the most common microorganism isolated in cattle, penicillin procainic is often the first choice antibiotic. In the presence of a severely contaminated wound, antibiotics against gram negative should be considered. In calves with septic joints, the antibiotic chosen should have an effect on *Mycoplasma* spp if no organism is isolated and the umbilicus is unlikely to be the cause specially if there is a history of *Mycoplama* spp on the farm or clinical signs associated to it (otitis, pneumonia, arthritis, mastitis). The duration of the treatment should be 2 to 3 weeks after the beginning of clinical improvement. Other route has been described: intra-articular injection, intra-venous under tourniquet and antibiotics incorporated in a slow release medium. Removal of infected tissue, debris and inflammatory mediators in the joint is essential for normal return to previous function. The goals of joint lavage are to remove debris and dilute the abnormal constituent in the joint. Joint lavage is performed in different way: tidal, through and through and arthroscopy. The size of the needle used are 16 G to 14G in calves and 14G to 5mm canula in adult. Arthrotomy is performed if the medical treatment failed or the joint is filled up with fibrin or pus and through and through lavage is impossible. Sites of arthrotomy are the same as the arthrocentesis. The incision should be long enough to allow adequate drainage and introduction of a forceps to remove fibrin. More than one incision per joint is necessary to access the entire cavity and improve the debridement. The incisions are covered with a bandage or stents and additional lavage are performed if necessary. Arthrodesis is the final solution when no treatments were efficient or because of the chronicity of the disease, joint function will never be restored. Articulations of the distal limb are easily arthrodesed (fetlock, proximal and distal interphalangeal joints). Severe carpal infection has also been treated with arthrodesis.

Prognosis: In cattle, prognosis is generally good for a return to previous function and productivity. It will depends of the time of presentation, radiographic evaluation (bone lysis and proliferation), and degree of extracapsular ankylosis. If more than 2 joints are infected, the prognosis is poor. Animal with chronic septic arthritis with bony lesions do not have a good prognosis for complete recovery and becoming a productive animal.

Biotechnology

K80

Simplified superstimulation programs for in vivo and in vitro embryo production in cattle

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Knowledge of follicular wave dynamics obtained using real-time ultrasonography and the development of the means by which follicular wave dynamics can be controlled have provided practical approaches for the *in vivo* and *in vitro* production and transfer of embryos in cattle. Two very important factors influencing variability in superstimulatory response are the intrinsic number of antral follicles in donors, and the stage of follicular development at the time of initiating FSH treatments. Response can be predicted by antral follicle counts done with ultrasonography, or the measurement of circulating concentrations of anti-Müllerian hormone (AMH). High antral follicle counts have been associated with more ovulations and a greater number of transferable embryos following superstimulation with FSH than low antral follicle counts. Furthermore, the elective control of follicular wave emergence and ovulation has had great impact on the application of on-farm embryo transfer, especially when large groups of donors need to be superstimulated at the same time. Although, estradiol and progestins have been used for many years, practitioners in countries where estradiol cannot be used have turned to alternative treatments, such as follicle ablation or the induction of ovulation by the administration of GnRH for the synchronization of follicle wave emergence. Initially, attempts to synchronize follicular wave emergence for superstimulation with GnRH were unsuccessful because of failure to induce ovulation consistently when administered at random stages of the estrous cycle, but subsequent field data were more promising. In these cases, GnRH was administered 1.5 to 3.0 days after the insertion of an intravaginal progestin device which may have increased the probability of an LH-responsive follicle at the time of treatment with GnRH. Indeed, we have reported on the strategic use of PGF2α, a progestin device and GnRH to induce ovulation prior to initiating FSH treatments. Basically, a persistent follicle was induced by treatment with PGF2α at the time of progestin device insertion; following administration of GnRH 7 days later, ovulation occurred in more than 95% of animals. Superstimulation initiated 36 hours after GnRH (with the P4-device remaining in place) resulted in a superovulatory response that did not differ from controls superstimulated between Days 8 and 12 of the estrous cycle. More recently, a study performed with Angus donors reported no difference in superovulatory response whether GnRH was administered 2 or 7 days after insertion of a P4-device with FSH treatments initiated 2 days later.

In vitro embryo production (IVP) also benefits from the synchronization of follicle wave emergence prior to oocyte recovery. As *Bos indicus* cattle have high antral follicle populations, large numbers of oocytes can be obtained by ovum pick-up (OPU) without superstimulation. However, synchronization of follicular wave emergence and superstimulation is necessary

to obtain high numbers of oocytes by OPU and blastocysts following *in vitro* fertilization (IVF) in *Bos taurus* donors. In Bos taurus breeds of cattle, especially those with low AFC, oocyte recovery and blastocyst production have been improved by superstimulatory treatments with FSH. There is also clear evidence that superstimulation increases oocyte and embryo developmental competence and thus, blastocyst production rates. Although FSH treatment protocols have involved twice daily treatments followed by a period of coasting to optimize follicle and oocyte development competence traditionally, more recent protocols with a single administration of Folltropin-V in 0.5% hyaluronan 48 or 72 hours before OPU have been very efficacious, making protocols user-friendly, while minimizing errors in compliance. FSH-treated beef and dairy cows and heifers had a greater percentage of medium-sized follicles (6 to 10 mm), a greater blastocyst production rate and more transferable embryos per OPU session. Finally, it has been shown that it is possible to obtain embryos in vivo and in vitro sequentially in the same donor. Although no significant differences in superovulatory response were found, initiation of FSH treatment 3 days after OPU resulted in a greater number of grade 1 embryos than when the protocol was initiated 2 days after OPU. All these protocols demonstrate that it is possible to obtain embryos in vivo and in vitro in commercial beef or dairy herds using efficacious protocols that are easily implemented by farm personnel.

K81

Factors affecting oocyte quality for embryo production in cattle

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The use of ovum pick up (OPU) associated with in vitro embryo production (IVEP) has a great potential to disseminate selected genetics, diminishing the interval of generations (use of young donors) and improving herds genetic gain. However, several factors appear to be critical to oocyte quality such as environmental factors, genetic background, age and lactation status of donor animals. The aim of this review is to highlight some critical areas that can help veterinary practitioners to enhance OPU efficiency and successfully implement IVP into their routine practice. The animal category is an important factor that affects oocyte quality. Our group used 120 Holstein donors of four animal categories, as follows: prepubertal heifers (n= 30), pubertal heifers (n= 30), lactating cows (n= 30) and nonlactating cows (n= 30). Donors were submitted to OPU without previous synchronization of the follicular wave. No difference was observed between experimental groups, regarding total number of aspirated follicles (P = 0.08). Despite a similar number of total recovered oocytes (P = 0.12), prepubertal heifers had an intermediate quantity of viable oocytes, and non-lactating cows produced more viable oocytes (P= 0.03), when compared to lactating cows. Still, prepubertal donors had lower cleavage rate (P< 0.0001) and lower blastocyst rate (P< 0.0001) compared to other categories. We studied the effect of donor pregnancy at the moment of OPU on oocyte quality and IVP in 3 Holstein categories: prepubertal heifers (8 to 10 month; n = 60), pubertal heifers (10 to 12 month; n = 60) and pregnant heifers (14 to 18 month; n=59). Pubertal heifers had a greater number of recovered oocytes as well as COCs cultured compared to other categories. In contrast, cleavage rate was similar between pubertal and pregnant heifers. Interestingly, pregnant heifers had a greater number of embryos produced per OPU and greater blastocyst rate when compared to other heifer categories. It has been reported that IVEP is more efficient in *Bos indicus* breeds than in *Bos taurus* breeds. The greater antral follicle population (AFP) found in *Bos indicus* cattle would appear to result in a greater number of suitable oocytes for *in vitro* culture. In this context, *Bos indicus* (Nelore and Gir) are reported to have greater number of visualized follicles and to produce greater number of total oocytes per OPU session, cultured COC and blastocyst rates than *Bos taurus* (Holstein). In dairy cows, the lactation period and interactions with insulin resistance may influence oocyte quality. Our group studied Holstein cows that were either at early or late days in milk (DIM) at the moment of OPU-IVP. Results showed that insulin resistance associated with late lactation period can disrupt oocyte quality, promoting lower efficiency of IVP. The number of blastocysts, as well as blastocyst rates, were greatly reduced in cows at later lactation. In addition, a number of apoptotic genes were upregulated in cows with greater days in milk. One factor related to the poor IVEP yields in *Bos taurus* cattle can be partly attributed to the heat stress. A previous seasonal experiment demonstrated that once the pool of ovarian oocytes is damaged by heat stress, two or three estrous cycles are required (after the end of heat stress) to restore the follicular pool and oocyte quality (Roth et al., 2001). However, our study (Torres-Júnior et al., 2008) showed a carry-over effect of heat stress on blastocyst production up to 105 days after the end of the heat stress. Factors that affect oocyte quality for embryo production have to be taken into account to increase the efficiency of ET in cattle.

K82

Factors affecting pregnancy rates in beef embryo recipients

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The main objective of implementing embryo transfer in beef operations is to accelerate the rate of genetic progress in the herd. Among the main factors that affect the use of these technologies are related to nutrition, management and estrus synchronization. Although prostaglandin F2α (PGF2α) has been used most commonly for synchronization of estrus, the requirement for estrus detection and the variability in the interval from treatment to estrus and ovulation has adversely affected its performance in embryo transfer programs. To avoid limitations associated with estrus detection, treatments that synchronize the time of ovulation, which were developed originally for fixed-time AI, have been utilized f/or FTET. These

treatments are generally divided into those that are GnRH-based and those that are estradiol-based. In either case, the recipient protocols include the insertion of a progesterone (P4) releasing device for 5 to 8 days, depending on the protocol. Recent studies with GnRH-based protocols have suggested that reducing the length of exposure of the P4-releasing device insertion to 5 days and increasing the interval from P4-device removal to GnRH and fixed-timed AI to 3 days may improve pregnancy per AI (P/AI) as compared to the traditional 7-day GnRH/P4 device protocol in beef cattle. Furthermore, it was suggested that a reduction in the length of the growth phase of the ovulatory follicle prior to ovulation, as occurs in some animals treated with the conventional 7-day protocols, alters the steroidogenic capacity of the dominant follicle prior to ovulation and the resulting CL, and decreases the ability of the uterus to support embryo development. Similar pregnancy rates per embryo transfer (P/ET) were obtained with in vitro-produced embryos to those of recipients synchronized with two PGF2α treatments 14 days apart and estrus detection and those synchronized using a modified 5-day Co-Synch+CIDR protocol (no GnRH at P4 device insertion, PGF2α at P4 removal on Day 5 and GnRH on Day 8). Based on these findings, we evaluated the effectiveness of an estradiol/P4 treatment protocol in which the exposure to P4 device was reduced to 6 days and proestrus was lengthened by the administration of GnRH 72 h after P4 device removal instead of ECP at device removal. The protocol for FTAI was named J-Synch. This treatment protocol has resulted in higher P/AI rates in beef heifers compared to the conventional protocol in which the P4-device is removed on Day 7 and ECP is given at that time. Furthermore, in a series of experiments that were conducted recently to evaluate the performance of the J-Synch protocol in embryo transfer programs the P/ET rate was greater in the J-Synch (49.4%) than in the conventional synchronization protocol (41.0%; P<0.05).

Although the previously described protocols have performed adequately for several years, recent attention has been directed to the effect of estrus expression and estradiol concentrations during growth of the preovulatory follicle on embryo growth and pregnancy. In recipients showing estrus, we have shown a significantly (P<0.05) greater P/ET (48.3% vs 30.1%) and lower pregnancy losses (25,6% vs 66.7%) than in recipients not showing estrus. Therefore, use of tail-paint or estrus detection patches in recipients would help identify animals showing estrus by simply running them through the chute at the appropriate time, without the necessity of labor intensive estrus observations. These modifications can be easily implemented in recipient synchronization programs and should result in overall higher pregnancy rates.

K83

Improving fertility in dairy herds using in vivo and in vitro produced embryos

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Embryo transfer is a reproductive technology commonly used around the world to reproduce animals with high genetic merit. However, the application of embryo transfer (ET) technology can also improve the reproductive performance of dairy herds artificially inseminated. Environmental conditions are important factors affecting oocyte developmental competence and embryo production in both *in vivo* and *in vitro* production systems. Heat stress (HS) has a deleterious effect on fertility in dairy herds around the world, especially in tropical areas in which summers are hot and humid. Studies have shown that embryo transfer is an effective tool to increase fertility during heat stress because it bypasses the damage to the oocyte and early stages of embryonic development caused by hyperthermia. Therefore, a useful management tool to maintain high pregnancy rates throughout the year would be to produce embryos during the cooler months and use them for ET during the periods of heat stress. A retrospective analysis of data from a large commercial herd in Brazil was performed in Holstein cows submitted to ET or AI. Pregnancy per ET (P/ET) was higher along the year than P/AI, but the differences were more pronounced in the warmer months than in the cooler months of the year. Furthermore, the transfer of embryos to repeat breeder cows resulted in increased pregnancy rates compared to AI, supporting that the fertility problem repeat-breeder cows may be associated with oocyte quality and/or failure of early embryo development. The seasonal profile of *in vitro* embryo production (IVEP) has been reported in non-tropical and in tropical areas. In USA (Wisconsin), the *in vitro* blastocyst production was reduced during mid-to late-summer, preceded by increased production starting during mid-to late-spring. Winter and fall months were characterized by stable, high yields of blastocysts, showing an evident effect of heat stress on IVEP in an area where summer is milder. *In vivo* embryo production (SOV and uterine flushing) in Holstein heifers and lactating cows was also shown to be affected by HS in the tropics (Brazil). Data from 1,562 SOV procedures indicated that the fertilization rate, proportion of transferable and freezable embryos and the number of embryos produced per SOV attempt (4.4 ± 0.4 vs 2.8 ± 0.3) was reduced during the warmer season compared with the cooler season. In a tropical environment, the number of ova/embryos produced after superovulation (SOV), fertilization rate and percentages of transferable embryos and freezable embryos were reduced during the warmer season when compared with the cooler season. Similarly, we observed in the tropics that during the hottest season of the year, oocytes obtained from lactating Holstein cows and heifers had a lower *in vitro* developmental competence compared to the coolest season. Although HS had an overall detrimental impact on *in vivo* embryo production and quality, its negative effects were more pronounced in lactating cows than in heifer donors. For example, during the hot season the decline in the

number of fertilized ova and in the rates of fertilization, transferable embryos and freezable embryos were greater in donor lactating cows compared with donor heifers. In addition, lactating cows generate more body heat and suffer greater hyperthermia when exposed to similar environmental temperatures compared with growing heifers and non-lactating cows, which may be related to the high metabolic energy associated with milk production. Regarding the embryo production technique, studies suggest that IVEP technology can be a viable solution to increase embryo production within a short period of time. The use of OPU-IVEP may be a valuable alternative to produce a large number of embryos and pregnancies within a reduced time period. The IVEP can also be associated with the use of sexed semen to increase the production of calves of a specific sex, which would benefit dairy industries worldwide. In summary, although the embryo transfer technology has been used primarily to reproduce animals with high genetic merit, this technology can be used to resolve reproductive problems such as the reduced fertility found during heat stress.

Pharmacology & Therapeutics

K84

Antimicrobial susceptibility testing and its clinical applications in bovine practice

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Objective: Antibiotic resistance is currently at the forefront of human and animal health and has been for many years. Antimicrobial susceptibility testing is commonly used in modern veterinary practice and so much so that many veterinarians submitting for this type of testing likely do so without thoughtful consideration in regard to the testing procedures or the interpretation of the testing results. However, in order to continue to be judicious with the use of antibiotics, it is essential that the clinician be familiar with the testing procedures and its interpretation in order to increase the chance of treatment success. The objective is threefold; 1) to re-familiarize the bovine practitioner with the most common methods of susceptibility testing performed in laboratories, 2) to educate on what goes into the determination of susceptible “S”, intermediate “I”, and resistant “R”, and 3) to exercise application of susceptibility testing to case management / treatment selection in the clinical setting.

Materials and Methods: The preferred methods of antimicrobial susceptibility testing and interpretive criteria are described in published standards documents to ensure that all laboratories are performing the testing procedures and interpretation in the same “standardized” fashion. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is the functioning body for such standardization in Europe and the Clinical and Laboratory Standards Institute (CLSI) functions as such in North America. For the purposes of this presentation the CLSI methods and definitions will be used; however, the themes presented will be applicable to all clinicians regardless of geography.

Results: Unfortunately, there is not a perfect correlation between antimicrobial susceptibility testing and clinical case outcome. Testing that yields bacteria that are “susceptible” to the prescribed antibiotic may have a poor outcome and those with “resistant” infections may have a positive outcome. In both cases, the ultimate outcome of the treatment may have been influenced by factors beyond just those of the “bug-drug” relationship, such as immune status, environmental conditions, individual pharmacokinetic differences, etc.

Conclusions: A clinician that thoroughly understands the basic methods of antimicrobial susceptibility testing, its interpretation, and its limitations will make sound clinical decisions and more judiciously select and utilize antimicrobials in their practice.