Naturally occurring mastitis disrupts developmental competence of bovine oocytes

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ABSTRACT

We examined the effects of naturally occurring mastitis on bovine oocyte developmental competence in vitro. Specifically, we investigated the effects of intramammary infection on the ovarian pool of oocytes (i.e., follicle-enclosed oocytes) and their ability to undergo in vitro maturation, fertilization, and further development to the blastocyst stage. Culled Holstein cows ($n = 50$) from 9 commercial dairy farms in Israel were allotted to 3 groups according to somatic cell count (SCC) records of the last 3 monthly milk tests as well as of quarter samples collected before slaughter: (1) low SCC ($n = 7$), (2) medium SCC ($n = 16$), or (3) high SCC ($n = 27$). Means of SCC values differed among low-, medium-, and high-SCC groups: 148,000, 311,000 and 1,813,000 cell/mL milk, respectively. Milk yield and days in milk did not differ among the 3 groups. Bacterial isolates included coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus dysgalactiae*, or no bacteria found. Ovaries were collected at the abattoir and brought to the laboratory. Cumulus oocyte complexes were recovered separately from each cow and subjected individually to in vitro maturation and fertilization, followed by 8 d in culture. The number of aspirated oocytes did not differ among groups, with a range of 17 to 21 oocytes per cow. The proportion of oocytes that cleaved into 2- to 4-cell-stage embryos ($86.1 \pm 3.4\%$) did not differ among groups. In contrast, mean percentages of embryos developed to the blastocyst stage on d 7 and 8 after fertilization were less in both medium- and high-SCC groups than in the low-SCC group (5.6 ± 2.3 and 4.1 ± 1.8 vs. $18.1 \pm 4.6\%$, respectively). Additional analysis indicated that cleavage and blastocyst-formation rates did not differ among the bacterial types in the low-, medium-, and high-SCC groups. These are the first results to demonstrate that naturally occurring mastitis disrupts the developmental competence of the ovarian

pool of oocytes, (i.e., oocytes at the germinal vesicle stage). The disruption was associated with elevation of SCC rather than bacterial type. The results may provide a partial explanation for the low fertility of cows that have contracted mastitic pathogens before insemination.

Key words: oocyte, mastitis, blastocyst, cow

INTRODUCTION

Mastitis is a major disease in dairy cows worldwide, which causes heavy economic losses because of reduced milk production, altered milk quality, and increased risk of culling (Bascom and Young, 1998). In the United States, for instance, the annual cost of mastitis has been estimated to be approximately \$2.0 billion (Wells et al., 1998). Mastitis is also detrimental to the reproductive performance of dairy cows. Epidemiological studies have shown that clinical IMI occurring either before or after AI suppresses reproductive performance (Santos et al., 2004; Hertl et al., 2010). Subclinical IMI also disrupts fertility (Schrack et al., 2001) and may reduce the probability of conception even more than clinical IMI, due to its long-term, chronic nature (Lavon et al., 2011a). Nevertheless, the effect of naturally occurring mastitis on the ovarian pool of oocytes, and the specific effect associated with bacterial type (i.e., gram negative or gram positive) have only been partially characterized.

Several in vitro and in vivo experimental models have been used to study the association between mastitis and ovarian function. These include induction of acute clinical mastitis by intramammary administration of an endotoxin of *Escherichia coli* origin (LPS), or in vitro exposure of ovarian tissues to endotoxin or cytokines (Suzuki et al., 2001; Sakumoto et al., 2003). It has been suggested that mastitis-induced elevation in cytokine deleteriously affects reproduction through activation of the hypothalamus-adrenal axis (Daniel et al., 2003). Induction of acute clinical mastitis altered blood concentrations of hormones (Hockett et al., 2000, 2005) and was associated with delayed ovulation (Suzuki et al., 2001; Lavon et al., 2008). Subclinical IMI before

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AI disrupts preovulatory follicle function: about 30% of mastitic cows expressed impaired steroidogenic function of the preovulatory follicle, resulting in low concentrations of estradiol in the follicular fluid and circulation; the latter was associated with a low and delayed LH surge, which in turn led to delayed ovulation (Lavon et al., 2010, 2011b). Other studies have shown that short-term culture of thecal or granulosa cells with LPS or tumor necrosis factor α (TNF α) reduces steroid production (Spicer and Alpizar, 1994; Herath et al., 2007).

In cows, mastitis-induced low fertility can be partially explained by early embryonic loss (Hansen et al., 2004), presumably due to increased concentrations of PGF $_{2\alpha}$ (Giri et al., 1990) or nitric oxide (Blum et al., 2000), both known to negatively affect corpus luteum function and early embryonic development. Given the potential impairments of the oocyte microenvironment, including the follicular fluid content of steroids and other substances, it is reasonable to assume that the oocyte also might be impaired upon mastitis-induced activation of the immune system. Short-term in vitro exposure of oocytes to LPS or other proinflammatory cytokines such as TNF α , mimicking the acute phase of clinical IMI, disrupted both oocyte maturation and embryonic development (Soto et al., 2003a,b). Recent studies have indicated short-term effects of mastitis on the function of the preovulatory follicle (Lavon et al., 2011b). Other studies have shown that chronic mastitis impairs ovarian follicular growth (Rahman et al., 2012) and follicular steroidogenesis (Lavon et al., 2008, 2011b). Nevertheless, to the best of our knowledge, the possible disruptive effects of naturally occurring mastitis on the developmental competence of the ovarian pool of oocytes have never been documented.

With respect to IMI-causing pathogens, epidemiological studies have indicated that reproductive performance of mastitic cows does not differ between those infected with gram-positive and gram-negative bacteria (Schrack et al., 2001). Similarly, clinical mastitis induced by both gram-negative and gram-positive bacteria reduces first-AI conception rates (Santos et al., 2004). Intramammary administration of gram-negative LPS of *E. coli* origin or mammary inoculation with gram-positive *Streptococcus uberis* altered endocrine events before ovulation (Suzuki et al., 2001; Hockett et al., 2005; Lavon et al., 2008). Intramammary administration of gram-positive *Staphylococcus aureus* extract (Younis et al., 2003) reduced steroid concentrations in the follicular fluid of the preovulatory follicles (Lavon et al., 2011c). Taken together, it seems that both gram-positive toxin and gram-negative endotoxin disrupt reproductive system function. Nevertheless, the effect of naturally occurring mastitis associated with differ-

ent bacteria on oocyte developmental competence has never been examined in cows.

The aim of the present study was to examine the effects of naturally occurring mastitis on the developmental competence of the ovarian pool of oocytes. It was hypothesized that mastitis induced by both gram-positive and gram-negative bacteria deleteriously affects the germinal vesicle (GV)-stage oocytes enclosed in small- to medium-size follicles, which is further expressed in their reduced ability to undergo maturation, fertilization, and development to blastocysts.

MATERIALS AND METHODS

SCC and Bacterial Analyses

Culled multiparous Holstein cows ($n = 50$) from 9 commercial herds in Israel were used for this experiment. Data on SCC were obtained from the last 3 routine monthly milk test samples collected before slaughtering (Israeli Herd Book). The SCC was determined using a Fossomatic 360 instrument (Foss Electric A/S, Hillerød, Denmark). In addition, aseptic milk samples were taken from each of the 4 quarters within 1 to 3 d before slaughter, and analyzed for SCC as well as for bacteriological determination. Each teat was cleaned and dried thoroughly with a towel and the teat end was sanitized with 70% ethanol swabs. Samples were collected into individual tubes and kept at 4°C. The SCC of each quarter was determined with a Coulter cell counter (Z1 Coulter Electronics, Luton, UK) as previously described (Younis et al., 2003). Bacterial culture and identification were performed as previously described (Oliver et al., 2004; Lavon et al., 2010). Briefly, tubes containing milk samples were swirled and 10 μ L from each sample was plated on one half of a plate containing bovine blood agar. Plates were then incubated at 37°C, and bacterial growth was recorded at 24-h intervals for 2 d. Bacteria isolated on culture medium were identified according to colony morphology, hemolytic characteristics, Gram stain reaction, and catalase and coagulase tests.

Experimental Protocol

The study was conducted during January through June to eliminate the additional effect of summer heat stress on the ovarian pool of oocytes. The minimum and maximum air temperatures and relative humidities were 12.4 and 23.7°C and 41 and 87%, respectively. Culled cows were assigned to 3 groups according to SCC records: (1) low SCC: monthly and quarter samples were below the cutoff of 200,000 cell/mL milk, (2) medium SSC: SCC in at least 1 quarter sample or 1

monthly sample was >200,000 and <600,000 cell/mL, or (3) high SCC: SCC in at least 1 quarter sample or 1 monthly sample was >600,000 cell/mL.

Ovaries were obtained from the local abattoir and transported to the laboratory within 60 to 90 min in physiological saline solution at 37°C with 50 µg/mL of penicillin-streptomycin. In the laboratory, ovaries were washed with fresh saline, cut through the center and placed over a transillumination stand for easy visualization of follicles (Arav, 2001). Cumulus oocyte complexes (COC) were aspirated from 3- to 8-mm follicles with an 18-gauge needle attached to a 10-mL syringe. Only COC with at least 3 layers of cumulus surrounding a homogeneous cytoplasm were selected for further processing. The COC from each cow were recovered separately and individually subjected to in vitro production of embryos.

In Vitro Production of Embryos

Unless otherwise indicated, all chemicals were obtained from Sigma-Aldrich Co. (Rehovot, Israel). Oocyte collection medium consisted of tissue culture medium 199 (TCM-199) with Hanks' salts and L-glutamine, 2% (vol/vol) fetal calf serum (Biological Industries, Israel) and 0.35 g/L of NaHCO₃. Oocyte maturation medium consisted of TCM-199 with Earle's salts supplemented with 10% fetal calf serum, 0.2 mM sodium pyruvate, and 50 µg/mL of gentamicin. HEPES-Tyrodé's lactate (TL), in vitro fertilization (IVF)-TL, and sperm-TL were used to prepare HEPES-Tyrodé's albumin lactate pyruvate (TALP), IVF-TALP, and sperm-TALP, respectively.

In vitro production of embryos was performed as previously described by Gendelman et al. (2010) with some modifications. Briefly, COC obtained from each cow were treated and incubated separately. The COC from each cow were washed 3 times in HEPES-TALP and transferred to a 50-µL droplet of oocyte maturation medium overlaid with mineral oil. The COC-containing droplets were incubated in humidified air with 5% CO₂ for 22 h at 38.5°C. Matured COC were washed 3 times in HEPES-TALP and transferred to 4-well plates containing 600 µL of IVF-TALP and 25 µL of PHE (0.5 mM penicillamine, 0.25 mM hypotaurine, and 25 µM epinephrine in 0.9% NaCl) per well. Percoll-purified spermatozoa (~1 × 10⁶) from frozen-thawed semen, from the same bull, were used for fertilization. Spermatozoa were co-incubated with COC for 18 h at 38.5°C in a humidified atmosphere with 5% CO₂. After fertilization, putative zygotes were removed from the fertilization wells, denuded of cumulus cells by gentle vortexing in HEPES-TALP containing 1,000 U/mL of hyaluronidase, and then placed in 25-µL droplets of

potassium simplex optimized medium (KSOM). All embryo droplets were overlaid with mineral oil and cultured for 8 d at 38.5°C in an atmosphere of humidified air with 5% CO₂ and 5% O₂. For each cow, the percentage of oocytes that were fertilized and cleaved to the 2- to 4-cell stage at 42 to 44 h after fertilization and the number of embryos developed to the blastocyst stage on d 7 and 8 after fertilization were recorded separately for each examined day, to determine possible delay in development. For each day, the proportions of embryos were calculated per whole cultured oocytes, and per cleaved oocytes.

Data Analysis

Milk yield, DIM, SCC, and number of oocytes per cow, as well as cleavage rates and blastocyst-formation rates for the low-, medium-, and high-SCC groups, were analyzed by one-way ANOVA (SAS Institute Inc., Cary, NC). When significant differences were found, means were separated by the Tukey-Kramer test. In addition, because not all bacterial types were present in all SCC groups, the effects of bacterial type (CNS, *E. coli*, *Streptococcus*, and no bacteria) on cleavage and blastocyst-formation rates were analyzed separately (one-way ANOVA) within each of the 3 SCC groups. Data are presented as means ± SEM.

RESULTS

Cow Features, Bacterial Characterization, and SCC

Milk yields and DIM were similar in all 3 groups (Table 1). The SCC among groups differed ($P < 0.05$). The means of the maximal SCC values recorded for the whole udder in the monthly milk tests, and the maximal values of the 4 quarters are presented in Table 1. For the low-SCC group, the average maximal value of the monthly and quarterly samples was 148,000 cell/mL. In 5 out of 7 low-SCC cows, no bacteria were detected, and in the remaining 2 cows, CNS were detected in a single quarter sample. Nevertheless, SCC in the corresponding quarters of the 2 CNS cows did not exceed the 200,000 cell/mL cutoff, and the average SCC of the monthly milk test sample in those 2 cows was as low as 116,500 cells/mL. For the medium-SCC group, the average maximal value was 311,000 cells/mL (Table 1). Seven of the cows in this group were infected by CNS, 5 cows were identified with *E. coli*, and in 4 cows, no bacteria were found (Table 2). For the high-SCC group, the average maximal value was 1,813,000 cells/mL (Table 1). In 21 out of the 27 high-SCC cows, the SCC of at least 1 monthly or quarterly

Table 1. Characteristics of the low-, medium-, and high-SCC groups¹

Item	Low SCC	Medium SCC	High SCC
Number of cows	7	16	27
Milk yield (kg/d)	24 ± 0.5	23.1 ± 0.9	26.6 ± 2.2
DIM	582 ± 54	580 ± 93	558 ± 53
Mean maximal SCC (× 10 ³ cells/mL)			
Monthly milk test ²	126 ± 29 ^a	282 ± 42 ^b	1,425 ± 268 ^c
Quarter milk samples ³	174 ± 19 ^a	340 ± 26 ^b	2,008 ± 415 ^c

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Data are presented as mean ± SEM.

²Samples were taken from the whole udder.

³Samples were collected 1 to 3 d before oocyte collection.

milk sample ranged between 10⁶ and 5 × 10⁶ cells/mL (upper counting limit); in the remaining 6 cows, the average maximal SCC value was 752,000 cells/mL. Of the high-SCC cows, 12 out of 27 were infected with CNS, 3 with *E. coli*, 6 with *Strep. dysgalactiae*, and in 6 cows, no bacteria were found.

Cleavage and Blastocyst-Formation Rates

The average number of aspirated oocytes did not differ between the low-, medium-, and high-SCC groups (Table 3). The average cleavage rate to 2- to 4-cell-stage embryos did not differ among SCC groups and was 86.1 ± 3.4% (Table 3). In contrast, the proportion of oocytes that developed to blastocysts on d 7 after fertilization was less ($P < 0.05$) in both the medium- and high-SCC groups than in the low-SCC group (Table 3). Similarly, blastocyst-formation rate on d 8 was also less ($P < 0.05$) in the medium- and high-SCC

groups than in the low-SCC group (Table 3). Calculation of blastocyst-formation rate per cleaved oocytes was similar to that determined per whole recovered oocytes. In other words, a greater ($P < 0.05$) percentage of blastocyst formation was detected in the low-SCC group than in the medium- and high-SCC groups on d 7 and 8 (Table 3).

Additional analyses were performed to determine the effect of bacterial type on cleavage and blastocyst-formation rates. Bacterial type (no bacteria: n = 15, CNS: n = 21, *E. coli*: n = 8; and streptococci: n = 6) within the low-, medium-, and high-SCC groups did not affect the proportion of cleaved oocytes or blastocyst developmental rate on d 7 and 8 (Table 2). An additional analysis across cows with no detected bacteria indicated that the proportion of blastocysts formed on d 7 and 8 was 4.2 times greater ($P < 0.04$; Table 2) in the low-SCC group than in the medium- and high-SCC groups.

Table 2. Developmental competence of oocytes in low-, medium-, and high-SCC groups analyzed by bacterial type¹

Item	No bacteria	CNS	<i>Escherichia coli</i>	Streptococci	P-value
Low SCC (n = 7)					
Number of cows	5	2	—	—	
Cleavage ² (%)	84.7 ± 5.2	90.4 ± 4.0	—	—	NS
d-7 blastocyst ³ (%)	15.4 ± 4.7	26.7 ± 17.6	—	—	NS
d-8 blastocyst ⁴ (%)	13.7 ± 4.3	27.0 ± 4.8	—	—	NS
Medium SCC (n = 16)					
Number of cows	4	7	5	—	
Cleavage ² (%)	89.9 ± 3.9	92.7 ± 2.7	76.9 ± 10.5	—	NS
d-7 blastocyst ³ (%)	4.7 ± 3.0	7.4 ± 4.0	4.4 ± 3.4	—	NS
d-8 blastocyst ⁴ (%)	7.7 ± 4.6	7.8 ± 5.5	0.0 ± 0.0	—	NS
High SCC (n = 27)					
Number of cows	6	12	3	6	
Cleavage ² (%)	91.5 ± 3.1	83.4 ± 4.7	78.3 ± 14.2	85.1 ± 11.8	NS
d-7 blastocyst ³ (%)	0.0 ± 0.0	4.7 ± 2.7	9.3 ± 4.9	0.0 ± 0.0	NS
d-8 blastocyst ⁴ (%)	4.4 ± 1.4	7.2 ± 3.5	9.3 ± 4.9	0.0 ± 0.0	NS

¹Data are presented as mean ± SEM.

²Proportion of oocytes cleaved to 2- to 4-cell-stage embryos 42 to 44 h after fertilization.

³Blastocyst-formation rate on d 7 after fertilization, relative to cultured oocytes.

⁴Blastocyst-formation rate on d 8 after fertilization, relative to cultured oocytes.

Table 3. Developmental competence of oocytes aspirated from low-, medium-, and high-SCC groups¹

Item	Low SCC	Medium SCC	High SCC
Number of cows	7	16	27
Aspirated oocytes (no.)	152	335	480
Aspirated oocytes per cow (no.)	21.7 ± 1.4	20.9 ± 1.8	17.7 ± 2.7
Cleavage ²	86.3 ± 3.9	87.1 ± 3.8	85.0 ± 3.3
Blastocysts/total oocytes ³ (%)			
d 7	18.7 ± 5.4 ^a	5.8 ± 2.1 ^b	3.4 ± 1.5 ^b
d 8	17.5 ± 4.0 ^a	5.3 ± 2.7 ^b	4.9 ± 1.9 ^b
Blastocysts/cleaved oocytes ⁴ (%)			
d 7	21.4 ± 5.7 ^a	6.4 ± 2.3 ^b	4.3 ± 1.9 ^b
d 8	20.2 ± 4.3 ^a	5.9 ± 3.0 ^b	6.0 ± 2.3 ^b

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

¹Data are presented as mean ± SEM.

²Proportion of oocytes cleaved to 2- to 4-cell-stage embryos 42 to 44 h after fertilization.

³Blastocyst-formation rate, calculated separately for d 7 or 8 after fertilization, relative to cultured oocytes.

⁴Blastocyst-formation rate, calculated separately for d 7 and 8 after fertilization, relative to cleaved oocytes.

DISCUSSION

The current study demonstrates for the first time that naturally occurring mastitis disrupts developmental competence of the ovarian pool of oocytes. The main finding of this study is that the follicle-enclosed oocyte (i.e., GV-stage oocyte) is highly sensitive to mastitis because oocytes obtained from mastitic cows manifested a reduced proportion of embryos developing to the blastocyst stage following fertilization. The reduced embryonic development on both d 7 and d 8 after fertilization, in both medium- and high-SCC groups, indicates that the impairment was mostly related to oocyte quality rather than to delayed embryonic growth.

The disruptive effect of IMI on blastocyst development was associated with elevated SCC values, which were individually determined for each cow for a period of 3 mo before oocyte collection. It should be noted that the SCC values varied markedly among cows. For instance, individual cows exhibited high SCC ($>10^6$ cell/mL), whereas others exhibited medium or low SCC throughout the period preceding the study (i.e., from 90 to 1 d before to oocyte collection). Although the exact type of mastitis (clinical or subclinical) was not documented in most of the examined cows, it can be cautiously assumed that the high-SCC group of cows experienced clinical IMI at some time point preceding oocyte collection, whereas the medium SCC cows (i.e., mean maximal SCC of around 300,000 cells/mL) were most likely subjected to subclinical IMI. Given that blastocyst formation was similarly reduced in the medium- and high-SCC groups, it seems that the deleterious effect of mastitis on oocyte developmental competence is not directly related to the type of mastitis or to the degree of SCC elevation above the 200,000 cell/mL milk cutoff (i.e., medium- and high-SCC groups). A recent large epidemiological study showed that subclinical IMI

cows that experienced mild or moderate SCC elevation around the time of AI exhibit a similar magnitude of depressed fertility (Lavon et al., 2011a).

In the current study, all bacterial types deleteriously affected blastocyst formation to a similar degree, as determined by analyzing the data by bacterial type within SCC groups. An important finding was that CNS, which in several studies has been defined as a minor pathogen (Taponen et al., 2006), disrupted oocyte developmental competence to a similar extent as major pathogens such as gram-negative *E. coli* or gram-positive streptococci in both medium- and high-SCC groups. Nevertheless, cows infected with CNS in the low-SCC group manifested a normally high proportion of developing blastocysts, reflecting the large variability among individual cows that had contracted the CNS family of bacteria. The above findings should be treated with caution due to the small number of replications per bacterial type.

Two more points regarding the effect of bacterial type on blastocyst formation are worth noting. First, cows in the low-SCC group in which no bacteria were found most likely represented uninfected individuals, whereas no detectable bacteria in the medium- and high-SCC groups most likely represented cows that had been infected with *E. coli* before oocyte collection, because this bacterium is known to be cleared from the mammary gland within days after contraction. Blastocyst-formation rate was normally high in the former and significantly low, reaching 0 on d 7 of culture in the latter. The second point, although not relevant for cows infected with *E. coli* or CNS, is that cows in the high-SCC group infected with *Strep. dysgalactiae* did not manifest any blastocyst formation at all on either d 7 or 8; this finding warrants further study. Collectively, the above findings suggest that the magnitude of the inflammatory response (i.e., amount of SCC), rather

than bacterial type, is associated with reduced oocyte developmental competence. Moreover, variable types of infections induced by either gram-positive or gram-negative bacteria have a similar deleterious effect on oocyte developmental competence. In agreement with this, Lavon et al. (2010) reported that subclinical mastitis attenuates follicular steroidogenesis irrespective of the isolated bacteria, either CNS or *Streptococcus*. Furthermore, epidemiological data indicate that reproductive performance of mastitic cows does not differ with the type of pathogen isolated from them (Schrack et al., 2001; Santos et al., 2004).

The mechanism by which mastitis affects the follicle-enclosed oocyte is unclear. Oocyte competence is acquired in a progressive manner during follicle growth and includes a variety of molecular and cellular modifications that require completing meiosis, successful fertilization, and further pre- and postimplanted embryonic development (Coticchio et al., 2004). Therefore, any disruption during follicular growth might impair the functioning of its enclosed oocyte. Mastitis has been recently associated with delayed ovulation of the preovulatory follicles in about 30% of mastitic cows (Lavon et al., 2010) in association with impaired steroidogenesis in the preovulatory follicles (Lavon et al., 2011a). Others have shown deleterious effects of cytokines, known to increase during inflammation, on steroid production by both theca and granulosa cells (Spicer, 1998; Sakumoto et al., 2003). Thus, it is reasonable to assume that attenuation of follicular steroidogenesis, in particular that in the preovulatory follicle, impairs the microenvironment of the enclosed oocyte, which in turn compromises the competence of oocytes to undergo maturation, fertilization, and further embryogenesis. Given that in the current study, the oocytes were collected from small- to medium-size follicles (i.e., low- or non-steroidogenically active follicles), it is highly likely that other factors stemming from the inflammatory response, rather than impaired steroidogenesis, are involved. In this respect, short-term exposure to inflammatory factors such as LPS or cytokines (TNF α) during the processes of in vitro maturation and zygote formation disrupts both oocyte maturation and subsequent embryonic development (Soto et al., 2003a,b; Hansen et al., 2004). Nevertheless, the interaction between cytokines and steroidogenesis cannot be ruled out because steroidogenic cells obtained from small follicles have been found to be more susceptible to TNF α than those obtained from large follicles (Spicer and Alpizar, 1994).

The current study not only supports the notion that the ovarian pool of GV-stage oocytes is highly susceptible to IMI, but also emphasizes the delayed effect of IMI on subsequent embryonic development. In

other words, mastitis-induced alterations in the follicle-enclosed oocyte are further expressed throughout embryonic development. The similarity in the number of oocytes aspirated from the different SCC groups and the lack of a group effect on cleavage rate suggest IMI-induced modification of the GV-stage oocyte associated with embryonic development rather than its ability to undergo maturation and fertilization. Such a delayed effect might explain, in part, the reduced fertility recorded in cured cows, characterized by high SCC before and low SCC after AI (Lavon et al., 2011c), suggesting that the ovarian pool of oocytes is highly sensitive to mastitis. A similar deleterious delayed effect of summer heat stress on GV-stage oocytes has been suggested to underlie the reduced fertility of lactating cows during the autumn (Roth et al., 2001) and has been recently associated with alterations in the expression of Proto-oncogene serine/threonine-protein kinase mos (*C-MOS*), growth differentiation factor 9 (*GDF-9*), and octamer-binding transcription factor 4 (*OCT-4*) genes, which are involved in oocyte maturation and early embryonic development (Gendelman and Roth, 2012). Similarly, Rahman et al. (2012) reported that oocytes collected from chronic mastitic cows are characterized by low *GDF-9* expression. Recently, we designed an experimental model to further characterize the effect of induced mastitis on oocyte maturation and embryonic development (Asaf et al., 2012). Understanding the cellular and molecular mechanisms underlying mastitis-induced disruption is highly important for developing new strategies to improve fertility in mastitic cows.

CONCLUSIONS

The current study provides first evidence for impairment of developmental competence of the ovarian pool of oocytes by naturally occurring IMI. The disruptive effect on the GV-stage oocyte was related to SCC elevation (medium or high) and not to the type of bacteria involved (gram negative or positive) or mastitis severity (clinical or subclinical). The results provide a partial explanation for the low fertility of cows that have contracted mastitic pathogens before insemination.

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