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The impact of sanity on the fertility of cows submitted to reproductive biotechnics

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Introduction

In Brazil, the cattle breeding is one of the main highlights of agribusiness being responsible for important and profitable segments in the meat and milk production chain. The productivity in herds is directly associated with reproduction since for dairy cattle each lactational cycle starts from pregnancy (Lucy, 2001). For beef cattle, only in Brazil 90 x 10^6 females are destined to reproduction annually, in the search for the efficiency of producing a calf/year (Anualpec, 2012).

Reproductive failures have been causing major financial losses in these sectors. For beef cow-calf producers, this is considered the main cause of economic loss (Berg, 2010). Likewise, the reproductive performance is essential for the dairy cattle system because the lactation cycle is dependent on pregnancy (Lucy, 2001). Several factors have a direct influence on reproductive performance, including genetics, nutrition, zootechnical management and sanitation (Vanroose et al., 2000). Special attention should be given to sanity programs since 37-50% of gestational losses in the herd are caused by infectious diseases leading to an increase in maternity fund rates (Khodakaram-Tafi and Ikede, 2005; McEwan and Carman, 2005).

Among such diseases are BVD, IBR, Brucellosis, Leptospirosis, Campylobacteriosis, Mycoplasmosis, Neosporosis, and Trichomoniasis. They cause reproductive failures that are usually characterized by anestrus, non-conception, early and late embryonic death, fetal/perinatal/neonatal mortality and lead to increased interval between deliveries. To prevent pregnancy losses in the properties, some management techniques such as hormonal manipulation, thermal comfort, and nutritional management are implemented (Lucy, 2001; Geary, 2005). However, immunization strategies are still not receiving the necessary attention (Littel-Van der Hurk, 2006). Thus, it is a need to know the main causes of the reduction of the indices of animal production, among them animal sanity.

Biotechnology of reproduction: improving the reproductive efficiency

The Brazilian cattle herd is composed of 210 million animals that serve to foment the development of the productive chains of meat and milk in the country. In 2015, according to data released by the Brazilian Institute of Geography and Statistics (IBGE), agribusiness generated R$ 1.26 trillion for the national economy, representing 21% of the total Brazilian intern product. In this scenario, livestock production reached R$ 400.7 billion, 30% of agribusiness. In order to keep cattle breeding on the rise, investment has become increasingly necessary in the means of breeding and production of these animals. In this way, new technologies developed for the improvement in the field have gained their application on a large scale, aiming the genetic improvement, zootechnical indexes with high productive and reproductive efficiency of the herd.

These improvements include animal breeding biotechnology, such as protocols for timed artificial insemination (TAI), superovulation of donors for collection and transfer of embryos (SOV/TE), in vitro embryos production (IVEP), cryopreservation of gametes and embryos and, also, sexed semen (Rufino et al., 2006; Vieira, 2012).

The TAI is a method used to increase the number of cows inseminated in the herd, eliminating the need for the detection of estrus (Baruselli, 2004). The protocols for synchronization of follicular wave emergence and ovulation of females are based on the use of the progesterone-releasing intravaginal device and the application of the hormone Gonadotropin Releasing (GnRH) or estradiol (Bó et al., 2016). With the use of TAI, it is possible to program the insemination of cows and the birth of calves, to obtain a uniform and genetically improved herd. Also, this technique makes the reproductive management more efficient, especially considering the particularities of bovine subspecies prevalent in Brazil and the nutritional challenges that the herds suffer.

Based on the same principle of manipulation of follicular wave through hormonal protocols, the SOV/ET is a biotechnology that increases the genetic gains and fertility of the herds (Nogueira et al., 2007; Bó et al., 2008). SOV consists of the stimulation of the ovary of the donor females with the use of exogenous hormones. The SOV aimed to provide the growth and maturation of several ovarian follicles simultaneously, to maximize the number of ovulated oocytes and viable embryos per donor (Rumpf et al., 2000). Approximately 6 to 8 days after artificial insemination the embryos are collected through uterine washes and subsequently transferred to the recipients.

The in vitro embryo production (IVEP) consists of the use of immature oocytes for the production of embryos. The laboratory procedure is divided into three sequential stages called in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC). Approximately 90% of the oocytes recovered by OPU reach metaphase II with the expulsion of the first polar corpuscle when passing through the IVM stage. But, which 80% are fertilized and begin to cleave, and only 25% to 40% of probable zygotes reach the stage of Blastocyst at day 7 of the culture stage (CIV). The effectiveness of the production is evaluated from the final rate of blastocysts, which is calculated on day 7 of the culture when the embryos can be transferred to the recipient or cryopreserved.

Embryos generated in vivo are cryopreserved by the slow freezing method. In the slow freezing method, the temperature is reduced gradually causing the water from the extracellular medium to undergo crystallization, by osmotic pressure the water passes from the intracellular medium to the extracellular medium (Leibo, 1977).

The use of sexed semen has made reproductive biotechnology an advantageous alternative for cattle breeding because it reduces the number of undesired calves in the herd. Despite the favorable results in other biotechnology, the use of sexed semen has its greater efficiency when employed in IVEP (Pontes et. al, 2010) due to the greater control in the fertilization conditions. Furthermore, the sexed semen decreases the number of receptors necessary to achieve the desired amount of females born from embryos produced in vitro.

Reproductive diseases of the cow and fertility

Several factors may interfere with reproduction rates. In this context, many strategies are applied in Brazil and in other countries to correct factors related to genetics, zootechnical management, and herd nutrition. Among the strategies are the breeding techniques, hormonal manipulation, and nutritional management.

The sanitary management of the herd, particularly in respect to infections that compromise the reproductive tract of females and embryo/fetus is very importance in the reproductive performance of beef or dairy cattle. These diseases result in lower economic losses directly due to a decrease in productivity or, indirectly, such as expenses for control sanitary programs.

However, the implantation of immunization strategies to control the susceptibility of animals to the effects and losses caused by reproductive diseases, often receive less attention and are neglected aspect.

On this topic, we will make a brief explanation of infectious viral, bacterial and parasitic diseases that cause abortions or decreases in fertility, citing their etiology and clinical signs.

Viral diseases

Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BoHV-1) - currently called alphaherpersvirus 1 are the protagonists of bovine viral diarrhea - BVD and rhinotracheitis (IBR) infectious, respectively.

These diseases are the main causes of reproductive viruses in cattle. They are classified as an infection listed in the OIE (OIE, 2017) because of their relevance and importance for socioeconomic losses in livestock, as well as international trade in animals and their products. BVDV is a RNA virus belonging to the family *Flaviviridae*, genus *Pestivirus*. Studies have reported that BVDV have two major species, BVDV1 and BVDV2, and for each specimen, there are genetically distinct viral isolates (Neill et al., 2011). Therefore, the great genetic variability is a challenge for one to be overcome for optimal protection and vaccine efficacy (Gribel et al., 2015).

The causative agent of IBR, bovine alphaherpesvirus 1 (BoHV-1), is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae*. BoHV-1 is known to promote late-onset abortion. It is described that the abortion usually occurs within a few weeks after viral exposure but can be postponed, reaching three to four months after exposure to the virus, if the pathogen is latent in the placenta (Radostits et al., 2007). Thus, making the diagnosis of this disease more difficult.

In general, as a consequence of BVDV and BoHV-1 infections, there are abortion outbreaks or more subtle reproductive losses, impaired conception, premature or dead births and early embryonic death. Especially in the case of BVDV, we can also highlight the birth of offspring with persistent infection (PI). The animal PI, constantly releases large amounts of virus, thus serving as a source of infection to animals that may come into contact with their secretions (Grooms et al., 2007).

Bacterial diseases

One of the most important bacterial diseases affecting the reproductive tract of cattle is brucellosis. Bovine brucellosis is zoonosis caused by *Brucella abortus* and negatively impacts livestock productivity. Many countries since the beginning of the twentieth century have succeeded in promoting the eradication of brucellosis in animal populations (Poester et al., 2009). Thereby, diminished with production losses and risks to human health.

This bacterium has developed mechanisms to live intracellularly for prolonged periods in its hosts, among them, the bovine species. In cattle, is largely associated with reproductive disorders both male and female. Abortions, low fertility and birth of weak calves are often observed in females (Jones et al., 2000; Silva et al., 2005).

Another important worldwide zoonosis, the leptospirosis, is determined by pathogenic spirochaetes that belong to the genus *Leptospira* (Adler, 2015). *Leptospira* infection belonging to serovar *hardjo* is the main responsible for causing leptospirosis in cattle. Moreover, other common causes of leptospirosis in this specie include serovars *pomona* and *grippotyphosa*.

In bovine, the leptospirosis is mainly characterized by infertility, increasing the number of services per conception, abortion, prolonged calving intervals, abortion besides stillbirths and weak offspring (Ellis, 2015). It should be noted that abortions caused by serovar *hardjo* tend to occur sporadically, in contrast to recurrent abortions observed as result of infection with serovars *pomona* or *grippotyphosa* (Grooms, 2006). The main site of colonization is the kidneys, where infected animals - namely carriers, through urine leads shedding of live leptospires functioning as a source of infection within the herd (Adler, 2015).

Therefore, other bacterial infections that affect the bovine reproductive tract, but due to the difficult clinical and laboratory diagnosis are neglected, are those caused by *Campylobacter fetus*, *Mycoplasma bovis* and *Mycoplasma bovigenitalium*.

Campylobacter fetus is a gram-negative bacterium in the form of spiral or S-shaped, which induces campylobacteriosis. In livestock, there are two species of Campylobacter fetus relevant: *Campylobacter fetus subspecie fetus* and *Campylobacter fetus subspecie venerealis* (Dekeyser, 1984). The species *Campylobacter fetus venerealis* resides exclusively in the genital tract of cattle (Iraola et al., 2013). Whereas *Campylobacter fetus fetus* usually inhabits the intestine, but due to an ascending genital infection or even venereal route, it can migrate to the genital tract via (Garcia et al., 1983).

The primary mode of transmission of *Campylobacter fetus venerealis* is during the coitus. owever, with more evident clinical signs in females. *Campylobacter fetus venerealis* are usually clinically asymptomatic and this bacterium can survive in raw and processed bull semen, and therefore, be transmissible via artificial insemination (Eaglesome, et al., 1995). Finally, likewise *Campylobacter fetus*, both mycoplasmas, *Mycoplasma bovis* and *Mycoplasma bovigenitalium* have also been isolated from semen and can be transmitted by natural breeding and by artificial insemination (Bielanski et al., 2000). *Mycoplasma bovigenitalium* is the most problem-related bovine species. Signs typically found in the herd are turbid or mucopurulent discharge indicating vaginitis, granular vulvar lesions, and reproductive failures such as early miscarriage or recurrence of estrus indicating early or late embryonic death (Rebhun, 2000).

Parasitic diseases

Bovine neosporosis is caused by the protozoan *Neospora caninum* (Apicomplexa: Coccidia), which is an obligate intracellular tissue cyst-forming coccidian belonging to the phylum Apicomplexa (Gondim, 2006). It is recognized worldwide as an important infectious cause of abortion in, primarily, cattle and clinical disease in dogs (Dubey and Schares, 2011).

The animal may be contaminated either by ingestion of the oocyte (when the protozoan is in the feces of dogs and coyotes) or congenitally. In the case of cattle, the abortions follow three main patterns (sporadic, endemic and epidemic abortions) where the model of the epidemic, cows who aborted for a short period are the most devastating and costly (Dubey et al., 2007). Also, fetuses may die in the uterus or be eliminated, for example, or otherwise arrive with clinical or clinically normal but persistently infected (Dubey et al., 2007).

Tritrichomonas fetus, the causative agent of trichomoniasis is an extracellular flagellated protozoan parasite that inhabits the prepuce of bulls (Parsonson et al., 1974). Thus, characterizing trichomoniasis as a venereal disease.

In bulls, *Tritrichomonas fetus* is usually clinically asymptomatic. However, in cows are characterized by genital infection which can cause abortion (Rhyan et al., 1988). Trichomoniasis is described as negatively impacting the reproductive performance of the herd by resulting in fewer pregnant cows and subsequently fewer calves (Ondrak, 2016). But, in view of the difficulty in obtaining a consistent clinical and laboratory diagnosis, many cases are not reported.

Importance of sanitary management to avoid economic losses

In livestock, beef or milk, animal production is structured from the triad: productivity, quality, and sustainability. However, it is necessary to work with the reproductive efficiency of the herd, which can be affected by nutrition, reproductive management, genetics, and sanity.

Among these factors, sanitary management is still largely neglected in properties. Special attention should be given to sanity programs since 37-50% of gestational losses in the herd are caused by infectious diseases leading to an increase in maternity fund rates (Khodakaram-Tafi and Ikede, 2005; McEwan and Carman, 2005).

As previously described in this review, breeding biotechniques may provide an increase in production and improvement of genetic merit of the herd (Hansen, 2014; Bó et al., 2016). However, a study by Aono et al. (2013) demonstrated that the application of the same TAI protocol in different properties obtained varied results. In Nelore herds vaccinated against BVD, BoHV-1 and *Leptospira* spp., pregnancy rates were higher when compared to unvaccinated herds, due to lower gestational losses. Similar results were described for dairy cattle, in a study conducted by the same group of studies (Pereira et al., 2013).

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Final comments

The reproductive biotechnology has collaborated with the development of livestock chain in the country. An increase in the productivity and genetic improvement of the national herd has been obtained in a progressive way in search of the best efficiency. However, negligence involving sanitary aspects is detrimental to the control of infections of the reproductive tract of females, with regard to animal reproduction, should be considered that zero risk does not exist. Therefore, it is extremely important to routinely implement herd programs that include adequate management and vaccination, in order to minimize the reproductive failures in the herds.

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