of non-productive disorders, the symptoms are pretty inapparent, and the clinical diagnosis is challenging. Ultrasonography is an advantageous diagnostic technique that can be easily applied in farm conditions. The etiological diagnosis can be made in living animals by bronchoalveolar lavage or after necropsy with an aseptic sampling of affected lung tissue and mediastinal lymph nodes.

K24

mRNA therapy to induce passive immunity in ruminants

Amelia R. Woolums[1], Merrilee Thoresen[1], E. Heath King[1], Santiago Cornejo[1], Hannah E. Peck[2], Daryll Vanover[2], Philip J. Santangelo[2].

[1]Mississippi State University, USA; [2]Emory University, USA.

Objectives: Our objective was to determine whether messenger RNA (mRNA) treatment of a ruminant mucosal surface could lead to rapid expression of antibody, which could provide passive immunity to infection within hours of treatment.

Materials and Methods: Initial studies were conducted in sheep. Yearling non-pregnant Kathadin-cross ewes were treated intravaginally with a topical spray of mRNA encoding a broadly neutralizing antibody to human immunodeficiency virus. Subsequently, Holstein calves weighing 127 – 172 kg were treated by respiratory aerosol with mRNA encoding a neutralizing antibody to influenza. At 24 hours or later times post treatment, animals were euthanized to evaluate expression of antibody on treated mucosal surfaces.

Results: In both sheep and cattle, expression of neutralizing antibody on treated mucosal surfaces was confirmed within 24 hours after treatment. In cattle treated by respiratory aerosol, expression was widely evident in the treated lung. Microscopic evaluation indicated that mucosal epithelial cells were transfected by the topically applied mRNA and then expressed antibody. Treated animals showed no adverse reactions during treatment, and no evidence of inflammation in treated tissues was evident postmortem.

Conclusions: While studies to date have evaluated expression of antibodies to human pathogens, they nevertheless demonstrate that ruminants can be induced to produce specific neutralizing antibodies rapidly after topical treatment of mucosal epithelia with mRNA. This approach merits further investigation as a method to provide rapid local protection from a variety of infectious agents.

Reproduction in Dairy Cattle

K27

The implications of spontaneous versus synchronized ovulations on the reproductive performance of high-producing dairy cows

Paul Fricke.

University of Wisconsin – Madison, USA.

In species classified as induced ovulators, the LH surge is induced by the act of coitus thereby precisely timing insemination relative to ovulation. Lactating dairy cows are spontaneous ovulators. Thus, the LH surge is induced by a cascade of endocrine events beginning with an increase in follicular estradiol in the absence of luteal progesterone, a GnRH surge from the hypothalamus is then followed by an LH surge from the anterior pituitary, followed by ovulation. Increased estradiol in the absence of progesterone causes behavioral estrus which is manifested by increased physical activity and standing to be mounted. Detection of behavioral estrus either visually, through the use of estrus detection aids, or via automated activity monitoring systems is widely used to determine timing of AI. Cow-related factors that limit service rates include the association of high milk production and duration of estrus, ovulation failure after estrus, ovulation in the absence of behavioral estrus, and anovular conditions which affect 20% to 30% of dairy cows at the end of the voluntary waiting period. One factor that limits fertility to spontaneous estrus is the high variation among cows in the timing of ovulation relative to increased activity resulting in imprecise timing of AI relative to ovulation. Another factor is the high rate of hepatic metabolism of estradiol and progesterone associated with high feed intake in high-producing cows. Under this endocrine milieu, development of preovulatory follicles from deviation until ovulation occurs in a prolonged low-progesterone environment marked by high frequency LH pulses that overstimulate the oocyte and compromise fertility similar to a persistent follicle. Development of the Ovsynch protocol turned dairy cows into induced ovulators, which allowed for increased service rates and precise timing of AI relative to ovulation. Subsequent modifications of the Ovsynch protocol, including presynchronization strategies and complete induction of luteal regression, lead to fertility programs that yield greater fertility than that of a detected estrus in high-producing dairy cows.

K29

Optimizing use of sexed and beef semen in dairy heifers

Paul Fricke.

University of Wisconsin – Madison, USA.

Our objective was to evaluate reproductive management programs for submission of Holstein heifers for first insemination with sexed semen. Nulliparous Holstein heifers (n = 736) from three commercial farms were randomized within farm to one of three treatments for first AI with sexed semen: 1) **CIDR5** (d -6, GnRH +CIDR; d -1, PGF_{2a} -CIDR; d 0, PGF_{2a}; d 2, GnRH+TAI); 2) **CIDR6** (d -6, GnRH +CIDR; d -1, PGF_{2a}; d 0, PGF_{2a} -CIDR; d 2, GnRH+TAI); and 3) **EDAI** (PGF_{2a} on d 0 followed by once daily estrus detection and AI). Heifers detected in estrus 24 h before TAI (d 1) were inseminated and the protocol was discontinued. All heifers were inseminated with sexed semen (ABS Sexcel™ Sexed Genetics, DeForest, WI) from sires that were randomly allocated between treatments within each farm, and AI technicians were blind to treatment at AI. Heifers were followed for 84 d after first service to determine days to AI and pregnancy. Actual farm costs (US$) were used for hormonal treatments, detection of estrus, semen and AI, pregnancy diagnosis, and feed ($1.70/heifer/d) to calculate cost per pregnancy. Feed costs for nonpregnant heifers or heifers moved to a bull pen during the 84-d breeding period (n=112) were allocated to the feed costs for heifers that became pregnant during the 84-d breeding period. Pregnancy outcomes were analyzed using the GLIMMIX procedure of SAS with farm included as a random effect in the model. Costs were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and farm as a random effect in the model. Delaying CIDR removal decreased early expression of estrus before scheduled TAI (0.004%vs. 27.8%); however, CIDR5 heifers tended to have more P/AI at 35 (52.9% vs. 45.3% vs. 45.8%) and 64 (51.8% vs. 44.8% vs. 44.9%) d after AI than CIDR6 and EDAI heifers, respectively. Overall, CIDR5 and CIDR6 heifers had fewer days to first AI and pregnancy than EDAI heifers which resulted in less feed costs than EDAI heifers due to fewer days on feed until pregnancy. Despite greater hormonal treatment costs for CIDR5 heifers, costs per pregnancy were $16.66 less for CIDR5 than for EDAI heifers. In conclusion, delaying CIDR removal by 24 h within a 5-CIDR-Synch protocol suppressed early expression of estrus before TAI, but tended to decrease P/AI for heifers inseminated with sexed semen. Further, submission of heifers to a 5-d CIDR-Synch protocol for first AI tended to increase P/AI and decrease the cost per pregnancy compared to EDAI heifers.

Udder Health and Mastitis

K30

How the milking machine influences mastitis

Ian Ohnstad.

The Dairy Group (UK).

Objectives: A fully functioning milking machine, correctly installed, maintained and operated will milk the vast majority of cows effectively, efficiently and with minimal adverse effects on udder health and cow behaviour.

Although we continually improve our understanding of the relationship between the cow, machine and operator, there remains room for improvement. Although the milking machine is often blamed for high somatic cell counts and clinical mastitis only 6 to 20% of new mastitis infections were related to the affects of the milking machine, either directly or indirectly (Mein, 2004).

Materials and Methods: However, poorly operating milking equipment can lead to sub-optimal teat conditions and, but not always, lead to udder health problems (IDF Bulletin, 1994). In addition, our scientific knowledge of the impact of machine milking and its use on udder health has often not been passed down to the milking staff.

The essential elements of a milking machine are the creation of vacuum, regulation of that vacuum at an appropriate level for the milking system and a means to alternate vacuum and atmospheric pressure in the pulsation chamber.

Continual exposure to vacuum leads to teats becoming congested with circulatory fluids leading to cow discomfort and compromised milk let down. The former leads to lowered resistance of the teat canal to bacterial invasion (Teat Club International, 2001). When atmospheric pressure is applied to the pulsation chamber the milking liner closes around the teat and vacuum at the teat end is relieved and the teat is massaged, resulting in the maintenance of blood circulation and minimising congestion. However, the closing liner applies a compressive load on the teat that can lead to excess keratin production near the teat end (Reinemann, ????), which in itself can impair the first line of the udder´s defence against bacteria.

When vacuum is applied to the pulsation chamber the liner opens and milk flows from a positive to negative pressure. A complete pulsation cycle is from the start of liner opening to the end of liner closing. The frequency varies between manufacturers, being typically 55 to 62 cycles per minute for milking cows.

Regardless of whether cows are milked in a high- or low-level system, the vacuum level at the teat end during peak flow rate should be in the range of 32.0 – 42.0 kPa (ISO 5707: 2007) to milk cows gently and efficiently.

How the milking machine influences udder health

Although the machine can cause short, medium or long-term changes in teat condition, environmental factors can also induce same. In practical situations, it will often be the combination of both that leads to increased levels of sub- and clinical mastitis.