Effects of follicular fluid of preovulatory follicles of repeat breeder dairy cows with subclinical endometritis on oocyte developmental competence

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ABSTRACT

The aims of the present study were to determine the concentrations of lipopolysaccharide (LPS), hormonal progesterone, estradiol-17β, insulin growth factor (IGF-1) and magnesium in the serum and the preovulatory follicle follicular fluid (FF) in repeat breeder (RB) cows without (nSCE) or with subclinical endometritis (SCE), and further to examine the effects of this FF on developmental competence of cattle oocytes. In Experiment 1, 13 of 23 clinically healthy Holstein RB cows were identified (uterine PMNs) to have SCE. The cows were estrous synchronized, and 6–12 h after detection of standing estrus, FF and blood of the preovulatory follicles were collected. The mean (± SD) LPS (862.3 ± 148.1 compared with 1063.4 ± 262.8 EU/ml, $P = 0.04$) and estradiol-17β (188.9 ± 15.8 compared with 162.0 ± 31.5 ng/ml, $P = 0.02$) concentrations of FF was different between nSCE and SCE cows. In Experiment 2, FF of RB cows with relatively lesser (nSCE, $n = 4$) and greater (SCE, $n = 4$) percentages of uterine PMNs was separately added to the oocyte maturation medium for in vitro embryo production. Addition of FF from SCE cows to the oocyte maturation medium resulted in a lesser rate of development to the blastocyst stage than that of the nSCE cows (21.9 ± 1.8 compared with 27.8 ± 2.5%, $P < 0.05$). Results of the present study indicate greater FF LPS concentration may result in a lesser quality microenvironment milieu for the final stages of oocyte maturation in RB dairy cows with subclinical endometritis. In addition, supplementation of oocyte maturation medium with FF of preovulatory follicles from RB cows with subclinical endometritis resulted in a lesser potential of in vitro oocyte developmental competence.

1. Introduction

Repeat breeding (RB) is a classification when a cow does not conceive after at least three consecutive breeding attempts. The etiology is multifactorial and may include uterine infections, hormonal imbalances, nutritional factors and genetic causes (Perez-Marin et al., 2012). Salasel et al. (2010) indicated that 52.7% of repeat breeder cows have subclinical endometritis (SCE). Furthermore, uterine infections, and more specifically subclinical endometritis, are important risk factors for a lesser reproductive performance of dairy cows (Gilbert, 2011; López-Helguera et al., 2012; Pascottini et al., 2017). On the contrary, Pothmann et al. (2015) observed that SCE has a minor role as a cause for the occurrence of RB cows.

For an optimal microenvironment of the ovulatory follicle, follicular fluid (FF), has an important function in the final growth of

the follicle and the oocyte. Inferior quality FF in ovulatory follicles adversely affects the conception rate when there is metabolic dysfunctions in lactating cows (Leroy et al., 2008) and cows with uterine infections (Williams et al., 2007; Cheong et al., 2017). The developmental competence of the oocyte is further disrupted when the FF of ovulatory follicles of repeat breeder Holstein heifers is added to the oocyte maturation media (Kafi et al., 2017). Proteomic analysis of the preovulatory FF of less fertile cows differed in some protein contents compared to that of the fertile cows (Zachut et al., 2016). Furthermore, Tanaka et al. (2013) changes in FF constituents and growth factors because of liver diseases resulted in dysfunctions of nuclear maturation of oocytes and blastocyst development in cattle.

In lactating cows, uterine (Sheldon et al., 2002) or mammary gland infections (Lavon et al., 2011) were associated with suppressed follicular growth, reduced intra-follicular estradiol-17β production and consequently disrupted ovarian functions and estrous cyclicity. Bromfield et al. (2015) reported that after the disappearance of clinical signs of uterine infections, the diseased cows may continue to be less fertile. The lesser fertility in cows with uterine diseases was associated with the large amount of lipopoly-saccharides (LPS) released from uterine Gram-negative bacteria which is then transported to FF of follicles from which ovulation occurs (Mateus et al., 2003; Herath et al., 2007). Asaf et al. (2014) reported that experimentally induced mastitis by the Gram-negative endotoxin of *Escherichia coli* origin could negatively alter the quality of preovulatory FF leading to a lesser oocyte developmental competence. There was a possible role of LPS in development of ovarian cysts reported for cattle (Shimizu et al., 2018). The presence of LPS in the FF of preovulatory follicles occurred in cows with mastitis (Asaf et al., 2014) and endometritis (Herath et al., 2007). There is, however, little information on the association of LPS concentration in FF of preovulatory follicles in cows with subclinical metritis with other FF components. There is a lack of information as to the effect of FF in preovulatory follicles of RB dairy cows with SCE on the developmental competence of cattle oocytes. The present study was conducted, therefore, to examine the composition of serum and FF of preovulatory follicles in RB dairy cows with or without SCE (Experiment 1), and the effects of preovulatory follicle FF from these cows, with and without SCE, on the developmental competence of cattle oocytes (Experiment 2).

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

The Ethical and Research Committee of the School of the Veterinary Medicine, Shiraz University (95GCU2M1251), Iran, approved this study. The experiment was conducted in a large commercial dairy farm located in Karaj, Alborz, Iran (35° 82′ N, 50° 97′ E). A total of 2000 Holstein cows were milked three times per day. The cows were maintained in free-stall barns and fed a total mixed ration that consisted of 40% forage including corn silage and alfalfa hay as well as 60% concentrate (corn, barley, wheat bran, soybean meal, and commercial concentrate for lactation with trace minerals and vitamins). Thirty-six RB Holstein dairy cows with at least three artificial inseminations in which pregnancy did not result were used in this experiment. Additionally, based on the previous routine examinations of the reproductive system, the cows did not have any detectable clinical signs of reproductive diseases. Means (± SD) days in milk, parity and number of previous artificial inseminations of the RB cows used in the present study were 277.9 ± 82.8, 3.3 ± 1.7 and 5.2 ± 1.6, respectively.

2.1.2. Time of ovulation synchronization protocol

The timing when ovulation occurred among cows was synchronized using an injection of gonadotropin-releasing hormone (200 μg Gonadorelin, Gonabreed, Parnell Laboratories, Alexandria, NSW, Australia) on Day 0 (Day of GnRH administration) and then an injection of PGF2α (500 μg Cloprostenol sodium, Estroplan Parnel Living Science) on Day 7. Cows received a second injection of gonadotropin-releasing hormone on Day 9, and then were observed for detection of standing estrus. The presence of a corpus luteum was confirmed by transrectal ultrasonography (CTS-800 V; 5 MHz rectal linear probe, SIUI Company, China) on Day 7 after the first gonadotropin-releasing hormone administration. There was intentional use of one dose of PGF2α in the Ovsynch protocol to induce luteolysis in cows with a corpus luteum to minimize any possible induction of handling stress or confounding factors. This approach was also used to minimize the probability of changes in the status of the uterine environment and composition of FF at the time of preovulatory follicle aspiration. Four cows did not have a corpus luteum on Day 7.

2.1.3. Detection of subclinical endometritis

Uterine secretory samples were collected using a 20 x 6 mm cytobrush (Heinz Herenz, Hamburg, Germany) technique on Day 7 if the cows had a mature corpus luteum. The brush was contained within an infusion pipette while a double-guarded cover was used to prevent vaginal contamination of the brush. The cover was punctured in the external os of the cervix. The infusion pipette was inserted into the uterus, endometrial secretions were collected by rotating the brush against the uterine wall, and the brush was retracted into the infusion pipette and removed from the cow. The brush was rolled onto a clean microscopic slide, fixed (immersion in alcohol 90% for 1 min) and stained using Giemsa. A total of 500 cells were counted to determine percentage of polymorphonuclear cells (PMNs). Cows were then categorized into two groups: RB cows without (nSCE, $n = 13$) and with (SCE, $n = 10$) subclinical endometritis based on the percentage of PMNs. A cut-off point of $\geq$ 3% PMNs was used for diagnosis of subclinical endometritis (Salasel et al., 2010; Pascottini et al., 2017). Immediately after cytobrush sampling, cows were administered PGF2α (500 μg Cloprostenol sodium, Estroplan Parnel Living Science) to induce estrus and to collect FF from preovulatory follicles.

2.1.4. FF aspiration and blood sampling

Cows were observed five times per day for a minimum of 20 min to detect standing estrus. The FF from the preovulatory follicles was aspirated 6–12 h after detection of standing estrus. Transrectal ultrasonography was performed to confirm the presence of the preovulatory follicle 14–17 mm in diameter. The FF was aspirated using a long fine-needle using caudal epidural anesthesia (2% Lidocaine hydrochloride; Pasteur Institute, Iran, 0.2 mg/kg) as previously described by Kafi et al. (2017). After follicular fluid aspiration, a blood sample was also collected *via* tail venipuncture. Serum was extracted by centrifugation at $750 \times g$ for 10 min. Special care was taken not to disturb the cows at the time of FF and blood sampling. The FF and serum samples were stored at $-20\,°C$ until assayed for LPS, magnesium, progesterone, estradiol-17β and insulin like growth factor (IGF-1).

2.1.5. Assay of LPS

The LPS concentrations in serum and FF of preovulatory follicles were quantified using a Bovine ELISA Kit (Hangzhou Eastbiopharm CO., LTD, China). The intra-assay coefficient of variation (CV) was < 10% and the inter-assay CV was < 12%. Assay range was 10–3000 EU/ml. The sensitivity of the assay was 3.8 EU/ml.

2.1.6. Hormone analyses

Serum and FF concentrations of progesterone were determined using an ELISA kit (IBL International, Hamburg, Germany). The intra- and inter-assay CV were 5.4% and 8.6%, respectively. The sensitivity of the assay was 0.1 ng/ml. Cows with serum progesterone concentrations of less than 1 ng/ml were considered in standing estrus. Estradiol-17β in serum and FF were determined using an ELISA kit (IBL International, Hamburg, Germany). The intra- and inter-assay CV for estradiol-17β were 8% and 10.8%, respectively, with a sensitivity of 20 ng/ml. The IGF-1 concentrations were also assayed using an ELISA kit (OCTEIA, IDS, Boldon, UK) in serum and FF samples. Intra-assay and inter-assay CV values were < 6.5% and < 7.5% respectively. The sensitivity of the assay was 3.1 ng/ml.

2.1.7. Magnesium measurements

Concentrations of serum and FF magnesium were determined by conducting a chemical photometric method using a Pars Azemoon kit, Iran. Intra- and inter-assay CV were 0.92% and 1.12%, respectively. The sensitivity of the assay was 0.05 mg/dl.

2.2. Experiment 2

2.2.1. In vitro maturation

The FF from eight repeat breeder cows with a relatively lesser (nSCE; < 3%, $n = 4$) and greater (SCE; > 8%, $n = 4$) percentage of uterine PMNs were used as the source of serum supplementation for the oocyte maturation media in the laboratory. The source of serum supplementation for the control group *in vitro* maturation was fetal calf serum (FCS). Unless otherwise stated, all chemicals were purchased from Sigma. Ovaries were collected from a local slaughterhouse and transported to the laboratory at 32 to 25 °C in physiological saline within 2–3 h of collection. Upon arrival at the laboratory, the ovaries were washed three times in pre-warmed (37 °C) sterile phosphate buffered saline. Immature cumulus oocyte complexes (COCs) were collected by aspirating 2–8 mm diameter follicles with a 10 ml syringe that had a 20 G needle attached. Only COCs classified to be of good quality with at least three complete layers of cumulus cells and finely granulated homogeneous ooplasm were selected and used for *in vitro* maturation as previously described (Lorenzo et al., 1994). The selected COCs were washed three times in drops of equilibrated oocyte washing medium consisting of HEPES-buffered TCM-199 (Sigma, M4530-500ML, St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS). A total of 1505 COCs were randomly allocated into three groups. Group 1 COCs (control, $n = 380$) were matured in TCM-199 supplemented with 10% heat-treated FCS and with 0.1 IU/ml recombinant human FSH (Follitrope, LG Life Sciences, South Korea), 5 IU/mL highly purified hCG (Karma, Pharmatech GmbH, Germany) and 10 ng/ml epidermal growth factor. In Group 2 (nSCE FF, $n = 545$), COCs were matured in TCM-199 supplemented with 10% filtered FF of preovulatory follicles of nSCE cows. In Group 3 (SCE FF, $n = 580$), COCs were cultured in TCM-199 supplemented with 10% filtered FF of preovulatory follicles of SCE cows. No hormones or growth factors were added to the maturation media in nSCE and SCE groups. The rationale for adding no hormones to the maturation media was to mature oocytes in a relatively similar follicular environment to that of preovulatory follicles of RB cows. Gentamicin (50 mg/ml) was used in the maturation media of all experimental groups. Groups of 30–40 COCs were cultured in 500 ml equilibrated maturation media in four-well culture dishes (Nunc™, Denmark) for 24 h at 38.5 °C in 5% CO_2 under maximum relative humidity.

2.2.2. Sperm capacitation

Frozen/thawed semen of a bull with proven *in vitro* fertility was used for *in vitro* fertilization using the swim up technique described by Parrish et al. (1995). Sperm capacitation medium contained modified Ca^{2+} free Tyrode's added to 2 mg/ml sodium bicarbonate, 1.12 mg/ml sodium lactate, 1 mg/ml sodium pyruvate, 0.1 mg/ml caffeine, 50 μl/ml gentamycin and 6 mg/ml bovine serum albumin. Briefly, the semen straw was thawed in 37 °C for 40 s and placed in a pre-warmed 15 ml-falcon tube. Semen (90 μl) was added to warm sperm capacitation medium and incubated for 45 min. The upper layer of media containing highly motile spermatozoa was then collected into a pre-warmed 15 ml falcon tube and centrifuged for 10 min at $300 \times g$. The supernatant was discarded and remaining pellet was added to the fertilization medium.

2.2.3. In vitro fertilization

In four-well dishes containing 500 μl fertilization medium, 30–40 matured COCs and highly motile spermatozoa were incubated at 38.5 °C, 5.5% CO_2 and 90% humidity. At 22 h after sperm addition, zygotes were mechanically denuded by repeated pipetting and transferred to synthetic oviductal fluid (SOF) medium. The SOF medium was refreshed every second day. Fertilization medium contained modified Tyrode's medium supplemented with 1 mg/ml sodium pyruvate, 2.2 mg/ml sodium bicarbonate, 1.12 mg/ml sodium lactate, 0.27 mg/ml caffeine, 10 μl/ml heparin, 50 μl/ml hypotaurine, 50 μl/ml gentamicin and 6 mg/ml BSA. Sperm capacitation and *in vitro* fertilization were conducted with all experimental groups.

2.2.4. In-vitro culture of presumptive zygotes

At the end of the fertilization period (18–20 h post insemination), the presumptive zygotes (control = 365, nSCE = 520 and SCE = 566) were completely denuded by repeated pipetting, washed in serum-free modified synthetic oviductal fluid (mSOF) supplemented with 4 mg/ml fatty acid-free BSA and cultured in the same medium in four-well dishes under mineral oil at 39 °C for 8 days in a water-saturated atmosphere of 5% CO_2 (Fouladi-Nashta et al., 2005). The embryo culture medium was renewed every 48 h. Embryo cleavage rate was determined at 48 h post insemination. The cleavage rate was defined as the oocytes cleaved to the two- to four-cell stage embryo divided by total number of oocytes fertilized (Asaf et al., 2014). Stage of embryo development was recorded using a stereomicroscope on the 7th and 8th day post insemination. The experiment was performed in 12 replicates.

2.3. Statistical analysis

Nine cows were excluded from the statistical analyses due to either having progesterone concentrations (> 1 ng/ml) at estrus or unsuccessful FF aspiration. The results were statistically analyzed using SPSS software (SPSS for Windows, version 23, SPSS Inc, Chicago, Illinois). The data were analyzed for normality of distribution using the Shapiro-Wilk test. Percentages of PMNs and concentrations of estradiol-17β, P4, IGF-1 and LPS of FF in SCE and nSCE groups were compared using the Independent Sample T test. Comparison between LPS in serum and FF was conducted using the Paired Samples T test. The associations between LPS and IGF-1 and LPS and magnesium in FF were assessed by determining the Pearson correlation coefficients. The associations between IGF-1 and magnesium in FF, as well as magnesium in serum and FF were evaluated using the Spearman's rho correlation coefficient.

Differences in cleavage and rate of blastocyst development between the control and repeat breeder cows were analyzed by Independent Sample T test. Differences between cleavage and rate of blastocyst development in control, nSCE and SCE groups were compared using the One Way ANOVA. Relationships between cleavage rates and LPS, IGF-1, magnesium, estradiol-17β, progesterone, and the percentage of uterine PMNs were assessed using the Pearson correlation coefficient. This analysis was repeated for rate development to the blastocyst stage. The replicate effect (three replicates for each FF) was not significant among replicates for each experimental group; therefore, Experiment 2 data were pooled. A significant difference was considered to exist where $P \leq 0.05$. All data were expressed as the mean ± SD.

3. Results

3.1. Experiment 1

3.1.1. Uterine cytology

Results of the uterine cytology indicated that 13 of 23 (56.5%) RB cows had cytological signs of SCE while ten of 23 (43.5%) had no cytologic signs of SCE.

3.1.2. LPS concentrations in serum and FF

The LPS concentrations in FF were greater in the SCE group than that of the nSCE group (1063.4 ± 262.8 compared with 862.3 ± 148.1 EU/ml, $P = 0.04$; Table 1). Serum LPS concentrations were not, however, different between SCE and nSCE groups (1448.9 ± 612.3 compared with 1509.2 ± 699.0 EU/ml, $P = 0.8$). Serum LPS concentration was greater than that of LPS concentration in FF of all cows ($n = 23$; 1475.1 ± 636.6 compared with 976.0 ± 238.8 EU/ml, $P = 0.004$). There was a positive correlation between the percentages of PMNs and LPS concentration in FF ($n = 23$, $r = 0.4$, $P = 0.06$).

Table 1
Results of uterine cytology and follicular fluid composition in repeat breeder (RB) cows without subclinical endometritis (nSCE) and with subclinical endometritis (SCE).

	nSCE ($n = 10$)	SCE ($n = 13$)	P value
PMN (%)	1.1 ± 0.9	8.7 ± 5.2	< 0.001
LPS (EU/ml)	862.3 ± 148.1	1063.4 ± 262.8	0.04
E2 (ng/ml)	188.9 ± 15.8	162.0 ± 31.5	0.02
P4 (ng/ml)	48.9 ± 20.7	58.6 ± 21.9	0.29
IGF-1 (ng/ml)	80.7 ± 17.0	94.6 ± 45.7	0.37

Values are Mean ± SD.
PMN, Polymorphonuclear cells; LPS, Lipopolysacchride; E2, Estradiol-17β; P4, Progesterone; IGF-1, Insuline like growth factor-1.

Table 2

Mean (± SD) percentages of cleavage and blastocyst yield following *in vitro* maturation of oocytes in follicular fluids collected from RB cows without (nSCE) and with subclinical endometritis (SCE) (Experiment 2, 12 replicates).

	Oocytes fertilized	Cleaved, *n* (%)	Blastocysts, *n* (%)
Control	365	277 (76.0 ± 6.6)[a]	106 (38.5 ± 6.2)[a]
nSCE	520	318 (61.3 ± 7.6)[b]	88 (27.8 ± 2.5)[b]
SCE	566	314 (55.5 ± 17.0)[b]	69 (21.9 ± 1.8)[c]

[a,b,c] Different superscript letters in the same column mean differ ($P < 0.05$).

3.1.3. Follicular fluid estradiol-17β, progesterone and IGF-1 concentrations

Estradiol-17β concentration in FF of cows with SCE was less than that of cows with no SCE (162.0 ± 31.5 compared with 188.9 ± 15.8 ng/ml, $P = 0.02$). There was no difference in follicular fluid progesterone concentrations and IGF-1 between SCE and nSCE groups (Table 1). There was a negative correlation between the percentages of PMNs and FF estradiol-17β concentrations ($n = 23$, $r = -0.45$, $P = 0.03$).

3.1.4. Serum and FF magnesium concentrations

Magnesium concentration was not different between SCE and nSCE groups in both FF (2.3 ± 0.4 compared with 2.2 ± 0.1 mg/dl, $P = 0.2$) and serum (2.6 ± 0.3 compared with 2.5 ± 0.1 mg/dl, $P = 0.6$). The FF magnesium concentration was positively correlated with serum magnesium ($n = 23$, r = 0.5, $P = 0.03$). Magnesium concentration was greater in serum than that of the FF in nSCE cows (2.5 ± 0.1 compared with 2.2 ± 0.1 mg/dl; $P = 0.001$), while there was no difference between serum and FF magnesium concentrations in cows with SCE (2.6 ± 0.3 compared with 2.4 ± 0.4; $P = 0.13$). The FF LPS and magnesium concentrations were positively correlated ($n = 23$, $r = 0.61$, $P = 0.004$). In addition, there was also a positive correlation between FF IGF-1 concentrations and magnesium ($n = 23$, $r = 0.50$, $P = 0.02$). There was also a positive correlation between FF LPS and IGF-1 concentrations ($n = 23$, $r = 0.50$, $P = 0.02$).

3.2. Experiment 2

3.2.1. Oocyte developmental competence

The data for results of the *in vitro* development of embryos are shown in detail in Table 2. The percentage of the cleaved zygotes differed between the control and nSCE group (76.0 ± 6.6 compared with 61.3 ± 7.6%, $P = 0.02$) as well as the control and SCE group (76.0 ± 6.6 compared with 55.5 ± 17.0%, $P = 0.001$). There was, however, no difference in the cleavage rates between the nSCE and SCE cows (61.3 ± 7.6 compared 55.5 ± 17.0%, $P = 0.4$). There was a difference in the percentage of cleaved zygotes that developed to the blastocyst stage among the control, nSCE and SCE groups (38.5 ± 6.2 compared with 27.8 ± 2.5 compared with 21.9 ± 1.8%, respectively, $P = 0.03$).
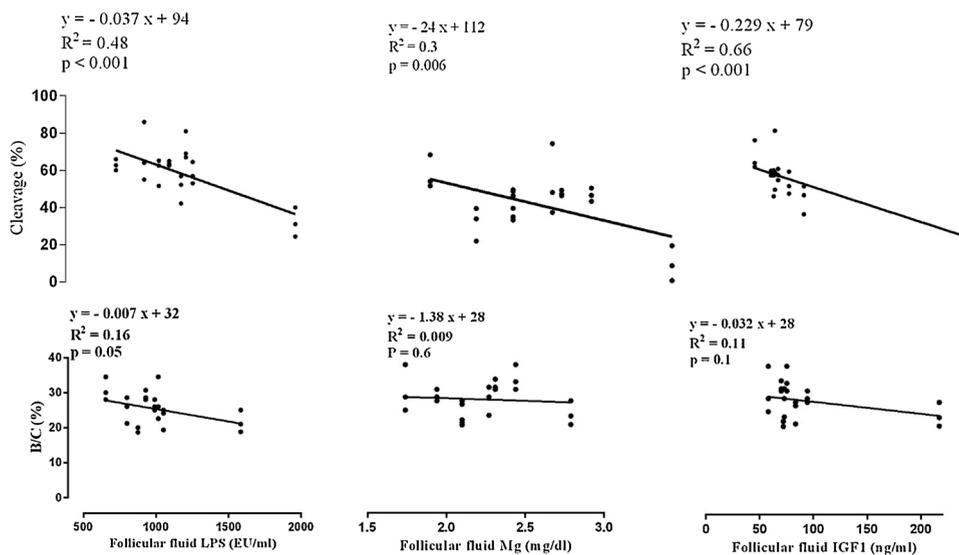

Fig. 1. Correlations between LPS, Magnesium, IGF-1 of follicular fluid and zygote cleavage rates and rate of development to the blastocyst stage of cleaved zygotes.
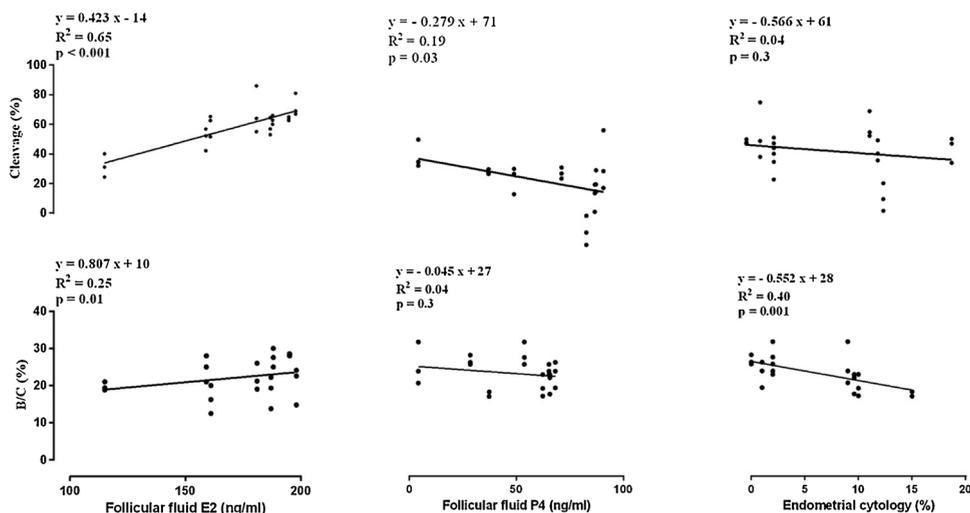
Fig. 2. Correlations between estradiol-17 β (E2) and progesterone (P4) of follicular fluid, endometrial cytology and zygote cleavage rates and rate of development to the blastocyst stage of cleaved zygotes.

3.2.2. Correlations of the composition of FF of preovulatory follicles and cleavage and blastocyst rates

Data for correlations among composition of preovulatory FF and cleavage, blastocyst rate are shown in Figs. 1 and 2. There was a negative correlation between LPS concentrations in FF and the cleavage rate ($r = -0.7$, $P < 0.001$) as well as LPS concentrations in FF and blastocyst rate ($r = -0.4$, $P = 0.05$). Further, there was a negative correlation between the percentage of uterine PMNs and the blastocyst rate ($r = -0.63$, $P = 0.001$). The FF magnesium concentration was negatively correlated with cleavage rate ($r = -0.55$, $P = 0.006$).

4. Discussion

The results of this study indicate that LPS concentration in the FF of preovulatory follicles in RB cows with SCE was greater than that of the RB cows with no SCE. Supplementation of oocyte maturation medium with the FF of preovulatory follicles of cows with SCE resulted in a lesser developmental competence of in vitro-matured cattle oocytes. Using a dose-response experiment, there was a detrimental effect of LPS on oocyte developmental competence in cattle (Magata and Shimizu, 2017). In the present study, there was an attempt to use an approach that would result in an intra-follicular milieu similar to what occurs naturally in the final stages of intra-follicle oocyte development by culturing cattle oocytes in vitro using the FF of preovulatory follicles of cows with SCE. Results in Experiment 1 further indicate that there are greater percentages of PMNs in cows with a greater mean concentration of FF LPS than that of the cows with lesser FF LPS concentration. Further, SCE cows with greater LPS concentrations in FF had less FF estradiol-17β than that of the cows with no SCE. The FF estradiol-17β concentration in the preovulatory follicle was less than that reported by Sood et al. (2017). This could be due to the earlier time of FF sampling in the present study compared with that of Sood et al. (2017). Findings in the present study provided insights as to why there were some of the results in the previous studies of Salasel et al. (2010) and Cheong et al. (2011) where there was lesser reproductive performance in multiparous dairy cows with SCE compared with those that did not have this disorder. Conversely, Sood et al. (2017) reported that RB cows had a greater FF estradiol-17β concentration in preovulatory follicles than control non-RB cows. The inconsistency in the previous findings and those in the present study could be due to the fact that in the presents study there was comparison of RB cows with or with no SCE. Results of the present study are consistent with those of Magata et al. (2014) where a greater LPS concentration in the FF affected follicle theca and granulosa cell function, resulting in less estradiol-17β production and, as a consequence, less accumulation of progesterone in FF. In addition, SCE (Green et al., 2011), and in particular LPS (Peter et al., 1990; Bromfield and Sheldon, 2011), can initiate an inflammatory response in granulosa cells of cattle, which impairs FF steroidogenesis. The inhibitory effect of LPS on follicular estradiol production occurred primarily because of an impaired luteinizing hormone secretion in less fertile dairy cows (Cheong et al., 2017). The relatively lesser luteinizing hormone secretion during the final stages of development of ovulatory follicles results in impairment of oocyte competence. The lesser fertility in repeat breeder cows with SCE, therefore, could be attributed to the relatively greater concentration of LPS in the FF of preovulatory follicles.

One important consideration is that values for blood quantitates of LPS should be interpreted with caution. Various researchers (Williams et al., 2007; Guerville and Boudry, 2016; Razavi et al., 2019) have unexpectedly observed insignificant differences in serum LPS concentration between treatment groups. In the present study, there was no difference in serum LPS concentration between the nSCE and SCE cows. Similar to the findings in the present study, Williams et al. (2007) reported that there was no difference in LPS concentrations in peripheral plasma between cows with relatively greater and lesser uterine pathogen density while the LPS concentration in the uterine content was 64 times greater than the peripheral concentration. These findings occur because with use of some LPS assay kits there is quantification of the endotoxin biological activity and not the concentration of LPS (Guerville and

Boudry, 2016). In addition, with use of most LPS assays there is not a distinguishing between lipopolysaccharide-binding protein bound to LPS or the unbound LPS (Bidne et al., 2018). The concentration of LPS in the ovarian follicles has been reported to be unrelated to the severity of the uterine inflammation (Magata et al., 2015). These findings may indicate that there is a long-term accumulation of LPS in FF following the recovery of uterine inflammation. In addition, the possibility should not be ignored that there could be selective LPS accumulation in some follicles.

Results of Experiment 2 indicate that supplementation of oocyte maturation medium with the FF from preovulatory follicles of cows with SCE resulted in a lesser development to the blastocyst developmental stage *in vitro*. The results also indicate that there is a marked inverse association between the rate of development to the blastocyst stage and FF LPS concentration. There is convincing evidence that the quality of the oocyte and its microenvironment are the principal factors determining the blastocyst yield (Lonergan and Fair, 2016). Using an experimental dose-response approach Magata and Shimizu (2017), found the addition of LPS to the oocyte maturation media reduced nuclear and cytoplasmic maturation of cattle oocytes, cleavage of zygotes and formation of blastocysts *in vitro*. More specifically, addition of LPS to oocyte maturation medium decreased rates of first polar body extrusion, increased rates of abnormal spindle and altered apoptotic indices of cultured cattle oocytes (Zhao et al., 2017). Although there should be caution when considering the importance of correlations, however, results from the present study further indicate that there is a negative correlation between FF LPS concentrations and the rate of development embryos to the blastocyst stage. In Experiment 2, there was a negative correlation between the percentage of PMNs in uterine cytology and rate of development of embryos to the blastocyst stage. Roth et al. (2013) reported that naturally occurring mastitis could lead to chronic exposure of oocytes to LPS in FF and disrupt the developmental competence of cattle oocytes. Results of the present study are consistent in that there is an important effect of the FF quality on the final stages of oocyte maturation and indicate there is a lesser quality of preovulatory follicle FF in RB dairy cows with SCE.

The results of the present study also indicate there is a positive correlation between serum and FF magnesium concentrations. The LPS and magnesium concentrations of FF were also positively correlated. Results of several studies indicate the anti-inflammatory properties of magnesium could attenuate LPS action in different organs in humans (Dowling et al., 2012; Suzuki-Kakisaka et al., 2013) and mice (Jiang et al., 2017). Dowling et al. (2012) reported that treatment with MgSO4 decreased placental inflammation when there was LPS-induced maternal infections. More specifically, Suzuki-Kakisaka et al. (2013) reported that MgSO4 administration could attenuate LPS-mediated production of pro-inflammatory agents such as IL-1β, IL-8, and TNF-α in umbilical cord blood mononuclear cells. The anti-inflammatory effects of MgSO4 were related to the magnesium rather than the sulphate moiety (Sugimoto et al., 2012). To the best of our knowledge, there is no study where there was assessment of the relationship between LPS and magnesium concentrations in FF in preovulatory follicles of repeat breeder dairy cows. The positive correlation between LPS and magnesium in FF may imply that magnesium has a function in attenuating the inflammatory actions of LPS in preovulatory follicles. Further research is needed to examine the dose-response relationship between LPS and magnesium when there is culturing in the presence of granulosa cells.

5. Conclusions

In conclusion, the greater FF LPS concentrations may lead to development of an inferior quality microenvironment for the final stages of oocyte maturation in preovulatory follicles of dairy cows with SCE. In addition, supplementation of oocyte maturation medium with FF from preovulatory follicles of cows with SCE resulted in a lesser *in vitro* developmental competence of cattle oocytes.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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