes after ET. However, the mean weight of placentome/ewe was greater ($P<0.05$) for ewes after ET (5.43±1.96 g) than for the naturally bred ewes (8.51±0.53 g). The total weight of placentomes/ewe tended to be greater ($P<0.1$) for ewes after ET (0.66±0.05 kg) than for the naturally bred ewes (0.48±0.03 kg).

Gravid uteri parameters evaluated in ewes after ET for single, twin and triplet pregnancies along with the correlation between the fetal number and the specific parameters are shown in Table 2.

Table 2: Parameters of gravid uteri on day 140 for ewes after ET produced in vitro for single, twin and triplet pregnancies.

Parameter	Single pregnancy	Twin pregnancy	Triplet pregnancy	Correlation coefficient (r) between number of fetuses and specific parameter
Number of pregnant ewes	8	7	3	
Weight of gravid uterus/ewe (kg)	9.58±0.34 [a]	14.98±0.81 [b]	22.03±0.62 [c]	0.948; P<0.001
Weight of fluid /ewe (kg)	1.92±0.24 [a]	3.09±0.57 [a]	6.49±1.05 [b]	0.769; P<0.001
Weight of uterus plus fetal membranes/ewe (kg)	1.69±0.07 [a]	2.24±0.13 [b]	3.04±0.30 [c]	0.840; P<0.001
Weight of fetal membranes/ewe (kg)	0.32±0.02 [a]	0.48±0.03 [b]	0.76±0.16 [c]	0.802; P<0.001
Total weight of placentomes/ewe (kg)	0.51±0.04 [a]	0.71±0.06 [b]	0.95±0.09 [c]	0.783; P<0.001
Total weight of placentomes/fetus (kg)	0.51±0.04 [a]	0.35±0.03 [b]	0.32±0.03 [b]	-0.652; P<0.003
Total number of placentomes/ewe	67.0±4.2 [a]	87.0±4.1 [b]	85.7±3.7 [b]	0.600; P<0.01
Total number of placentomes/fetus	67.0±4.25 [a]	43.5±2.06 [b]	28.5±1.23 [c]	-0.868; P<0.001
Mean weight of placentome/ewe (g)	7.67±0.55 [A]	8.32±0.88 [A]	11.16±1.23 [B]	0.508; P<0.03
Mean weight of fetus (kg)	5.97±0.18 [a]	4.82±0.24 [b]	4.17±0.21 [c]	-0.800; P<0.001
Total fetal weight/ewe (kg)	5.97±0.18 [a] (8 fetuses)	9.64±0.48 [b] (14 fetuses)	12.50±0.63 [c] (9 fetuses)	0.938; P<0.001
Range of fetal weight (kg)	5.0-6.4	3.85-6.35	2.95-5.05	

[a,b,c] P<0.01; [A,B,C] P<0.06; Values with different superscripts differ within a row.

Significant differences were seen for several uterine parameters in the single, twin and triplet pregnancies (Table 2). Weight of the gravid uterus, weight of uterus with fetal membranes, weight of fetal membranes, total weight of placentomes/ewe, and total weight of fetus/ewe were greatest (P<0.001) for triplet, less for twin and least for single

pregnancies. The total number of placentomes/fetus (Fig. 1A) and the mean weight of fetus (Fig. 1B) were greatest (P<0.001) for single pregnancy, less for twin and least for triplet pregnancies. The total weight of placentomes/fetus (Fig.1C) and total number of placentomes/ewe were greater (P<0.01) for single than twin or triplet pregnancies, which were similar. Mean weight of placentomes was similar for single and twin pregnancies and lower (P<0.06) for triplet pregnancy (Fig.1D).

For single, twin and triplet pregnancies, the number of fetuses per ewe was negatively correlated with the weight of placentomes/fetus (P<0.003), the number of placentomes/fetus (P<0.001) and the weight of the fetus (P<0.001) and positively correlated with the weight of individual placentome (P<0.03) (Table 2).

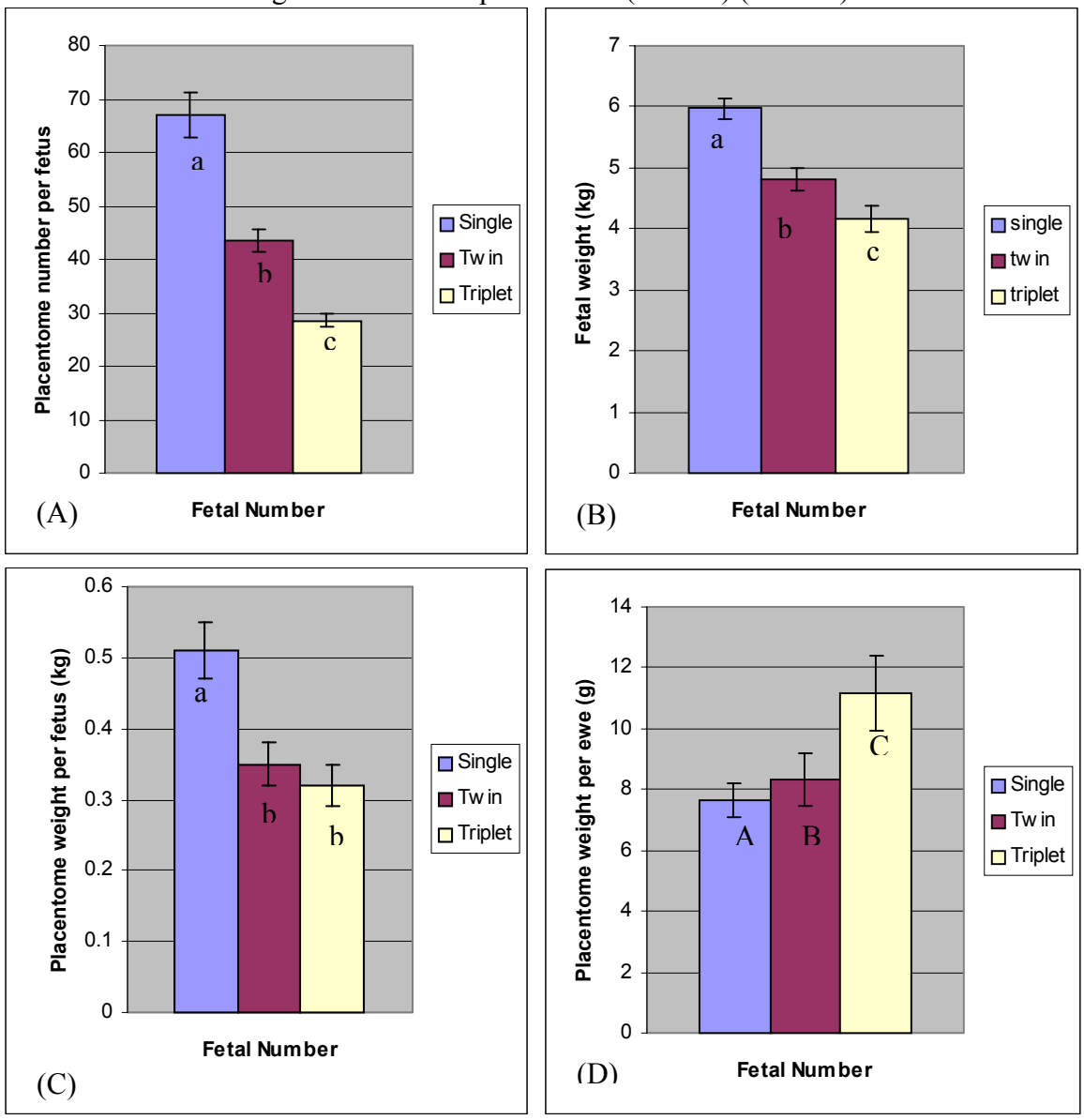

Figure 1. Placentome number per fetus (A), fetal weight (B), the placentome weight per fetus (C), and placentome weight per ewe (D) for single, twin and triplet pregnancies. ^{a,b,c} P<0.01; ^{A,B,C} P<0.06; Values with different subscripts differ.

Discussion

In the present experiment, all uterine parameters were similar for naturally bred ewes and ewes after ET, except the weight of individual placentomes, which was greater for ewes after ET than for naturally bred ewes. The range of weights of fetuses for naturally bred ewes and for ET ewes were similar. Therefore, in the present study we did not observe any symptoms of LOS. Numerous studies have indicated an increase in birth weight or large offspring syndrome in in vitro produced embryos compared with those produced by natural breeding in offspring from cattle and sheep (Young et al., 1998; Walker et al., 1996; Thompson et al., 1997; Farin et al., 2001; Bertolini et al., 2002). For cows, Bertolini et al. (2002) observed an increased size of cotyledons in pregnancies after transfer of in vitro produced embryos compared with embryos produced in vivo, but they did not weigh these cotyledons. Our data confirm the increase in individual placentome weight, but demonstrate no significant differences in birth weights between in vivo and in vitro produced embryos, indicating the culture conditions we used were optimal for production of ovine embryos.

The single, twin and triplet pregnancies affected the uterine parameters evaluated. The close relationship between fetal weight and placental size has been well established (Ford et al., 1984; Metcalfe et al., 1988; Ferrel, 1989; Ferrel and Reynolds, 1992). The association of multiple fetuses with low birth weight and low placental weight also has been reported for several species (Ibsen, 1928; Warwick, 1928; Alexander, 1964). However, individual fetal weight, the number of placentomes/fetus and the weight of placentomes per fetus were greater for single pregnancy than for twin or triplet pregnancies. The total fetal weight per ewe, weight of fetal membranes per ewe, total weight of placentomes per ewe and total number of placentomes per ewe increased with the increase in fetal number. Thus, although twin and triplet pregnancies support an overall increase in fetal and placental mass, the size of individual fetuses and their placentas is reduced. The reduced number of placentomes per fetus and the reduced weight of placentomes/fetus seen in multiple pregnancies appear to be compensated for by the increase in the total number of placentomes and the weight of individual placentomes per ewe.

In summary, the gravid uterine parameters were not affected by the method of embryo production but were affected by the number of fetuses present. Multiple fetuses are known to have a lower birth weight, increased neonatal mortality, reduced growth rate and poor performance in subsequent stages of life (Huffman et al., 1985; Alexander, 1974). These offspring never achieve their complete genetic potential, which has a negative impact on subsequent genetic selection processes in the flock. Studies are underway to examine fetal growth and placental development in multiple fetuses.

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