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Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows

D. Rizos,* F. Carter,† U. Besenfelder,‡ V. Havlicek,‡ and P. Lonergan†[1]
*Departamento de Reproducción Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain
†School of Agriculture, Food Science and Veterinary Medicine, College of Life Sciences, University College Dublin, Belfield, Dublin 4, Ireland
‡Reproduction Centre-Wieselburg, University of Veterinary Medicine Vienna, Veterinärplatz 1, A-1210, Austria

ABSTRACT

Infertility in dairy cattle is a multifactorial problem that may be linked to follicle development and the quality of the ovulated oocyte, to sperm transport and fertilization, to the reproductive tract environment, or to a combination of these factors. Using a state-of-the-art endoscopic embryo transfer technique, the aim of this study was to compare the ability of the reproductive tract of postpartum dairy cows and nulliparous heifers to support the development of early embryos to the blastocyst stage. Bovine embryos of 2 to 4 cells (n = 1,800) were produced by in vitro maturation and fertilization of oocytes derived from the ovaries of slaughtered cattle. The estrus cycles of nulliparous Holstein heifers (n = 10) and postpartum Holstein cows (n = 8, approximately 60 d postpartum) were synchronized using an 8-d controlled internal drug release device coupled with prostaglandin injection. On d 2, one hundred 2- to 4-cell embryos were endoscopically transferred to the oviduct ipsilateral to the corpus luteum. Five days later, on d 7, the oviduct and uterus were flushed nonsurgically to recover the embryos. The number of embryos developing to the blastocyst stage was recorded immediately at recovery and following overnight culture in vitro. A representative number of blastocysts from heifers and cows were stained to assess cell number. Progesterone concentrations were lower in cows than in heifers on d 5, 6, and 7 (d 7 = 2.39 ± 0.33 vs. 5.34 ± 0.77 ng/mL, respectively). More embryos were recovered from heifers than cows (79.0 ± 7.0 vs. 57.2 ± 11.4%). Of the embryos recovered, 33.9 ± 3.6% had developed to the blastocyst stage in the heifer oviduct compared with 18.3 ± 7.9% in the postpartum cow oviduct. There was no evidence of a difference in blastocyst quality as evidenced by total cell number in the blastocysts (71.2 ± 5.7 vs. 67.0 ± 5.3, respectively). In conclusion, the reproductive tract of the postpartum lactating dairy cow may be less capable of supporting early embryo development than that of the nonlactating heifer, and this may contribute to the lower conception rates observed in such animals.

Key words: oocyte, embryo, infertility, embryo transfer

INTRODUCTION

Low reproductive efficiency is a worldwide problem affecting the dairy industry and was the subject of recent reviews (Lucy, 2001; Leroy et al., 2008a,b). Although in some locations this situation is exacerbated by problems of heat stress in summer (Hansen, 2007), even in more moderate climates a steady decline in fertility has been noted. There is evidence of an association between high milk production and the lower conception rate observed in cows (25–40%) compared with heifers (55–65%; Pursley et al., 1997; Lucy, 2001).

Infertility in dairy cattle is a multifactorial problem that may be linked to suboptimal follicle development associated with reduced estrus exhibition and, consequently, low detection, suboptimal oocyte quality, altered sperm transport, problems at fertilization, a suboptimal reproductive tract environment to support embryo development, or a combination of these factors. One of the obstacles to achieving a better understanding of the causes of reduced fertility is the difficulty in identifying the respective contribution made by each of these factors.

One approach to understanding the causes of infertility or identifying at which stage problems arise has been to flush embryos at different stages after AI to determine fertilization failure and timing of embryonic mortality. Fertilization rate is very high in beef heifers (>90%) and most embryonic mortality occurs by d 16 (Dunne et al., 2000). Sartori et al. (2002) compared lactating and nonlactating (either nulliparous heifers or dry cows) Holstein cattle in terms of fertilization rate and early embryo development after AI and found that fertilization was reduced only during summer in

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[1]Corresponding author: pat.lonergan@ucd.ie

lactating dairy cows; however, lactating dairy cows had reduced embryo development compared with nonlactating females. This finding was confirmed by Leroy et al. (2005), suggesting that the ability of the reproductive tract to support normal embryo development may be impaired in lactating cows. Oocyte quality cannot be ruled out as a contributing factor because it is clear from in vitro fertilization (**IVF**) studies, where typically 80% of inseminated oocytes cleave and 30 to 40% develop to blastocysts, that fertilization success is no guarantee of future development.

One way of experimentally separating potential issues surrounding the follicle and oocyte from issues related to the reproductive tract environment is to use ovum pickup coupled with IVF. Although several authors reported development of ovum pickup-IVF embryos in dairy cows, few have compared development from dairy cows and heifers directly. Rizos et al. (2005) found no difference in the proportion of good-quality oocytes undergoing IVF and development to the blastocyst stage between lactating cows and heifers, whereas Snijders et al. (2000) observed that a lower proportion of oocytes recovered from dairy cows with a higher genetic merit for milk production underwent cleavage or developed to the blastocyst stage in vitro than those from cows of average genetic merit.

The use of embryo transfer allows the endogenous oocyte to be removed as a confounding factor and thus bypass the events of follicle development and oocyte quality. Conception rate was higher for embryo transfer (**ET**) than AI when fresh or frozen in vivo-produced embryos were used (Al-Katanani et al., 2002). Consistent with this, Demetrio et al. (2007) reported higher conception rates in lactating Brazilian dairy cows following the transfer of fresh embryos derived from nonlactating cows compared with after AI. In contrast, Sartori et al. (2006) compared ET with AI in dairy cows in Wisconsin at cooler times of the year and found no difference in conception rate.

The above studies involved routine ET at d 7 and tested only the ability of the reproductive tract to support development from d 7 onwards. None have examined the ability of the tract to support embryo development in the period encompassing the events between fertilization and d 7. The reproductive tract environment undoubtedly plays a significant role in determining developmental outcome, as evidenced by the described role of progesterone in modifying endometrial gene expression (Satterfield et al., 2006; Forde et al., 2009) and the association of progesterone in the days immediately following conception with subsequent conceptus elongation (Mann and Lamming, 2001; Satterfield et al., 2006; Carter et al., 2008) and pregnancy rate (Stronge et al., 2005).

In this study, the hypothesis was that part of the difference in fertility between heifers and postpartum lactating dairy cows could be explained by differences in the ability of the reproductive tract (oviduct and uterus) to support early embryo development and that this would be related to circulating progesterone concentration. To test this hypothesis in single-ovulating animals would be extremely challenging because of the numbers of animals required. Therefore, using a state-of-the-art endoscopic transfer technique, we transferred 1,800 in vitro-produced embryos to the oviducts of nulliparous Holstein-Friesian heifers and postpartum lactating Holstein-Friesian cows and assessed their development to the blastocyst stage following recovery on d 7.

MATERIALS AND METHODS

In Vitro Production of Bovine Embryos

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (Poole, UK). The techniques for producing embryos in vitro were described previously (Rizos et al., 2002). Immature cumulus oocyte complexes were obtained by aspirating follicles on bovine ovaries collected at slaughter. Cumulus oocyte complexes were matured for 24 h in TCM-199 medium supplemented with 10% (vol/vol) fetal calf serum (**FCS**) and 10 ng/mL of epidermal growth factor at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity. For IVF, matured cumulus oocyte complexes were inseminated with frozen-thawed, Percoll-separated bull sperm at a concentration of 1×10^6 spermatozoa/mL. Gametes were coincubated at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity.

At approximately 20 h post-insemination, presumptive zygotes were denuded, divided into groups of 40 to 50, and transferred to 500-μL culture wells. The basal medium for all embryo culture was synthetic oviduct fluid (**SOF**) supplemented with 5% FCS. Cleavage rate was recorded at 48 h post-insemination and only cleaved embryos were used for transfer.

Preparation of Recipient Animals and Embryo Transfer

All experimental procedures involving animals were licensed by the Department of Health and Children (Dublin, Ireland), in accordance with the Cruelty to Animals Act (Ireland 1897) and European Community Directive 86/609/EC, and sanctioned by the University College Dublin Animals Research Ethics Committee.

The estrus cycles of nulliparous Holstein heifers (n = 10; BW = 349.1 ± 12.21 kg; age = 500 ± 32 d) and