CHAPTER 27

MECHANISM RESEARCH AND TREATMENT OF REPEAT BREEDING SYNDROME IN COWS

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INTRODUCTION

Repeat breeding syndrome (RBS) is a critical reproductive disorder in repeated breeder cows, with pregnancy losses after three or more artificial inseminations but no anatomical or infectious abnormalities (Gunther 1981). The incidence of RBS in cow was reported to be 25% in Spain and Australia, 22% in USA and United Kingdom and 10% in Sweden (Bartlett et al. 1986; Eddy 1994; Gustafsson et al. 2002; Garcı´a-Ispierto et al. 2007; Canu et al. 2010). RBS is one of the most important factors affecting economic success in the dairy and beef industries, such as increasing intervals to conception, wasteful semen and insemination costs, increased veterinary examination and treatment costs, and additional maintenance charges (Casida 1961; Bartlett et al. 1986; Lafi et al. 1992; Perez-Marin et al. 2012). RBS contributes to declining fertility worldwide, which poses a severe threat to the breeding of cows. Unfortunately, RBS remains unmanageable mainly due to the lack in obvious factors. This chapter is focused on the mechanism research and clinical treatment of repeat breeding syndrome.

Mechanism Research

The physiological cause of repeat breeding syndrome is multifactorial. Nevertheless, many researchers prefer to focus on the following mechanisms: fertilization failure and early embryo death.

Failure of Fertilization

Failure of fertilization may result from inferior oocyte quality of female or unqualified artificial insemination (AI), including inopportune insemination. Poor oocyte quality can be caused by the following factors: chromosomal abnormalities, heat stress, and hormone disorders (Wiltbank et al. 2006; Sartori et al. 2010; Roberto et al. 2013; Sood et al. 2017). Plenty of research shows that repeat breeding cows have poorer oocyte quality and less quantity, which delays cytoplasmic maturation and obstructs fertilization (Kurykin et al. 2011; Kafi et al. 2017). During the research study in heifers directed by two scientists, seventy-four percent of the embryos generated by superovulation on day 7 after insemination were morphologically normal, and merely 28% were generated by the superovulated repeat breeding group (Gustafsson et al. 1983). A comparison of the amount in gene expression of repeat breeding cow and healthy cumulus-oocyte complex showed 178 genes were distinctly expressed. There were lower expression levels of ANXA1, Lactoferrin, HEM45, LOX-1, and GSTA4 gene in repeat breeding Holstein Friesian heifers, which provides reliable data for seeking the underlying candidate marker genes for RBS (Roberto et al. 2013). Heat stress, one of the major problems for cow fertility, has a harmful impact on the maturation of oocytes and the sustainable development of predatory embryos (Ferreira et al. 2011; Walsh et al. 2011; Silva et al. 2013). For instance, at the embryo development towards the blastocysts period, heat stress can increase the apoptotic index and disturb the expression of some genes in both *in vitro* fertilized and parthenogenetic embryos (Roberta et al. 2016). Hormone disorders is another main factor contributing to the failure of fertilization (Ariane et al. 2011). During the estrous cycle, hormonal asynchrony was found in repeat breeding heifers, with progesterone (P_4) concentration of approximately 0.5-1.0 nmol/L and lower plasma P_4 concentrations after AI (Albihn et al. 1991; Wiltbank et al. 2006). In addition, delayed LH peak, prolonged duration of estrus, and delayed ovulation were considered the evident clinical symptoms in some reports (Bage et al. 2002; Sood et al. 2017). Finally, fertilization failure can also be caused by unsuccessful AI, including in-accurate oestrus detection, incorrect time and unbefitting skill of AI, obstruction of fallopian tubes in females and inferior semen quality in males (Ayalon et al. 1984; Sartori et al. 2010; Yusuf et al. 2010).

Death of Early Embryo

Early embryo death, a major source of repeat breeding syndrome, is mainly generated by inferior oocyte quality and an inadequate uterine environment. The embryonic mortality of ruminants ranges between 20 and 50% (Humblot 2001; Diskin et al. 2008). The highest early embryo death rate is in the first week of a pregnant lactating cow (Walsh et al. 2011). Firstly, nutrition plays a vital role in the cytoplasmic maturation of

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oocytes in cows. Accumulation of cytoplasmic lipid deposits and their misdistribution have deleterious effects on oocyte maturation and quality (Adamiak et al. 2006; Leroy et al. 2008). A mass of oocytes with lipid droplets was an inescapable threat to their quality, mature capacities, and cryo-tolerances (Abe et al. 2002). It was reported that the reproductive performance of animals has a connection with their weight. As the body score surpasses 3.5, the cow has delayed initial oestrus, late conception and additional services to be pregnant (Crow et al. 2008). The body condition is inextricably linked to the daily feeding management, and several studies show deleterious influence on oocyte quality and blastocyst rate by immoderate intake (Humblot et al. 1998; Boland et al. 2001; Webb et al. 2004). Secondly, an inadequate uterine environment resulting from endometritis or other diseases can reduce embryo death by growth factors, bacterial toxins, and inflammatory mediators, including endometrial epidermal growth factor, prostaglandins, and reactive oxygen species, and cytokines (Sheldon et al. 2006; Wagener et al. 2017). Cytological endometritis has been reported to have deleterious effects on repeat breeder cows (Salasel et al. 2010; Janowski et al. 2013; Pothmann et al. 2015; Pascottini et al. 2016).

Treatment

Hormonal Treatments

Hormonal treatments, such as progesterone (P_4), gonadotropin-releasing hormone (GnRH), human chorionic gonadotropin (HCG), or insulin, have been widely used to improve the rate of conception for repeat breeding cows (Young et al. 1988; Thuemmel et al. 1992; Selvaraju et al. 2002; Kharche et al. 2007). During early pregnancy in the repeat breeding cows and heifers, the administration of P_4 (75 mg/d for cows, 40 mg/d for heifers, intramuscular injection on daily days 6-10 after AI) may increase plasma P_4 enough to reduce peripheral circulation demand for P_4 from the ovarian circulation, thus decreasing embryonic mortality due to insufficient P_4. However, it did not affect the conception rate (Thuemmel et al. 1992). Supplementation with PRID (1.55g of P_4) between days 5 and 19 after AI-enhanced the likelihood of pregnancy in 1-2 parity repeat breeding cows. It is worth mentioning that the proportion of abortions tended to be lower in PRID cows. However, there are some contradictory results regarding the advantage of GnRH therapeutic effects (Perry et al. 2009).

On the one hand, some reports indicated that the GnRH or synthetic analogues could improve the pregnancy rate in crossbred cows. There are several preparations of GnRH, such as gonadorelin, buserelin, cystorellin, and fertirelin, and the dose used for them ranges from 10 to 500μg in clinical treatments for several decades (Chenault et al. 1990; Stevenson et al. 1990; Ahuja et al. 2005). A study showed that there is a dose-effect relationship in RBS crossbred cow bred, which was previously exceeded 5 times reproductive procedure, particularly in the times of first service and conception rate (Kharche et al. 2007). On the other hand, it was shown that a dose of GnRH at the time of AI did not influence conception rates in beef cattle with obvious behavior of estrus (Heuwieser et al. 2011). Insulin and IGF-I can function as significant accommodators of follicular growth, reproductive hormone fluctuation, oocyte maturation, and embryonic development. Cows in the insulin treatment group were administrated subcutaneously with a long-acting purified form of bovine insulin (0.2 IU/kg/day on days 8, 9 and 10 of the estrous cycle). The results showed that P_4 concentration increased after insulin injection. However, the pregnancy rate had no first service pregnancy rate, and the overall pregnancy rate did not differ between the insulin injection group and the control group (Selvaraju et al. 2002).

Regulation of Fatty Acids by Diet Therapy

Fatty acids (FA) were not only closely related to the formation and metabolism of many important chemical compounds (hormone, ketosteroid and cholesterin) in reproductive health but also were essential factors for the structure and function of some organelle in germ cells (Abayasekara et al. 1999; Jump et al. 1999; Cheng et al. 2004; Leroy et al. 2005). Dietotherapy of FA can improve milking and reproductive performance, speculated by the possibility of harm reduction in the negative energy balance of high-producing dairy cows. Studies were conducted on diets with some individual fatty acids or FA groups. The results showed that ω-3 PUFAs could positively influence reproductive status, for instance, follicular development, persistent corpus luteum, and high fertility (Staples et al. 1998; Mattos et al. 2004). Results from dietary studies showed that ω-3 added to the ration of ruminants can prolong the presence of corpus luteum by reducing PGF_{2a} and increasing embryonic survival (Diskin et al. 2008; Gulliver et al. 2012). Ambrose and his colleagues verified that dietary supplementation of ALA (alpha-linolenic acid, a type of ω-3) was provided for Holstein cows for twenty-eight successive days before artificial inseminations caused larger preovulatory follicles and fewer embryonic losses (Ambrose et al. 2006). During the non-breeding season, a more significant proportion of saturated FA in cow oocytes could account for a smaller conception rate (Zeron et al. 2001). Feeding fish oil is rich in ω-3 (a blend of eicosapentaenoic acid-EPA and docosahexaenoic acid-DHA, two types of ω-3), from −2 to +2 weeks of breeding, and the experimental results indicated that the group of repeat breeding cows who achieved pregnancies fed by fish oil in the synchronization of estrus and artificial insemination, possessed a greater diameter of the preovulatory follicle and higher expression of mRNA of interferon-stimulated gene transcripts (Aamir et al. 2019).

Aquapuncture Therapy

Acupuncture, a simple, safe and effective technique, has been used successfully to treat reproductive disorders in cows, pigs, and women (Fung 1984; Lin et al. 1988; West 2000; Lin et al. 2001). As an alternative treatment, it is recognized gradually by the Oriental. Eighteen repeat breeding cows and heifers were treated by aquapuncture, in which 5 and 10 ml of 50% glucose solution were injected at Shenpeng and Baihui acupoints, respectively. The pregnancy rates after the treatment were 77.7 and 66.6% based on P_4 concentration and rectal palpation, respectively. However, the final rate obtained from the actual delivery of the fetus was 44.4%, which had a similar consequence to GnRH treatments (Lin et al. 2002).

Embryo Transfer Protocol

Embryo Transfer (ET) is diffusely applied to enhance the amount of excellent production performance animals and build superiority gene population. Many studies have investigated the function of embryo transfer for increasing breeding efficiency in repeat breeding cows (Tanabe et al. 1985; Son et al. 2007; Block et al. 2010; Yaginuma et al. 2019). Rodrigues and his colleagues used the conception rate to compare RBS Holstein cows by AI with those by ET. The conception rate was higher in the group by ET (41.7%) than that by AI (17.9%) (Rodrigues et al. 2007). However, there was no remarkable distinction in the pregnancy rates on two months between health and RBS (82% vs. 70%) (Tanabe et al. 1985). Timed artificial insemination superovulated donors and timed embryo transfer in embryo recipients is a choice to avoid oestrus detection. In a study, conception rates were enhanced subsequent CIDR TAI or TET, compared with the rates subsequent AI after a single injection of $PGF_{2\alpha}$ in the synchronization in RBS cows. There was a higher conception rate in the ET than in the appropriate AI group or the TAI (53.8% vs. 18.5% vs. 7.7%) group (Son et al. 2007). In a word, the embryo transfer protocol provides a capacity for mitigating the threat of inferior quality of oocyte and embryo and remedying the imperfect functionality of the uterine in repeat breeding cows.

Vaginal Treatment

Seminal plasma (SP) and milk osteopontin (mOPN) in the vagina are new approaches in recent years. The normalized endometrial Epidermal growth factor (EFG) profile and preserved fertility in RBS cows were studied (Dagvajiamts et al. 2020; Hay et al. 2022). A series of research have manifested there is a likelihood that SP enhances female reproductive performance by regulating some factors of uterine function and ameliorating prenidatory environment in mice, sows, horses, cows, and humans (WS et al. 1989; Gooneratne et al. 1989; Tremellen et al. 2000; Alghamdi et al. 2004; Robertson 2007; Dagvajiamts et al. 2020). EFG is a critical factor in controlling uterine function and embryo development, which is regulated by estrogen in mice and rats. The healthy cows have two peaks at days 2-4 and 13-14 of the estrus cycle with EGF concentrations of their uterine endometrium, and the RBS cows are deficient in these peaks relatively (Katagiri et al. 2004; Katagiri et al. 2013; Katagiri et al. 2016; Kafi et al. 2017). In a recent study, 5% SP with PBS and only PBS were infused into the vagina of two RBS cows' groups at day 3 of their estrous cycle, respectively (31 vs. 36). The results showed that about 60% of RBS cows had normalized two maximum values of endometrial EGF concentrations again and produced more pregnancies than the controls (44.4 % vs 19.4%) (Dagvajiamts et al. 2020). However, the mechanism of EGF concentrations in the uterus altered by SP infusion into the vagina remains unknown. Nowadays, studies have shown that SP improved females' fertility, which is likely associated with sperm motility and function or the direct mode of action on uteri found in other varieties (Katila et al. 2012; Bromfield 2016). OPN, a glycosylated phosphoprotein, was firstly separated from the bovine diaphyseal bone and widely detected in a variety of epithelial cells and seminal plasma, blood, milk, urine, oviduct, uterus and placenta (Franzen et al. 1985; Cancel et al. 1997; Sodek et al. 2000; Johnson et al. 2003; Tsuji et al. 2007; Goncalves et al. 2008; Schack et al. 2009; Dudemaine et al. 2014). Cow OPN has 262 amino acids and displays a molecular weight of 16- 60 kDa (Kumura et al. 2004; Christensen et al. 2016; Dagvajiamts et al. 2020). mOPN, a bountiful origin of OPN in cows, could promote sperm capacitation, cleavage, blastulation and embryonic development *in vitro* experiments (Killian et al. 1993; Christensen et al. 2016). Hay and his team separated mOPN from cow milk, identified 3 major protein bands of 31, 37 and 61 kDa and compared three vaginal treatments (mOPN vs. SP vs. PBS only) to normalize EFG profiles and conception rate of RBS cows. The result shows that mOPN regained the normal endometrial EGF profile (56.1% vs 58.1% vs 23.8%) and improved the conception rate (43.5% vs 40% vs 23.8%) in RBS cows. Besides, there are approximate levels between seminal plasma OPN and milk OPN (Hay et al. 2022).

Conclusion

In summary, researchers on mechanism research and treatments of repeat breeding syndrome generally pay more attention to endocrinology and genomics nowadays. We can utilize multi-omics in all kinds of fluids of RBS and healthy cows in the future. In addition, a high-throughput mass spectrometric discovery approach can be used to unravel new information and potential candidate proteins for repeat breeding syndrome. Finally, the complexity of exosome biology of RBS deserves more investigation, which is beneficial for discovering new treatments.

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