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Pregnancy rates and gravid uterine parameters in single, twin and triplet pregnancies in naturally bred ewes and ewes after transfer of in vitro produced embryos

Anna T. Grazul-Bilska[a,b,c,*], Disha Pant[a], Justin S. Luther[a,c], Pawel P. Borowicz[a,c], Chainarong Navanukraw[a], Joel S. Caton[a,c], Marcy A. Ward[a,c], Dale A. Redmer[a,b,c], Lawrence P. Reynolds[a,b,c]

[a] *Department of Animal and Range Sciences, North Dakota State University, Fargo, ND 58105, USA*
[b] *Cell Biology Center, North Dakota State University, Fargo, ND 58105, USA*
[c] *Center for Nutrition and Pregnancy, North Dakota State University, Fargo, ND 58105, USA*

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Abstract

The objectives of this study were to: (1) evaluate the pregnancy rates after transfer of embryos produced in the presence or absence of epidermal growth factor (EGF) during in vitro maturation, and (2) compare several variables of the gravid uterus on day 140 after fertilization in single, twin and triplet pregnancies in ewes ($n = 12$) bred naturally and in ewes ($n = 18$) after transfer of embryos produced in vitro. Oocytes collected from FSH-treated ewes ($n = 18$) were collected from all visible follicles and cultured in maturation medium with or without EGF. Oocytes were then fertilized in vitro by frozen-thawed semen. On day 5 after fertilization, embryos with $\geq$16 cells were transferred to recipient ewes ($n = 39$). In addition 12 ewes were bred naturally. Pregnancy was verified by real-time ultrasonography on day 45 or later after embryo transfer (ET) or breeding. On day 140 of pregnancy, the reproductive tract was collected from all ewes and the following parameters were determined: the number, sex, weight and crown to rump length (CRL) of fetuses, weights of gravid uterus and fetal membranes, and weight and number of placentomes. Presence of EGF in maturation medium increased ($P < 0.04$)

* Corresponding author. Tel.: +1 701 231 7992; fax: +1 701 231 7590.
E-mail address: Anna.Grazul-Bilska@ndsu.edu (A.T. Grazul-Bilska).

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cleavage rates (78% versus 59%) and percentage of ≥16 cell embryos on day 5 after fertilization (62% versus 40%). Pregnancy rates tended to be greater ($P < 0.1$) after transfer of embryos matured in the presence of EGF (52%) than in the absence of EGF (39%). EGF presence in maturation medium did not affect any variables of gravid uterus or fetal weight. For single pregnancies in naturally bred ewes and ewes after ET all uterine variables were similar. For twin pregnancies, weight of gravid uterus, weight of uterus plus fetal membranes, total weight of placentomes/ewe, mean weight of individual placentome, mean weight of fetus, total fetal weight/ewe and CRL were greater ($P < 0.0001$–0.04) for ewes after ET than for ewes bred naturally. The weights of gravid uterus, fluid, uterus plus fetal membranes, fetal membranes, total placentomes/ewe, mean weight of individual placentome and total fetal weight/ewe were greater ($P < 0.0001$–0.08) for triplet pregnancies in ewes after ET than single and twin pregnancies in ewes naturally bred or after ET. The number of placentomes/fetus was greatest ($P < 0.0001$–0.06) in single pregnancies in ewes bred naturally and after ET fewer in twin pregnancies in ewes bred naturally and after ET and fewest in triplet pregnancies in ewes after ET. The total number of placentomes/ewe was greatest ($P < 0.0001$–0.06) for twin pregnancies in ewes naturally bred, fewer in single pregnancies in ewes naturally bred and twin and triplet pregnancies after ET, and fewest in single pregnancies in ewes after ET. The mean weight of fetus was greater ($P < 0.0001$–0.07) in single pregnancies in ewes naturally bred or after ET than in twin or triplet pregnancies in ewes naturally bred or after ET. The CRL was the lowest ($P < 0.01$) in twin pregnancies in ewes bred naturally. For pregnancies after natural breeding and after ET, the number of fetuses/ewe was negatively correlated ($P < 0.03$–0.0001) with the weight of placentomes/fetus, the number of placentomes/fetus, the mean weight of the fetus and CRL, and was positively correlated ($P < 0.0001$–0.05) with weight of gravid uterus, the total number of placentomes/ewe and total fetal weight/ewe. These data demonstrate that the presence of EGF in maturation medium increases the rates of cleavage and early embryonic development, and has a tendency to enhance rates of pregnancy but does not affect variables of the gravid uteri in ewes after transfer of in vitro produced embryos. Transfer of embryos produced in vitro affected some uterine variables in twin but not single pregnancies to compare with pregnancies after natural breeding. In addition, culture conditions in the present experiment did not create large offspring syndrome. The low number of placentomes/fetus seen in triple pregnancies appears to be compensated for by the increase in the weight of each individual placentome.

Keywords: Epidermal growth factor; In vitro fertilization; Embryo transfer; Gravid uterus; Ewe

1. Introduction

In vitro production (IVP) of embryos and embryo transfer (ET) are used to augment conventional genetic improvement programs in agriculture and for research purposes (Ishwar and Memon, 1996; Earl and Kotaras, 1997; Loi et al., 1998). However, IVP of embryos results in a reduced percentage of transferable embryos compared to in vivo conditions (Thompson, 1997; Van Wagtendonk-de Leeuw et al., 1998). Therefore, numerous factors including gonadotropins, steroid hormones, growth hormone and several growth factors including epidermal growth factor (EGF) have been tested as supplements to maturation, fertilization and/or culture media in order to improve the efficiency of IVP systems (Watson et al., 1994; Lonergan et al., 1996; Guler et al., 2000). Epidermal growth factor has been demonstrated to enhance oocyte maturation and blastocyst formation in several species