Manipulation of ovarian and uterine function to increase conception rates in cattle

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Abstract

In cattle, embryonic and fetal losses are the major causes of reproductive failure. Losses associated with failure in the process of maternal recognition of pregnancy can amount to as much as 30%. Successful establishment of pregnancy depends on a delicate balance between luteolytic mechanisms inherent to the endometrium at the end of diestrus and antiluteolytic mechanisms, orchestrated by the conceptus to change endometrial function and, ultimately, block luteolysis. Antiluteolytic strategies are pharmacological, mechanical, nutritional and management manipulations of the process of maternal recognition of pregnancy that aim to increase the probability of successful gestations. Objective of the present paper is to review the recent literature on antiluteolytic strategies in cattle. Specifically, we focused on strategies to stimulate production of progesterone, strategies to reduce production of estradiol and other strategies. Future directions for research in this area are proposed.

Keywords: antiluteolytic strategies, cattle, conception rates, GnRH, gonadotropins, sex steroids.

Introduction

Reproductive efficiency is the factor that mostly affects productivity and profitability of a commercial cattle operation. In cattle, embryonic and fetal losses are the major causes of reproductive failure (see Santos *et al*., 2004b and Sartori, 2004 for reviews). Indeed, Humblot (2001) reported the incidence of 20.5 to 43.6% embryonic mortality up to day 25 post-insemination in French herds and Santos *et al*. (2004b) suggested that embryonic mortality is around 60%, 28 days after artificial insemination. Moreover, Kunz *et al*. (2002) reported embryonic mortality rates between 20 and 40% from insemination to day 22 of pregnancy.

It is generally accepted that the embryonic mortality that occurs in the initial seven days of gestation is associated with fertilization failure, genetic defects and impaired embryonic development to the blastocyst stage. Mortality rate at this stage is usually less than 10%, but can increase to as much as 40% in dairy cows under heat stress (Ayalon, 1978; Sartori *et al*., 2002; Sartori, 2004).

Measuring embryonic mortality in weeks two and three of gestation is much more challenging, as it requires interrupting gestations at more advanced stages. Therefore, direct measurements of mortality during this period were reported in a very limited number of publications. Diskin and Sreenan (1980) reported that embryonic mortality between 8 and 16 days of gestation approached 30% in *Bos taurus* type cattle. This period coincides with the maternal recognition of pregnancy, which is the process by which the conceptus signals its presence to the maternal unit by blocking the mechanisms involved in pulsatile release of prostaglandin F2α (PGF) and, consequently, luteolysis (Thatcher *et al*., 1995). Conceptus ability to inhibit PGF release requires adequate secretion of paracrine factors, such as interferon-tau (IFN; Binelli and Thatcher, 1999). Well elongated conceptuses, occupying most of the uterine horn ispilateral to the ovary containing the corpus luteum (CL) at the time of maternal recognition of pregnancy, are the most likely to effectively block luteolysis and maintain pregnancy (Mann *et al.*, 1999). Thus, successful establishment of pregnancy depends on a delicate balance between luteolytic mechanisms inherent to the endometrium at the end of diestrus and antiluteolytic mechanisms, orchestrated by the conceptus to change endometrial function and ultimately block luteolysis.

Antiluteolytic strategies are pharmacological, mechanical, nutritional, and management manipulations of the process of maternal recognition of pregnancy that aim to increase the probability of successful gestations. Such strategies aim to reduce the luteolytic capacity of the maternal unit as well as increase the antiluteolytic stimulus induced by the conceptus. Strategies include manipulations of follicular, luteal, endometrial, and conceptus functions, as well as changes in environmental factors. A number of specific strategies was proposed by Binelli *et al*. (2001): 1) stimulate growth and/or differentiation of the pre-ovulatory follicle to generate a CL with greater progesterone-producing capacity; 2) stimulate CL growth rate; 3) increase plasma progesterone concentrations in the initial three weeks after insemination; 4) decrease the effects of a dominant follicle during the critical period (i.e., days 15-19 after estrus); 5) increase the antiluteolytic stimulus provided by the conceptus; and 6) decrease uterine luteolytic capacity. Similar strategies were also proposed by Santos *et al*. (2004b) to enhance embryonic survival in cattle.

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The objective of the present paper is to review the recent literature on antiluteolytic strategies in cattle. Specifically, we focused on strategies to stimulate production of progesterone, strategies to reduce production of estradiol and other strategies. Then, future directions for research in this area are proposed.

Strategies to increase progesterone

Background and rationale

During initial development in the uterus, the bovine conceptus has no access to the maternal blood supply and is "free-floating" in the uterine lumen. Therefore, conceptus growth depends on an optimal composition of the luminal micro-environment. Composition of the luminal environment depends on uterine gland secretions released in the uterine lumen (Geisert *et al.*, 1988, Mann *et al.*, 1999). Progesterone modulates function of uterine glands, and thereby, conceptus growth (Spencer *et al.*, 2004). Indeed, there was a positive association between plasma progesterone concentrations during the initial three weeks of gestation and pregnancy rates. For example, conceptuses were recovered 16 days after artificial insemination and classified as poorly or well developed Mann *et al.* (1996, 1998). Low plasma concentrations of progesterone during early pregnancy were associated with poorly developed conceptuses. These data provided the motivation for working out systems to increase progesterone concentrations during early pregnancy. Strategies to increase pregnancy rates based on the increase of progesterone concentrations were reviewed previously by Thatcher *et al.* (2001) and Binelli *et al.* (2001) and are updated here.

Strategies to increase progesterone by manipulations conducted before ovulation

Mantovani *et al.* (2005) used intravaginal progesterone releasing devices before induced ovulation to generate large, persistent follicles in embryo recipient heifers. Rationale was that larger preovulatory follicles would generate larger corpora lutea with a greater capacity to produce progesterone and better support development of transferred embryos. On day of embryo transfer (day 7 after synchronized estrus), CL area (1.9 ± 0.1 vs. 2.3 ± 0.1 cm^2) and plasma progesterone concentrations (2.3 ± 0.2 vs. 3.8 ± 0.2 ng/ml) were greater for treated than for control heifers. However, pregnancy rates were unaffected by treatment (30% vs. 29.6% for control and treated animals, respectively). It is possible that detrimental effects of prolonged exposure to increased progesterone (i.e., from devices) and estradiol concentrations (i.e., produced by the persistent follicle) during the cycle previous to embryo transfer counterbalanced potential beneficial effects of the greater progesterone concentrations resulting from treatment

(Wehrman *et al.*, 1997; Shahan-Albalancy *et al.*, 2001).

Another approach to stimulate growth of the preovulatory follicle by aiming for increased production of a larger corpus luteum is to increase the time allowed for growth of the preovulatory follicle in a low progesterone environment (Vasconcelos *et al.*, 2000). In timed insemination protocols, this can be achieved by increasing the interval between the removal of endogenous progesterone (i.e., injection of PGF) and the induction of ovulation. Recently, Peres *et al.* (2008) synchronized ovulations of Nellore cows using a progesterone-releasing device for 9 days. Then, cows received a PGF injection on the day of device removal or 2 days before device removal and ovulations were induced in all cows by an injection of estradiol cypionate on the day of device removal. Diameter of the preovulatory follicle (11.5 ± 0.2 vs. 10.8 ± 0.2 mm) and concentrations of progesterone (3.05 ± 0.2 vs. 4.58 ± 0.2 ng/ml) measured on the day of device removal were different ($P < 0.05$) for cows that received PGF earlier in comparison to control cows, respectively. Resulting pregnancy rates were also higher for cows receiving PGF earlier in the synchronization protocol (52.0 vs. 36.4%, respectively). Finally, growth and final differentiation of the preovulatory follicle can be stimulated by strategic administration of equine chorionic gonadotrophin (eCG; please see Baruselli *et al.*, 2004 and Bó *et al.*, 2007 for reviews). Baruselli *et al.* (2003) and Cutaia *et al.* (2003) reported that 0 or 400 IU eCG were administered to cows on the day of progesterone-releasing device removal of a synchronization protocol. Ovulations were induced by an injection of estradiol benzoate and cows were timed-inseminated. The eCG treatment increased pregnancy rates in Bradford (26.7 vs. 34.6%), Nellore (38.9 vs. 45.7%) and Nellore cross-bred cows (46.8 vs. 59.1%). Their hypothesis was that increased pregnancy rates are due to a stimulated luteal function in the corpora lutea resulting from ovulation of follicles exposed to eCG. Indeed, we treated cows with a protocol similar to that of Baruselli *et al.* (2003) and showed that despite the similar size preovulatory follicle, both the CL size and plasma progesterone concentrations post-estrus were increased for cows receiving 400 IU vs. 0 IU eCG (Bergamaschi *et al.*, 2005). Notwithstanding, it is important to mention that eCG efficacy varies depending on body condition status, number of days post-partum and association with other management strategies, such as temporary weaning, as discussed by Bó *et al.* (2007).

Strategies to increase progesterone by manipulations conducted after ovulation

Early researchers detected beneficial effects of supplementing progesterone, by daily injections, on maintenance of pregnancy (Wiltbank *et al.*, 1956). More recently, plasma concentration of progesterone was increased by supplementation using progesterone-

126

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

releasing intravaginal devices (Van Cleeff *et al.*, 1991; Marques, 2002; Stevenson *et al.* 2007). An alternative way to increase progesterone is by inducing the formation of accessory corpora lutea by the ovulation of the first wave dominant follicle. This can be achieved by injections of GnRH (Schmitt *et al.*, 1996; Howard *et al.*, 2006; Stevenson *et al.*, 2007; Machado *et al.*, 2008), LH (Goissis *et al.* 2004; Kastelic and Ambrose, 2004) or hCG (Schmitt *et al.*, 1996; Santos *et al.*, 2001; Stevenson *et al.*, 2007) or combinations of these hormones (Bergamaschi *et al.*, 2006; Machado *et al.*, 2006a, b). In general, efficiency to increase progesterone concentration is greatest when accessory CL is induced by hCG. Beneficial effects of accessory corpora lutea and supplemental progesterone were reported in some (Santos *et al.*, 2001; Marques, 2002; Stevenson *et al.*, 2007), but not all studies (Howard *et al.*, 2006; Machado *et al.*, 2006b).

What needs further understanding about progesterone supplementation

Reasons for why progesterone supplementation improves fertility in some, but not all situations has not been tested critically. For example, high fertility heifers bearing hCG-induced accessory corpora lutea had pregnancy rates similar to untreated heifers (63 vs. 65% for controls and hCG-treated, respectively; Schmitt *et al.*, 1996). Also, Machado *et al.* (2006b) induced accessory corpora lutea using strategic injections of both GnRH and hCG in grazing embryo recipient heifers in Brazil. During the dry season (low feed availability), treated heifers had a 10% greater pregnancy rate than untreated (50 vs. 40.5%, respectively). However, pregnancy rates were similar during the rainy season (59.6 vs. 60.4% for control vs. treated heifers). Summarizing a large dataset (n = 1984), Bó *et al.* (2007) indicated that fixed-time artificial insemination protocols in which eCG stimulation was included increased pregnancy rates when animals presented body condition scores between 2 and 3. The eCG did not affect pregnancy rates of animals with body condition scores of 3.5 or above. One could argue that progesterone supplementation only benefits fertility when progesterone is limiting, but a precise diagnostic for this situation is lacking.

Another important aspect to consider when planning a strategy for progesterone supplementation is the ideal timing of supplementation. In an elegant work by Demetrio *et al.* (2007), pregnancy rates were analyzed as a function of progesterone concentrations measured on day 7 for animals receiving artificial insemination or embryo transfer. Conception rates were positively associated with progesterone concentrations for inseminated cows. Surprisingly, there was not such relationship in cows receiving embryos. Moreover, there was no association between pregnancy rates and progesterone concentrations measured on day 14 for

either inseminated or recipient cows. This is in agreement with data from Mann *et al.* (2006). These authors observed that progesterone supplemented via intravaginal progesterone-releasing devices stimulated conceptus growth when supplementation was between days 5 and 9 after estrus, but not between days 12 and 16. Altogether, it is suggested that progesterone supplementation is beneficial before the conceptus elongates. This is in contrast with data from Marques (2002). This author treated heifers on the day of embryo transfer (i.e., day 7) to induce an accessory corpus luteum with LH, GnRH or hCG, or administered supplemental progesterone via an intravaginal device. Because of the time needed for ovulation and luteinization in response to injections, increases in progesterone concentrations should take at least 48 h to be detected (Diaz *et al.*, 1998). Notwithstanding, progesterone supplementation increased conception rates in comparison with controls (28.6, 53.5, 57.0, 45.4 and 41.1% for untreated, GnRH-, hCG-, LH- or intravaginal device-treated heifers, respectively). In summary, effects of progesterone supplementation on maintenance of pregnancy vary among precise stages of early pregnancy. Defining the best stage for supplementation in each particular situation (e.g., nutritional status, management practices, breed, parity, etc) remains to be clarified.

Finally, it is important to consider indirect effects of manipulations to increase progesterone, such as effects in follicular dynamics and endometrial function. For example, when ovulation of the first wave dominant follicle was induced by an LH injection on day 6 of the estrous cycle, emergence of the second wave was hastened and all cows presented 3-wave cycles (Goissis *et al.*, 2004). This was also reported for heifers which received hCG injections on day 5 (Diaz *et al.*, 1998). Evidence from Ahmad *et al.* (1997) indicated that animals with three waves of follicle development during the period corresponding to an estrous cycle after breeding had greater pregnancy rates than animals with two waves. Furthermore, Santos *et al.* (2008b) reported that progesterone concentrations during the luteal phase of the estrous cycle may in fact increase PGF release in response to estradiol injections. However, progesterone effects in the PGF-releasing mechanisms during pregnancy have not been studied in cattle.

Strategies to decrease estradiol

Background and rationale

Inskeep (2004) indicated that estrogen secretion from a large follicle from days 14 to 17 of pregnancy may negatively affect embryo survival. Binelli *et al.* (2001) suggested the attenuation of luteolytic stimuli as a strategy to decrease early embryonic mortality. For example, it is known that ovarian follicles produce increasing quantities of

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

127

estradiol as they grow and become dominant (Komar *et al*., 2001). Moreover, this hormone has a central role in PGF production and luteolysis (Villa-Godoy *et al*., 1985; Thatcher *et al*., 1986; Salfen *et al*., 1999), via mechanisms not well understood in the cow. Thus, strategies resulting in the absence of dominant follicles, reduction of their steroidogenic capacity or reduction of endometrial responsiveness to estradiol during the period of maternal recognition of pregnancy should increase the probability of conceptus survival and pregnancy rates. Experimental manipulations to reduce estradiol concentrations or its actions include mechanical and pharmacological approaches.

Strategies to reduce production of estradiol and responsiveness to estradiol using mechanical manipulations

One simple way to reduce plasma concentrations of estradiol is to remove follicles by transvaginal ultrasound-guided aspiration of follicles. Bisinotto *et al*. (2006) collected blood samples and conducted aspiration of follicles of 6 mm and larger daily, from days 13 to 25 of the estrous cycle in *Bos taurus indicus* crossbred cows. Their hypothesis was that compared to a sham-aspirated control group, decrease in plasma progesterone associated with luteolysis is delayed in the aspiration group. Aspirations were successful in decreasing mean largest follicle size during the peri-luteolysis period (days 15 and 19 of the estrous cycle; 8.5 ± 0.2 vs. 6.4 ± 0.2 mm for control and aspirated cows, respectively; P < 0.01). Aspirations also tended (P < 0.1) to decrease plasma concentrations of estradiol between days 18 and 20 of the estrous cycle (4.3 ± 0.72 vs 2.95 ± 0.72. pg/ml for control and aspirated cows, respectively). However, despite such differences, day of luteolysis was similar for both groups (mean, 19.6 ± 0.4) In another study, with Holstein heifers, Araujo *et al*. (2008) aspirated follicles 4 mm and larger on day 9 (control group) or every other day, from day 9 to 21 of the estrous cycle (treatment group). Estradiol concentrations in plasma increased on day 16 for controls but not until day 22 for treated animals. Luteolysis was initiated on day 16 in controls, but was delayed to day 19 in treated cows. A possible explanation for the discrepancy in these results is that a greater reduction in mean follicular diameter and estradiol concentrations is necessary to inhibit luteolysis in *Bos taurus indicus* type cattle and their crosses. Compared to *Bos taurus taurus* type cattle (i.e., follicle diameter at deviation 8.5 mm; Beg *et al*., 2002), *Bos taurus indicus* cattle have smaller size follicles at the time of follicle deviation (6.0 to 6.5 mm; Gimenes *et al*., 2008) after which follicular estradiol production increases dramatically. However, a common aspect of both experiments is that it seems that there is an increasing sensitivity of the endometrium to estradiol at around the time of expected luteolysis. As a result,

luteolysis may be triggered by small quantities of estradiol secreted by small follicles. In that regard, our unpublished data indicate that the release of PGF in response to estradiol injections increased gradually on days 13, 15, 17, and 19 of the estrous cycle in Holstein cows. This information is not available for *Bos taurus indicus* cows, but a similar response is expected in these animals.

Experimental approaches aiming to reduce the sensitivity of the endometrium to estradiol as a means to delay luteolysis are scarce. Santos *et al*. (2008b) showed that aspiration of the first wave dominant follicle on day 6 of the estrous cycle decreased PGF release (as measured by plasma concentrations of its metabolite, PGFM) in response to an estradiol injection given on day 17 in comparison with non-aspirated controls. This data are intriguing, but further experimentation is needed to elucidate potential mechanisms involved.

Strategies to reduce estradiol using pharmacological manipulations

Follicular turnover and wave emergence can be manipulated using a variety of pharmacological approaches. Thus, one may take advantage of such approaches to reduce the amount of time within the period of maternal recognition of pregnancy in the presence of highly estrogenic follicles. Machado *et al*. (2008) injected Nellore cows (*Bos taurus indicus*) with 0 or 3000 IU hCG on day 5, and 0 or 5 mg estradiol-17ß on day 12 of a synchronized estrous cycle. In agreement with Bó *et al*. (1995), treatment with estradiol stimulated follicle turnover and emergence of a new wave of follicular growth approximately 4 days later. As a consequence, dominant follicles were not observed in the ovaries until day 19.4 for animals receiving hCG and estradiol vs. day 16.6 for control animals. Despite this fact, estradiol treatment anticipated onset of luteolysis 1.5 days on average in comparison with the control group. It was expected that luteolysis would in fact be retarded because of the absence of dominant follicles during the peri-luteolysis period (i.e., days 15 to 19 after estrus). It is possible that exposure to estradiol injected on day 12 sensitized the uterus and led to premature luteolysis. Next, Bergamaschi *et al*. (2006) injected GnRH on day 5 and hCG on day 13 of a synchronized estrous cycle. Rationale was that the GnRH injection would induce ovulation of the first wave dominant follicle and an accessory CL. Moreover, emergence of the second wave would be synchronous. As a result, dominant follicle of the second wave would have ovulatory diameter on day 13, when the hCG was injected. Injection of hCG would cause ovulation of the second wave dominant follicle inducing a second accessory CL as well as synchronizing emergence of the third wave of follicle development. The expected result would be a reduced number of days in the peri-luteolysis period in the presence of highly steroidogenic

128

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

follicles. In a low estrogen environment luteolytic stimuli should also be low. Indeed, animals receiving the GnRH-hCG treatment combination had a luteal phase extension of 6 days in comparison with controls (19 vs. 25 days), indicating that luteolysis was delayed in these animals. Furthermore, fetal losses between days 30 and 60 were less for treated vs. control animals (6.2 vs. 17.6%, respectively; Machado *et al.*, 2006b). Finally, the GnRH-hCG treatment combination induced an increase in plasma progesterone concentrations, which has beneficial effects on conceptus development and survival, as mentioned above. Therefore, this GnRH-hCG approach takes advantage of both the progesterone supplementation and the estradiol reduction strategies to modify uterine function and has the potential to affect positively pregnancy rates in cattle.

What needs further understanding about decreasing the effects of estradiol during early pregnancy

Control of estradiol effects on PGF production may be achieved by the control of estradiol production and the control of estradiol responsiveness. Current understanding of dynamics, endocrine regulation and manipulations of follicular development provided principles for manipulating timing and magnitude of follicular estradiol production. As mentioned above, such principles can be applied as a means to control production of PGF and, consequently, luteolysis. However, success of such approaches was only partial. If one could also control uterine responsiveness to estradiol, it is possible that production of PGF during the peri-luteolysis period could be manipulated with a greater precision and reduced further. However, acquisition of uterine responsiveness to estradiol is a poorly understood process. Evidence indicates that uterine sensitivity to estradiol increases dramatically towards the end of the luteal phase (Bisinotto *et al.*, 2006; Araujo *et al.*, 2008). In each study, follicles were kept at similar sizes through frequent aspirations and so were their estradiol secretory capacity. However, as the cycle progressed, the same plasma concentrations of estradiol gained the ability to induce PGF release and luteolysis, probably due to an increased sensitivity of the uterus. In the ewe endometrium, downregulation of progesterone receptors at mid-cycle allows for expression of estrogen receptors, that in turn activate oxytocin receptors which are key players in the regulation of pulsatile release of PGF and luteolysis (McCraken *et al.*, 1984). A better understanding of endocrine, cellular and molecular mechanisms regulating the dynamics of estrogen receptor expression and activity in the cow endometrium is lacking and deserves investigation.

Other antiluteolytic strategies

Besides manipulating ovarian steroidogenesis as a mean to decrease the luteolytic stimuli and increase conceptus survival, other strategies have targeted the uterus and the conceptus directly with the same objectives. Strategies include the use of antiinflamatory drugs, fat feeding and bovine somatotropin (bST) administration.

Synthesis of PGF results from a coordinated cascade of intracellular events. A rate limiting step in this cascade is the conversion of arachidonic acid to prostaglandin-H2 (PGH) by the enzyme prostaglandin-endoperoxidase synthase 2 (PTGS2 or COX-2; mechanism revised in Burns *et al.*, 1997). The PGH is subsequently converted to PGF. Strategies targeting the inhibition of PTGS2 activity, and consequently PGF synthesis, during maternal recognition of pregnancy should increase embryo survival and pregnancy rates. Guzeloglu *et al.* (2007) treated Holstein heifers with flunixin meglumine, a non-steroidal antiinflamatory drug which inhibits PTGS2 activity, on days 15 and 16 after insemination. They observed increased pregnancy rates on days 29 (76.9 vs. 50%; P < 0.04) and 65 of gestation (69.2 vs. 46.2%; P < 0.09) for treated vs. non-treated control animals. These results were in agreement with the reports by Merryl *et al.* (2007) and Pfeifer *et al.* (2007). It is possible that these treatments inhibited PGF synthesis temporarily, allowing for the development of conceptus antiluteolytic mechanisms (i.e., production of IFN) and resulted in lower embryonic mortality. It should be mentioned that PTGS2 inhibition must be achieved at precise moments within the maternal recognition of pregnancy process. Edmond *et al.* (2004) showed PTGS2 protein expression in conceptus tissues starting on day 18 of pregnancy. Therefore, inhibition of PTGS2 activity from that moment onwards may in fact be detrimental to conceptus development.

Fat feeding influences several aspects of reproduction in cattle (for an excellent review in this topic, see Santos *et al.*, 2008a). Specifically, feeding long chain fatty acids can modulate PGF production in the endometrium. Feeding the n-3 fatty acids attenuates PGF production (Mattos *et al.*, 2003, 2004), whereas the opposite effect was observed when n-6 fatty acids was fed to cattle (Pettit and Twagiramungu, 2004). Results from Bilby *et al.* (2006a, b) indicate that those beneficial effects of feeding n-3 fatty acids to lactating dairy cows may be through a decrease in the proportion of arachidonic acid in the endometrial lipid pools, which corroborates previous data from Burns *et al.* (2003). Decreasing substrates for PGF synthesis should result in an uterine environment less conducive to luteolysis, which should result in greater rates of embryonic survival. A summary of the effects of fatty acid feeding on cattle fertility reported by Santos *et al.* (2008a) indicated that pregnancy rates and embryonic loss responses varied among experiments according to

experimental conditions and type of fat included in the diet. For example, compared with groups of cows fed saturated fats, embryonic losses were decreased between 53 and 100% in three studies and not affected in two other studies in cows which received diets enriched in n-3 fatty acids.

Strategies aiming to stimulate growth of the conceptus should result in greater survival rates. Secretion of IFN is positively associated with conceptus size (Mann *et al.*, 1999), therefore, larger conceptuses should be better able to block PGF synthesis and luteolysis. One possible way to stimulate conceptus growth is through the administration of bST. For example, *in vitro* administration of recombinant bST increased fertilization rates, hastened embryo development and increased embryo quality (Moreira *et al.*, 2002b). In a subsequent study, placebo or bST was injected in superovulated embryo donors and embryo transfer recipients in a 2 x 2 factorial arrangement (Moreira *et al.*, 2002a). The bST treatment increased the proportion of transferable embryos, number of blastocysts present per flushing and pregnancy rates of recipients treated with bST, or receiving embryos from donors treated with bST. It was concluded that both maternal and conceptus components were affected by bST treatment. In subsequent studies, lactating dairy cows received bST injections or served as controls and were slaughtered on day 17 after insemination (Bilby *et al.* 2006b, c). The bST treatment increased conceptus length (45 vs. 34 cm) and IFN in uterine flushings (9.4 vs. 5.3μg) compared with controls. Consistent findings were reported by Santos *et al.* (2004a), which showed decreased embryonic losses in cows treated with bST.

Future research directions

It is expected that novel antiluteolytic strategies will emerge as investigators understand more the biology of pregnancy recognition. Specifically, new understanding should be gathered in the topics of measurements of early conceptus losses, uterine biology and conceptus biology.

Quantification of pregnancy losses associated with failure of maternal recognition of pregnancy is challenging. This is because there is no clear marker of the conceptus status (i.e., live, dead, healthy, unhealthy etc) in this period that can be measured non-invasively. For example, there is presently no variable that can be measured in blood samples taken from the mother which is an adequate predictor of conceptus status. However, potential new diagnostic tools are developing, such as exciting discoveries reported by Gifford *et al.* (2007). In that study, expression of interferon-stimulated genes was detected in peripheral blood leukocytes collected from pregnant cows, but not in blood collected from bred, non-pregnant cows. The association of conceptus-stimulated gene expression in blood leukocytes and conceptus status warrants further

investigation and may provide a unique tool to quantify embryo mortality during early pregnancy. Pregnancy diagnosis by ultrasonography has been used routinely. However, this technique only reached a 100% sensitivity on day 26 of gestation for heifers and day 29 for cows (Romano *et al.*, 2006) and these days are past the period of pregnancy recognition. This makes this method unsuitable for determination of early embryonic mortality. Collectively, the implication of these facts is that only a very limited number of studies reported early embryo losses (i.e., during the first three weeks of gestation; for a review see Diskin and Morris, 2008), and such studies relied on termination of pregnancy and visual inspection of the conceptus. Moreover, no such study has been conducted with *Bos taurus indicus* type cattle. Precise determination of timing of embryo losses is critical for the discovery of causal factors and the elaboration of more precise strategies for mitigation.

Because initial growth of the conceptus is highly dependent on the uterine luminal microenvironment, there must be an optimal composition of such microenvironmental factors needed for embryo and conceptus growth. Likewise, there must be factors which presence is detrimental to growth. Nature of such factors, remains largely unknown. Characterization of factors in the intrauterine milieu which stimulate/limit conceptus growth is urgently needed because they may: 1) provide the biological basis to explain mechanisms of action of strategies such as progesterone supplementation; 2) provide markers of fertility or infertility associated with individual animals (i.e., effects of genetics) or management systems (i.e., effect of the environment); and 3) provide novel models for stimulation/inhibition of specific factors to ultimately stimulate embryo survival. For example, considering that the beneficial effects of progesterone supplementation are through the stimulation of secretion of stimulatory factors to the uterine lumen, identification of such factors might provide clues to explain differential responses to increased progesterone early (i.e., before day 7; Mann *et al.*, 2006; Demetrio *et al.*, 2007) vs. later in the luteal phase (i.e., after day 7; Marques, 2002). Recent work has identified genes whose expression is modulated in the cattle endometrial tissue in response to increasing plasma concentrations of progesterone (Forde *et al.*, 2008), but changes in uterine fluid composition remain unstudied.

Finally, a better understanding of conceptus biology in relation to its ability to block luteolysis is needed. For example, besides IFN, little information exists about conceptus factors related to survival. Characterization of such factors and understanding their effects in the PGF synthesizing machinery is critical. Moreover, identification of such critical conceptus factors could provide the tools for evaluating the conceptus survival potential before embryo transfer, for example. New exciting data indicate that conceptus secreted factors may not only act in a paracrine fashion

130

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

but may actually leave the uterine lumen and have endocrine actions. Uterine venous blood had 500 to 1000-fold greater IFN bioactivity in pregnant ewes than uterine arterial blood (Oliveira *et al.*, 2008), whereas no differences were detected in bioactivity in cyclic ewes. In addition to changes in IFN bioactivity, they also reported a higher expression of IFN-stimulated genes, such as ISG15, in extrauterine tissues, such as the CL, which supports extrauterine, endocrine actions of a conceptus-produced factor. Changes in gene expression and, ultimately, in tissue function induced by conceptus secreted factors to support pregnancy remains to be established, and provide a promising venue of research.

Acknowledgments

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Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

131

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132

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009

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134

Anim. Reprod., v.6, n.1, p.125-134, Jan./Mar. 2009