Programming of development during embryonic and fetal life in cattle and its consequences for subsequent production

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Introduction – capacity for health and productive function begins to form *in utero*

At an early stage in their education, animal scientists are taught that an animal's phenotype depends on a combination of genetic and environmental effects. Indeed, efforts to increase the productive capacity of livestock have depended largely on two approaches – selection of allelic variants to genes that confer superior production and manipulating the environment to enhance the ability of animals to realize the potential of those genes. From a genetics point of view, environment includes everything that is not genetic including, as examples, nutrition, meteorological conditions, exposure to pathogens, and human interventions such as treatment with growth regulators or reproductive hormones, vaccinations and other veterinary procedures.

While the importance of the environment for optimal health and production is well recognized, it is less widely understood that the function of an adult organism depends not only on environmental determinants after birth but also on environmental conditions it was exposed to before birth during embryonic and fetal development. This concept has been variously termed developmental programming, fetal programming or developmental origins of health and disease.

For mammals, the prenatal environment of a developing embryo or fetus is established by its mother and changes in maternal environment can alter postnatal phenotype of the developing organism. We are only beginning to understand the degree to which developmental programming affects health and productive function of cattle. Experiments performed to date are indicative that developmental programming can occur throughout gestation, from the early stages of development of the preimplantation embryo to the final weeks of gestation. Moreover, developmental programming can shape many functions of adult cattle and in a way that influences economically-important traits.

The purpose of this review is to provide some examples of developmental programming in cattle to illustrate the wide range of influences the prenatal environment can have on postnatal function. Emphasis will not be on mechanisms although these are likely to include epigenetic modifications in the developing organism as well as in patterns of development of specific cell lineages that give rise to adult tissues. By providing examples of developmental programming, we can appreciate the potential for manipulating the embryo and fetus to positively or negatively affect health and production of the postnatal animal. The take-home message is that there is opportunity for animal managers and veterinarians to use information about developmental programming to increase the efficiency of milk and meat production.

Programming during the preimplantation period as revealed by the in vitro produced embryo

One of the most extreme examples of an abnormal environment during the preimplantation period is the in vitro produced (IVP) embryo. Such embryos are exposed to concentrations of nutrients, salts and growth factors that differ from those present in vivo and the substratum for culture (usually plastic) is different from that established by the oviductal and endometrial epithelium. Not surprisingly, then, the IVP embryo differs from the embryo produced in vivo in terms of morphology, gene expression, cryotolerance and competence to establish pregnancy (see review by Hansen, 2015 and recent data on competence to establish pregnancy by Ferraz et al., 2016).

Alterations in the characteristics of the embryo caused by IVP can result in offspring with altered characteristics in the postnatal period. This concept was first recognized by observations that calves born following IVP sometimes exhibit a range of developmental disorders termed the large offspring syndrome that is characterized most prominently by excessive body growth (Farin et al., 2006). Recently, it was reported that alterations in physiological function extend to adult life, at least when IVP embryos are produced using fertilization with reverse-sorted sperm (Siqueira et al., 2017). In this study, characteristics of females born from embryo transfer using embryos produced by IVP with conventional semen (IVP-conv; 345 heifer calves born alive and 218 with lactation records), IVP with reverse-sorted semen (IVP-RS; 685 heifers born alive and 430 with lactation records) and multiple-ovulation embryo transfer (MOET; 249 heifer calves born alive and 183 with lactation records) were compared to females born as a result of artificial insemination (AI; 3,465 AI females born alive and 2,037 with production records for first lactation). Lactational performance of the offspring was adjusted by using genomic estimates for milk, fat or protein as a covariate. The most striking difference between groups was in lactational performance during first lactation (Table 1). In particular, IVP-RS females produced less milk, fat and protein than cows of other groups. The difference in average projected actual milk yield between cows produced using IVP-sexed and cows produced by AI was 321 kg.

Table 1. Effects of technique used to produce a pregnancy on first-lactation milk yield of the resultant offspring after adjusting for genomic predicted transmitting ability for yield.[1]

	AI	IVP-conv	IVP-RS	MOET	*P*-value
Projected actual milk yield, 305 d (kg)	11038±31^{a}	10946±100^{ab}	10717±76^{b}	10891±149^{ab}	0.0014
Projected actual fat yield, 305 d (kg)	388.3±1.2^{a}	385.6±3.9^{ab}	377.1±3.0^{b}	384.7±5.8^{ab}	0.0072
Projected actual protein yield, 305 d (kg)	334.6±1.0^{a}	336.5±3.3^{a}	327.1±2.5^{b}	331.2±4.8^{ab}	0.0318

[1] The P-values are for the main effect of reproductive technique. Within a row, means without a common superscript differ at $P < 0.05$.

It should be noted that these results do not come from a controlled experiment and there could be unknown biases that affect the results including the fact that neither dams nor sires of the animals studied were assigned randomly to treatment. In mice, the specific culture system used to produce embryos can affect the nature of developmental programming (Fernández-Gonzalez et al., 2004; Donjacour et al. 2014; Rexhaj et al., 2015) and effects observed by Siqueira et al. (2017) might not be observed with other IVP systems. The fact that IVP was only associated with lower milk yield when reverse sorting was used for fertilization is suggestive that damage of the sperm during sorting may have been responsible for the observed programming.

There are two other examples where the specific culture conditions in which IVP embryos developed changed the characteristics of the resultant offspring. Addition of the cytokine colony-stimulating factor 2 (CSF2) to culture medium on day 5 of culture (when embryos are typically at the morula stage) resulted in changes in growth profiles of the resultant calves produced after embryo transfer (Kannampuzha-Francis et al., 2015). There were no differences in birth weight or withers height between heifers derived from IVP with or without CSF2. Subsequently, however, body growth (weight and height) through 13 mo of age was greater for the CSF2 group. In another experiment, addition of the WNT antagonist dickopf-related protein 1 (DKK1) to culture medium at day 5 of culture resulted in calves of lower birth weight than calves derived from embryos cultured without DKK1 (Tribulo et al., 2017). These results suggest that, for embryos produced in vivo, changes in maternal environment could conceivably result in changes in secretion of regulatory molecules like CSF2 or DKK1 from the maternal reproductive tract that alter the developmental program of the embryo. Studies to examine this hypothesis are warranted.

Effects of maternal protein nutrition on postnatal growth and reproduction

Experiments in Australia with pregnant beef heifers indicate that protein nutrition during pregnancy can affect prenatal and postnatal growth of male and female offspring (Micke et al., 2010a, 2010b, 2011). In these experiments, pregnant beef heifers were fed diets that were either high (240% of recommended intake) or low in crude protein (70% of recommended intake) during the first or second trimesters of gestation. There was no effect of maternal diet in the first trimester on calf birth weight but postnatal growth was affected in a sex-specific manner. For male offspring, those that were derived from dams receiving a low protein diet were heavier than those from dams fed a high protein diet. The opposite trend was apparent for female offspring. Cross-sectional area of the *semitendinosus* and *longissimus dorsi* muscles followed a similar pattern: low protein nutrition of the dam was associated with larger muscle size for males but not for females. Developmental programming caused by maternal protein nutrition during the second trimester was different than for programming during the first trimester. Calves from mothers fed a low protein in the second trimester had lower birth weights than calves fed a high protein diet. After 29 days of age, there was no effect of treatment on body weight or, in general, on muscle growth. Cross-sectional area of *longissimus dorsi* at slaughter was increased for male and female progeny from low protein dams although this effect was removed when area was adjusted for carcass weight.

There is a report by Martin et al. (2007) that fertility of female offspring can also be affected by maternal protein nutrition during the last trimester of gestation. Heifers born from dams that received a protein supplement three times per week were more fertile in their first breeding season than heifers from dams that were not supplemented. In particular, maternal diet did not affect birth weight or age at puberty but more heifers from supplemented dams became pregnant after a 45 day breeding season (93%) than heifers from non-supplemented dams (80%). Differences were particularly pronounced when examining the percent of heifers that became pregnant early in the breeding season. The percent of heifers that calved in the first 21 days of the breeding season was 77% for heifers from supplemented dams vs 49% for heifers from control dams.

Antral follicle count of heifers can be influenced by maternal energy intake of their dams

An important determinant of fertility in dairy cattle is the number of ovarian follicles recruited into the growing pool during a follicular wave. The size of the follicular pool is usually estimated as the number of follicles ≥ 3 mm in diameter (antral follicle count; AFC) or by measuring concentrations of antimullerian hormone (AMH) in the blood. Several studies in dairy cattle found that the size of the AFC was associated with fertility and productive herd life with a low or high AFC or AMH concentration being deleterious and with an intermediate AFC or AMH concentration being desirable (Mossa et al., 2012; Jimenez-Krassel et al., 2015, 2017).

One determinant of AFC could be the nutritional environment the embryo or fetus is exposed to during early- and mid-gestation. Mossa et al. (2013) individually fed beef heifers a diet that provided either 1.2 or 0.6 times the energy requirements for maintenance from 11 days before insemination until Day 110 of gestation. As illustrated in Table 2, heifers born from undernourished cows had a reduction in AFC from 7-86 weeks of age and in the number of visible antral follicles when the reproductive tract was collected at slaughter at 95 wk of age. In addition, circulating AMH concentrations from 7-86 wk of age were lower in heifers from undernourished cows.

Table 2. Characteristics of heifers as affected by energy intake of their dams from 11 days before insemination to 110 days of gestation (Mossa et al., 2013).

Trait	Control dams	Underfed dams
Birth weight (kg)	40.5 ± 1.0	39.5 ± 2.0
Placental weight at birth (kg)	5.6 ± 0.6	5.3 ± 0.5
Age at puberty (weeks)	95 ± 0.2	95 ± 0.2
Mean antral follicle ≥ 3 mm count, 86 wk of age	23.6 ± 19.9	15.8 ± 1.8*
Visible antral follicles at slaughter, 95 wk of age	47 ± 5	32 ± 6*

* Treatment effect, P<0.05

Conclusions and implications

This paper represents a sampling of experiments showing that events in the prenatal life of a developing bovine can have effects that alter the function of the animal after birth. Clearly, more work is needed to identify aspects of physiology of the pregnant female that cause long-term changes in its offspring and to identify mechanisms responsible for developmental programming. Doing so will generate new opportunities to maximize an animal's genetic potential for production by allowing us to shape postnatal function through manipulation of maternal environment during gestation.

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