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RESEARCH ARTICLE

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Use of endometrial cytology and metabolic profiles for selection of embryo donor cows

F. Ismael Fernandez-Sanchez[1], Monica Barrio-Lopez[2], Luis A. Quintela-Arias[2]*, Juan J. Becerra-Gonzalez[2], Ana I. Peña-Martinez[2], Daniel Martinez-Bello[3], Pedro J. Garcia-Herradon[2] and Carlos C. Perez-Marín[4]

[1] Xénese E.T.E. Barreiros, Sedes. 15596 Narón (A Coruña), Spain. [2] Departamento de Patología Animal. Facultad de Veterinaria de Lugo. Universidad de Santiago de Compostela. Campus Universitario. 27002 Lugo, Spain. [3] Embriovet S. L. Rois 18 K. 15165 Bergondo (A Coruña), Spain. [4] Departamento de Medicina Animal y Cirugía. Universidad de Córdoba. Campus de Rabanales. Córdoba, Spain

Abstract

The aim of this study was to evaluate the use of endometrial cytology and metabolic profiles for selection of donor cows in embryo transfer programmes. For this purpose, 69 clinically healthy Holstein cows were enrolled in the study. At the start of the superovulation procedure (Day 0), blood and endometrial samples were obtained to determine metabolic and uterine status, respectively. The cows were then subjected to porcine follicle stimulating hormone (pFSH) superovulation treatment, and embryos were recovered after 7 days. The mean number of embryos obtained per flush was 9.89 ± 8.21 (4.63 ± 5.34 viable embryos, 0.82 ± 2.01 degenerated embryos and 4.57 ± 6.44 unfertilized ova). The following statistically significant variables were entered in a regression model: beta-hydroxybutyrate, serum cholesterol, body condition, number of calvings and percentage of neutrophils. In almost all cases, the model explained some percentage of the variance: total number of embryos, 4.8% ($p < 0.05$); number of degenerate embryos, 4.2% ($p = 0.051$); and number of unfertilized ova, 14.2% ($p < 0.01$). Statistical models for the percentage of viable embryos and unfertilized ova accounted for 24.0% and 29.4% of the variance, respectively, and both were statistically significant ($p < 0.01$). The model for the percentage of degenerated embryos was statistically significant ($p < 0.05$) and explained 4.4% of the variance. In conclusion, we have demonstrated that positive energy balance and healthy uterus can improve ovarian response and the proportion of viable embryos in cows. Efficient tools for monitoring the metabolic and uterine status should therefore be used in bovine embryo transfer programmes.

Additional key words: dairy cattle; embryo transfer; serum chemistry parameters; endometritis; neutrophils.

Introduction

Variability in the response to superovulation (SOV) protocols and the time and effort required to administer treatments have affected the widespread application of embryo transfer (ET) in genetic improvement programmes for cattle (Bó *et al.,* 2008). Differences in the superovulatory response have been associated with gonadotrophin preparation, batch and total dose, duration and timing of treatment, and the use of additional hormones in the superovulatory protocol (Mapletoft *et al.,* 2002). Environmental and individual cow factors such as nutritional status, reproductive history, age, season, breed, ovarian status and repeated SOV treatments can also affect the superovulatory response (Mapletoft *et al.,* 2002).

Early studies of the metabolic profile and blood biochemistry in cows clarified the relationship between some blood components and physiological events (Payne, 1970). Metabolic profiles have been used to identify metabolic problems that may directly or indirectly affect fertility and productivity in cattle

* Corresponding author: luisangel.quintela@usc.es
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Abbreviations used: BHB (beta-hydroxybutyrate); ET (embryo transfer); LH (luteotrophic hormone); MOET (multiple ovulation and embryo transfer); NEB (negative energy balace); NEFA (non sterified fatty acid); pFSH (porcine follicle stimulating hormone); PMN (polymorphonuclear neutrophils); SOV (superovulation).

(Ramos *et al.,* 2007). Several studies have also demonstrated a relationship between metabolic disorders and reproductive efficiency and, subsequently, embryo production (Chorfi *et al.,* 2007; Ramos *et al.,* 2007; Pfeifer *et al.,* 2009).

A relationship between uterine health and reproductive performance has also been established (Gilbert *et al.,* 2005; Santos *et al.,* 2009; Senosy *et al.,* 2009). Subclinical endometritis is common in dairy cows, affecting as many as 50% of all cows from 7 weeks postpartum in high-producing dairy herds (Hammon *et al.,* 2001; Gilbert *et al.,* 2005; Sheldon *et al.,* 2006). This can severely impair subsequent reproductive performance (LeBlanc *et al.,* 2002; Kasimanickam *et al.,* 2004) by exerting a detrimental impact on embryo quality due to inflammatory mediators (Hill & Gilbert, 2008).

Useful information for diagnosing reproductive disorders in cows can be obtained by standard techniques: transrectal palpation (Azawi, 2008), vaginoscopy (McDougall, 2001; LeBlanc *et al.,* 2002), collection of vaginal mucus (McDougall *et al.,* 2007) and ultrasonography (Quintela *et al.,* 2012). However, these routine types of examination are not always very efficient. Cytological examination of endometrial samples from cows can be used to obtain an earlier and more precise diagnosis of subclinical endometritis (Gilbert *et al.,* 1998; Kasimanickam *et al.,* 2004; Azawi, 2008; Barlund *et al.,* 2008).

The aim of this study was to evaluate the influence of subclinical endometritis and several biochemical parameters on an embryo collection resulting from a multiple ovulation and embryo transfer (MOET) programme for dairy cows.

Material and methods

Animals and location

Forty-one high genetic merit Holstein donors at the Bovine Embryo Transfer Unit (FEFRIGA, A Coruña), in northwestern Spain, were used for this study. The cows were housed in individual stalls and were fed a total mixed ration (25.5 kg dry matter intake; 16.5 crude protein; 1.5 Mcal kg^{-1} dry matter intake net energy). Dry cows were fed a grass-based diet supplemented with rye-grass hay, in accordance with National Research Council (NRC, 2001) guidelines. The cows were clinically healthy, with an average number of calvings of 2.28 and mean body condition score of 3 (Ferguson *et al*., 1994).

Sample collection and serum determination

Donor cows were selected for SOV treatment. Blood samples were obtained by puncture of the coccygeal vein by using vacuum tubes without anticoagulant. The blood samples were centrifuged at 1500 g for 20 min, and serum was aliquoted and stored at –20°C until analysis. A digital photometer (Selecta MD200, Barcelona) was used for all analyses, except protein analysis, for which a portable refractometer was used. Glucose, total cholesterol, triglyceride and albumin concentrations were determined by a colorimetric endpoint method, with Biosystems reagents (Biosystems S. A., Barcelona); urea was also assayed by a colorimetric enzymatic method with Spinreact reagents (Spinreact, S.A.U., Sant Esteve de Bas, Spain), and non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) were analyzed by use of kinetic enzymatic kits from Randox Laboratories (Antrim, UK).

Endometrial sampling was performed with the aid of a small cytobrush (length 20 mm and diameter 0.6 mm) twisted onto the plunger of insemination catheter (Quicklock 2000, Minitube Iberica, Barcelona). The catheter and cytobrush were protected by an insemination sheath and surrounded by a plastic sanitary sleeve (Minitube Iberica, Barcelona). The catheter was passed through the vagina to the external cervical opening. At the cervix, the tip of the sanitary sheath was punctured with the insemination catheter and the device comprising catheter, cytobrush and insemination sheath was passed into the uterine body. The plunger was depressed to extend the cytobrush, and a sample was obtained by rotating the cytobrush against the adjacent uterine wall. Before being completely removed, the cytobrush was retracted into the catheter to prevent cellular contamination from the cervix or vagina. The cytobrush was removed and the sample was rolled onto a glass slide and allowed to air-dry. Slides were stained with Quick Panoptic reagents (Quick Panoptic kit, Química Clínica Aplicada S.A., Tarragona, Spain). The slides were then examined under an optical microscope (CHT, Olympus Iberia S. A.U., Barcelona, Spain), at 100× magnification, by one examiner who counted at least 150 cells (nucleated cells: epithelial cells, large vacuolated epithelial cells, neutrophils and lymphocytes) (Ahmadi & Nazifi,

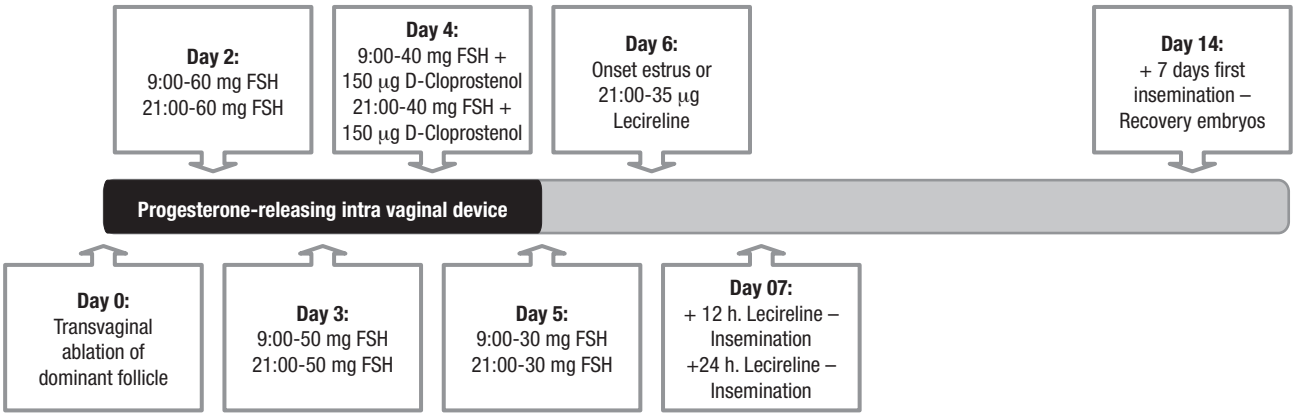

Figure 1. Schematic diagram of the superovulation (SOV) protocol with FSH (follicle stimulating hormone).

2006) for quantitative assessment of endometrial inflammation.

Superovulation and embryo collection

Sixty-nine SOV treatments were applied. The genital tract of each cow was thoroughly explored by rectal palpation, ultrasound and vaginoscopy, and any cows with signs of clinical endometritis or abnormalities were excluded from the study.

When a dominant follicle was present at the beginning of the SOV treatment (Fig. 1, Day 0 of the SOV protocol), transvaginal ablation was performed. A progesterone-releasing intravaginal device (PRID®, Ceva Salud Animal S.A., Barcelona) was then inserted. Treatment with pFSH (Folltropin®, Bioniche Animal Health Europe Ltd., Dublin, Ireland) was started on Day 2: a total dose of 360 mg was given over four days by administration every 12 h of a dose that was decreased daily (60, 60, 50, 50, 40, 40, 30 and 30 mg). Luteolysis was induced by intra muscular (i.m.) injection of 150 μg D-Cloprostenol (Dalmazin®, Fatro Ibérica, Barcelona) at the same time as the fifth and sixth doses of pFSH were administered. PRID® was withdrawn after the eighth dose of pFSH. At the onset of oestrus or 24 h later, donors were injected i.m. with 25 μg Lecireline (Dalmarelin®, Fatro Ibérica, Barcelona, Spain) and were inseminated twice, 12 h and 24 h later.

Embryos were recovered with the aid of a Foley catheter (18 Gauge for bovine). The catheter was guided, by a sterile gloved hand, through the vagina and cervix until it reached the uterine horn; the catheter balloon was then inflated with 10 mL of air and each uterine horn was flushed repeatedly with 0.5 L of phosphate buffered saline solution (Vigro™ Complete Flush, Bioniche Animal Health Europe Ltd., Dublin, Ireland). This was carried out 7 days after the first insemination. The flushed medium was collected into sterile embryo collection filters (EZ Way filter, SPI™, PETS, Canton, TX, USA) and transported to the laboratory. The filters were searched for embryos under a stereomicroscope (Leica MZ 95, Leica Microsistemas S.L.U., Barcelona). Finally, ova/embryos were placed in a Petri dish containing holding medium (Vigro™ Holding Plus, Bioniche Animal Health Europe Ltd., Dublin, Ireland) and were classified as unfertilized ova, degenerated embryos or viable embryos (IETS Manual; Stringfellow & Givens, 2010).

Statistical analysis

Statistical analysis was performed using the SPSS 19.0 software package (SPSS, Chicago, IL, USA). Data were expressed as means ± standard deviation (SD). Differences in means were considered significant at $p \leq 0.05$. Relevant clinical parameters (number of calvings, body condition score, percentage of polymorphonuclear neutrophils, calving-SOV treatment interval, glucose, total cholesterol, triglycerides, albumin, total proteins, urea, β-hydroxybutyrate and non-esterified fatty acids) were included as independent variables in a multiple linear regression model to determine independent effects on the number of total embryos, unfertilized ova, viable and degenerated embryos, percentage of viable and degenerated embryos and percentage of unfertilized ova (dependent variables). Variables selected by a stepwise were entered in a final

multivariate linear regression model. The R^2 coefficient was used to describe the percentage of variance in the dependent variables that was explained by the multivariate model.

Results and discussion

The SOV procedure yielded a total of 9.89 ± 8.21 embryos or ova per flush. The mean number of viable and of degenerated embryos was 4.63 ± 5.34 and 0.82 ± 2.01, respectively. We also collected per flush a mean of 4.57 ± 6.44 unfertilized ova.

Models obtained to evaluate factors (Tables 1, 2 and 3) related to SOV explained some of the variance for the total number of embryos (4.8%; $p < 0.05$), number of degenerate embryos (4.2%; $p = 0.051$), number of unfertilized ova (14.2%; $p < 0.01$), and the percentage of viable embryos (24.0%; $p < 0.01$), degenerated embryos and unfertilized ova (29.4; $p < 0.01$).

Table 1. Descriptive statistics for independent variables used in multiple linear regression analysis

Independent variables	Mean±SE
Number of calvings	2.28±0.13
Body condition score	3.00±0.05
Polymorphonuclear neutrophils (%)	0.86±0.31
Calving-superovulation treatment interval	354.78±48.89
Glucose (mg dL^{-1})	73.62±1.41
Total cholesterol (mg dL^{-1})	216.43±10.01
Triglycerides (mg dL^{-1})	16.60±1.26
Albumin (g L^{-1})	38.01±0.62
Total proteins (g L^{-1})	64.99±0.70
Urea (mg dL^{-1})	33.07±1.36
Beta-hydroxybutyrate (mmol L^{-1})	0.53±0.03
Non-esterified fatty acids (mmol L^{-1})	0.32±0.04

Table 2. Results of multiple regression analyses with total and degenerated embryos and unfertilized ova as outcome parameters

Dependent variable	Model variables	Non-standardized beta coefficient ± SE	Standardized beta coefficient	Significance level
Total embryos	Constant	4.266±1.745		0.017
	BHB	–1.147±0.576	–0.236	0.051
Degenerated embryos	Constant	10.575±2.776		0.000
	BCS	–2.245±1.103	–0.241	0.046
Unfertilized ova	Constant	6.373±2.101		0.003
	BHB	8.170±2.690	0.371	0.003
	Cholesterol	–0.028±0.009	–0.370	0.004

BHB: beta-hydroxybutyrate. BCS: body condition score. SE: standard error.

Table 3. Results of multiple regression analyses with percentage of viable and degenerated embryos and unfertilized ova as outcome parameters

Dependent variable	Model variables	Non-standardized beta coefficient ± SE	Standardized beta coefficient	Significance level
Viable embryos (%)	Constant	11.646±10.954		0.292
	Cholesterol	0.197±0.048	0.441	0.000
	PMN	–4.615±1.556	–0.316	0.004
Degenerated embryos (%)	Constant	10.575±2.776		0.000
	Nº of calvings	–2.245±1.103	–0.241	0.046
Unfertilized ova (%)	Constant	80.382±11.744		0.000
	Cholesterol	–0.256±0.053	–0.541	0.000
	PMN	5.221±1.589	0.337	0.002
	BHB	27.896±15.032	0.206	0.068

PMN: polymorphonuclear neutrophils. BHB: beta-hydroxybutirate. SE: standard error.

Variables entered in the models were related to energy balance (total cholesterol, BHB and body condition) as well as uterine health status (polymorphonuclear neutrophils [PMN]). The number of calvings affected the percentage of degenerated embryos, although the model only explained 4.4% of the variance.

In cows with negative energy balance (NEB) status, body fat is mobilized and the blood NEFAs increase and are metabolized in liver, to be transformed into ketone bodies (Wathes *et al.,* 2003) or to be stored as triglycerides, which can cause fatty liver (Knegsel *et al.,* 2007). BHB is the most stable ketone body, and serum values are used to diagnose subclinical ketosis (Oetzel, 2003). In the present study, we found a negative correlation between the level of BHB and the number of embryos collected. This suggests that low energy status induces a lower ovarian response during superovulatory treatment. NEB status in dairy cows is known to affect luteotrophic hormone (LH) secretion and, thus, ovulation (Jolly *et al.,* 1995; Buttler, 2003). In this study, glucose had no effect on embryo production and was also not a good indicator of energy balance in cows, because of the homeostatic control exerted on this sugar (Reist *et al.,* 2002). We also found positive correlations between BHB, the number of unfertilized ova, and the percentage of unfertilized ova in relation to the number of embryos collected (Tables 2 and 3). This suggests that the ability of oocytes to mature and be fertilized may be compromised by an increased concentration of BHB. Leroy *et al*. (2004) observed that changes in serum glucose concentration and BHB are reflected in the composition of dominant follicle fluid in bovine embryos cultured *in vitro*. These authors concluded that in cows experiencing clinical ketosis the biochemical environment prevents optimal oocyte maturation in follicular fluid and thus hampers developmental competence (Leroy *et al*., 2006). They also demonstrated that in cows with subclinical ketosis, in which the glucose concentration is moderately low, BHB aggravates the toxic effect of low glucose concentrations and induces a significant reduction in the ability of fertilized ova to develop into morulae or blastocysts. Gómez *et al*. (2002) demonstrated that early bovine embryos are capable of consuming BHB as an alternative energy source. However, BHB does not seem to be used as alternative source of energy in oocyte maturation when glucose concentration is low (Leroy *et al*., 2006). In cumulus cells, glucose is predominantly metabolized via the glycolytic pathway to produce pyruvate and lactate, the oocyte's preferred substrates for ATP production (Cetica *et al*., 2002). Pyruvate and lactate molecules cannot be produced from BHB, because BHB is inserted as acetyl CoA in the Krebs cycle (Stryer, 1995). In the oocyte, glucose is predominantly metabolized in the pentose phosphate pathway (PPP) (reviewed by Sutton *et al*., 2003), and BHB cannot be utilized as an alternative substrate in this pathway (Nehlig, 2004). NEB cows may suffer long-term effects on fertility due to a deleterious effect on follicular development and on oocytes (Britt, 1994). Animals with high BHB levels during SOV treatment may have suffered NEB in early postpartum, and this may affect maturation of oocytes.

In the present study, cholesterol also strongly influenced the number of unfertilized embryos and the percentage of viable and unfertilized embryos (Tables 2 and 3). As a component of serum lipoproteins, serum cholesterol is a good indicator of total lipoproteins. The decrease in cholesterol levels observed in peripartum cows with elevated NEFA concentrations has been associated with altered liver metabolism (Vasquez-Anon *et al.,* 1994) and with the occurrence of other diseases such as metritis (Lotthammer *et al.,* 1971; Kweon *et al.,* 1985, 1986). Although cholesterol did not affect the ovarian response —measured as the total number of embryos collected— it had a positive impact on the percentage of viable embryos. The number and percentage of oocytes were low in the cows under study, which suggests that the presence of cholesterol might improve the capacity of donor cows to produce oocytes that mature and become fertilized. In this respect, components of follicular fluid are known to affect granulose cells and oocyte, as they regulate follicle development and steroidogenesis (Brantmeier *et al.,* 1987). Cholesterol, through HDL and LDL lipoproteins, is the main precursor of steroid hormones (Ramos *et al.,* 2007). Herrera-Camacho *et al.* (2008) indicated that ovulation and fertilization are promoted when the oocyte develops and matures in a suitable endocrine environment. Staples *et al*. (1998) observed an increase in progesterone synthesis in follicular and luteal cells when the concentration of cholesterol increases as a result of dietary fat supplementation, producing a higher percentage of pregnancies in dairy cows. Although no increase in the number of viable embryos was observed in the present study (Table 2), other authors have observed that the number of transferable embryos was significantly higher in cows with ≥130 mg dL^{-1} of serum cholesterol than in cows with lower concentrations of cholesterol (Kweon *et al.,* 1985). In

a study carried out in crossbreed cows undergoing ovum pick-up, Pfeifer *et al*. (2009) obtained more follicles suitable for puncture in cows with cholesterol levels higher than 50 mg mL^{-1} than in cows with lower cholesterol levels, although the number and quality of recovered oocytes were not affected. Lucy *et al*. (1992) also found that the number and the size of follicles were higher in cows with higher blood cholesterol levels. In contrast, Chorfi *et al*. (2007) observed that serum cholesterol levels in superovulated cows did not significantly influence the number of transferable embryos.

Several authors (Green *et al*., 2009; Burke *et al*., 2010; Priest *et al*., 2013) have reported links between inflammation, liver function, subclinical endometritis and reproductive performance. Although these links are more likely to be associations that cause an effect, they constitute potential markers for subclinical endometritis in blood/serum. In a study of the association between liver proteins and subclinical endometritis diagnosed at day 21 and 42 postpartum, Green *et al*. (2009) found that cows with subclinical endometritis had lower plasma albumin and lower total protein concentration than control cows. However, we did not observe this relationship in the present study, and neither albumin or total protein index accounted for the variation in results (Tables 1 and 2), probably because the cows used in this study had calved for several months. Endometrial cytological sampling was used in this study to assess the presence of polymorphonuclear neutrophils, as previously described (Kasimanickam *et al.,* 2004; Senosy *et al.,* 2009). We observed that as the PMN increased, the percentage of viable embryos and fertilized ova decreased (Table 3). A lower number of fertilized ova and lower embryo quality associated with high uterine PMN may be affected by altered function of sperm and also by oocyte quality. It is well known that PMN can produce reactive oxygen species (Alóe *et al*., 2012) and that these may be damaging to sperm. Furthermore, Sheldon *et al*. (2009) concluded that uterine bacterial infections impair the function of the hypothalamus and pituitary and directly perturb steroidogenesis by ovarian granulose cells. Our results are consistent with those of a recent study by Carvalho *et al*. (2013), who reported a substantial reduction in embryo yield when the percentage of uterine PMN was increased before initiation of the superstimulation protocol. Moreover, an unfavourable uterine environment associated with inflammation might negatively affect embryo viability and development. Hill & Gilbert (2008) reported a reduction in blastocyst quality in bovine embryos cultured in vitro in media conditioned by fluid from an inflamed endometrium. These authors observed a lower number of total and trophectoderm cells, and a higher inner cell mass:trophectoderm ratio, although the number of degenerated or dead embryos did not increase, which suggests that aseptically-induced endometritis has a lower impact on embryo quality than expected for active bacterial endometritis. However, Drillich *et al*. (2012) did not find any difference in the quality of blastocysts produced referred to total cell number or apoptotic cell index, recovered from cows with or without high levels of PMN at insemination or flushing. They explained this on the basis of the relatively short time that embryos remained in the uterus cavity in comparison with the time that embryos remained in conditioned media. In this respect, Cerri *et al*. (2009) observed a lower fertilization rate in cows with subclinical endometritis, suggesting that decreased fertility is linked to decreased sperm transport or fertilizing capacity.

Our results show that the influence of parity was low. We found that as the number of calvings increased, the percentage of degenerated embryos decreased (Table 3).

In this study, we explored different factors (Table 1) that may influence the response to superovulatory treatments. These results can be improved by identifying negative energy balance (NEB) and by determining BHB and cholesterol levels. Endometrial cytological sampling can also be used to detect optimum uterine health status, as reflected by a low percentage of PMN, in order to select donor cows with a suitable uterine environment that allows the transport of spermatozoa, fertilization and the development of embryos.

In conclusion, we found that measurement of biochemical parameters (such as cholesterol and mainly BHB, which can be measured *in situ*) and the use of endometrial cytology, linked to previous exploration of the genital tract, can facilitate the selection of donor cows for *in vivo* embryo production.

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References

Ahmadi MR, Nazifi S, 2006. Evaluation of reproductive status with cervical and uterine cytology in fat-tailed sheep. Comp Clin Pathol 15: 161-164.

Alóe S, Weber F, Behr B, Sauter-Louis C, Zerbe H, 2012. Modulatory effects of bovine seminal plasma on uterine inflammatory processes. Reprod Domest Anim 47: 12-19.

Azawi OI, 2008. Postpartum uterine infection in cattle. Anim Reprod Sci 105: 187-208.

Barlund CS, Carruters TD, Waldner CL, Palmer CW, 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 69: 714-723.

Bó GA, Carballo Guerrero D, Adams GP, 2008. Alternative approaches to setting up donor cows for superovulation. Theriogenology 69: 81-87.

Brantmeier SA, Grummer RR, Ax RL, 1987. Concentrations of high density lipoproteins vary among follicular sizes in the bovine. J Dairy Sci 70: 2145-2149.

Britt JH, 1994. Follicular development and fertility: Potential impacts of negative energy balance. Proc Natl Reprod Symp, Pittsburgh PA Texas Agric Ext Serv, College Station, TX, pp: 103-112.

Burke CR, Meier S, McDougall S, Compton C, Mitchell M, Roche JR, 2010. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. J Dairy Sci 93: 5363-5373.

Butler WR, 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 83: 211-218.

Carvalho PD, Souza AH, Sartori R, Hackbart KS, Dresch AR, Vieira LM, Baruselli PS, Guenther JN, Fricke PM, Shaver RD, Wiltbank MC, 2013. Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. Theriogenology 80: 1074-1081.

Cerri RLA, Rutigliano HM, Lima FS, Araújo DB, Santos JEP, 2009. Effect of source of supplemental selenium on uterine health and embryo quality in high-producing dairy cows. Theriogenology 71: 1127-1137.

Cetica P, Pintos L, Dalvit G, Beconi M, 2002. Activity of key enzymes involved in glucose and triglyceride catabolism during bovine oocyte maturation in vitro. Reproduction 124: 675-681.

Chorfi Y, Lanevschi A, Dupras R, Girard V, Tremblay A, 2007. FERUM biochemical parameters and embryo production during superovulatory treatment in dairy cattle. Res Vet Med 83: 318-321.

Drillich M, Tesfaye D, Rings F, Schellander K, Heuwieser W, Hoelker M, 2012. Effects of polymorphonuclear neutrophil infiltration into the endometrial environment on embryonic development in superovulated cows. Theriogenology 77: 570-578.

Ferguson JO, Galligan DT, Thomsen N, 1994. Principal descriptors of body condition score in Holstein cows. J Dairy Sci 77: 2695-2703.

Gilbert RO, Shin ST, Guard CL, Erb HN, 1998. Incidence of endometritis and effects on reproductive performance of dairy cows. Theriogenology 49: 251.

Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M, 2005. Prevalenceof endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64: 1879-1888.

Gómez E, Duque P, Díaz E, Facal N, Antolin I, Hidalgo C, Díez C, 2002. Effects of acetoacetate and D-beta-hydroxybutyrate on bovine in vitro embryo development in serum-free medium. Theriogenology 57: 1551-1562.

Green MP, Ledgard AM, Beg MC, Peterson AJ, Back PJ, 2009. Prevalence and identification of systemic markers of subclinical endometritis in postpartum dairy cows. Proc New Zeland Society of Animal Production 69: 37-42.

Hammon DS, Holyoak GR, Jenson J, Bingham HR, 2001. Effects of endometritis at the beginning of the breeding period on reproductive performance in dairy cows. Am Assoc Bov Pract 34: 142-143.

Herrera-Camacho J, Aké-López JR, Ku-Vera JC, Williams GL, Quintal-Franco JA, 2008. Ovulatory response and embryo quality and development in superovulated Pelibuey ewe supplemented with polyunsaturated fatty accids in the diet. Tec Pecu Mex 46: 107-117.

Hill J, Gilbert R, 2008. Reduced quality of bovine embryos cultured in media conditioned by exposure to an inflamed endometrium. Aust Vet J 86: 312-316.

Jolly PD, McDougall S, Fitzpatrick LA, MacMillan KL, Entwistle KW, 1995. Physiological effects of undernutrition on postpartum anoestrus in cows. J Reprod Fertil 49 (Suppl): 477-492.

Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, Johnson WH, 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62: 9-23.

Knegsel ATM van, Brand H van den, Dijkstra J, Kemp B, 2007. Effect of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. Theriogenology 68 (Suppl 1): 274-280.

Kweon OK, Ono H, Yamashina H, Seike N, Mori K, Kanagawa H, 1985. Relationship between serum total cholesterol and progesterone levels and the number of transferable embryos in superovulated Holstein heifers and cows. Jpn J Anim Reprod 31: 231-235.

Kweon K, Ono J, Osasa K, Onda M, Oboshi K, Uchisugi J, Kurosawa S, Yamashina H, Kanagawa H, 1986. Factors affecting serum total cholesterol level of lactating Holstein cows. Jpn J Vet Sci 48: 481-486.

Kweon OK, Kanagawa H, Takahashi Y, Miyamoto A, Masaki J, Umezu M, Kagabu S,Iwazumi Y, Aoyagi, 1987. Plasma endocrine profiles and cholesterol levels in superouvalted cows. Theriogenology 27: 841-857.

LeBlanc SJ, Duffield TF, Leslie KE, Bateman KJ, Keefe GP, Walton JS, Johnson WH, 2002. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci 85: 2223-2236.

Leroy JLMR, Vanholder T, Delanghe JR, Opsomer G, Van Soom, Bols PEJ, Kruif A, 2004. Metabolite and ionic composition of follicular fluid from different-sized

follicles and their relationship to serum concentrations in dairy cows. Anim Reprod Sci 80: 201-211.

Leroy JLMR, Vanholder T, Opsomer G, Van Soom A, Kruif A, 2006. The in vitro development of bovine oocytes after maturation in glucose and beta-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod Dom Anim 41: 119-123.

Lotthammer KH, Benten KV, Hahas HE, 1971. Klinischchemise Bluntuntersuchung zur Frudiagnose und Grundlage der prophylaxe primar nicht infectioser Erkrankungen des Rindes in Puerperium. Prakt Tierarzt 13: 563.

Lucy MC, Savio JD, Badinga L, De la Sota RL, Thacher WW, 1992. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 70: 3615-3626.

Mapletoft RJ, Bennett Steward K, Gregg PA, 2002. Recent advances in superovulation in cattle. Reprod Nutr Dev 42: 601-611.

McDougall S, 2001. Effect of intrauterine antibiotic treatment on reproductive performance of dairy cows following periparturient disease. New Zeal Vet J 49: 150-158.

McDougall S, Macaulay R, Compton C, 2007. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cows. Anim Reprod Sci 99: 9-23.

Nehlig A, 2004. Brain uptake and metabolism of ketone bodies in animal models. Prostaglandins Leukot Essent Fatty Acids 70: 265-275.

NRC, 2001. Nutrient requirements of dairy cattle, 7th rev. ed. National Research Council, Natl. Acad. Sci., Washington DC, USA.

Oetzel GR, 2003. Herd-based biological testing for metabolic disorders. Adv Dairy Tech 15: 275-285.

Payne JM, Dew SM, Manston R, Faulks M, 1970. The use of metabolic profile test in dairy herds. Vet Rec 87: 150-157.

Pfeifer LFM, Pivato I, Rumpf R, Dionello NJL, Schneider A, Goulart MA, Correa MN, 2009. O nivel de colesterol influencia a quantidade de folículos na punçao follicular de vacas de corte. Arch Zootec 58: 153-156.

Priest NV, McDougall S, Burke CR, Roche JR, Mitchell M, McLead KL, Greenwood SL, Meier S, 2013. The responsiveness of subclinical endometritis to a non steroidal anti-inflammatory drug in pasture-grazed dairy cows. J Dairy Sci 96: 4323-4332.

Quintela LA, Barrio M, Peña AI, Becerra JJ, Cainzos J, Herradón PG, Díaz C, 2012. Use of ultrasound in the reproductive management of dairy cattle. Reprod Dom Anim 47: 34-44.

Ramos AF, Neves EF, Marques VS, Lima FPC, Drumond DL, Marques Jr AP, 2007. Efeito de diferentes protocolos de superovulação plasmática de progesterona e de metabólitos lipídicos de vacas Nelore. Arq Bras Med Vet Zootec 59: 273-279.

Reist M, Erdin DK, VonEuw D, Tschümperlin KM, Leuenberger H, Hammon HM, Morel C, Philipona C, Zbinden Y, 2002. Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. Theriogenology 59: 1707-1723.

Santos NR, Lamb GC, Brown DR, Gilbert RO, 2009. Postpartum endometrial cytology in beef cows. Theriogenology 71: 739-745.

Senosy WS, Uchiza M, Tameoka N, Izaike Y, Osawa T, 2009. Association between evaluation of the reproductive tract by various diagnostic tests and restoration of ovarian cyclicity in high-producing dairy cows. Theriogenology 72: 1153-1162.

Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO, 2006. Defining postpartum uterine disease in cattle. Theriogenology 65: 1516-1530.

Sheldon IM, Price SB, Cronin J, Gilbert RO, Gadsby JE, 2009. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reprod Dom Anim 44 (Supl 3): 1-9.

Stringfellow DA, Givens MD, 2010. Manual of the International Embryo Transfer Society (IETS), 4th ed. Champaign, IL: IETSStaples CR, Burke JM, Thatcher WW, 1998. Influence of supplemental fats on reproductive tissues and performance of lactating cows. J Dairy Sci 81: 856-871.

Stryer L, 1995. Biochemistry, 4th ed. W.H. Freeman and Company, NY, USA.

Sutton ML, Gilchrist RB, Thompson JG, 2003. Effects of in vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte development capacity. Hum Reprod Update 9: 35-48.

Vasquez-Annon SB, Luck M, Grummer RR, 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. J Dairy Sci 77: 1521.

Wathes DC, Taylor V, Cheng Z, Mann GE, 2003. Follicle growth, corpus luteum function and their effects on embryo development in postpartum dairy cows. Reproduction 61: 219-237.