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Correlations among oocyte quality, hepatic triacylglycerols, and some blood metabolites in Carora breed cows during early postpartum

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Abstract: The aim of this study was to assess the relationships of hepatic triacylglycerol (TAG), plasma glucose, plasma cholesterol, and plasma urea nitrogen (PUN) concentrations with the morphological quality of oocytes obtained from 20 Carora breed lactating cows at days 20 and 35 postpartum in a commercial farm. Oocytes were obtained through the technique of transvaginal ultrasound-guided follicle aspiration. Change in body condition score (0.35 vs. 0.44, P = 0.02) and mean plasma cholesterol (3.59 vs. 4.35 mmol/L, P = 0.01) significantly differed between the 2 periods, whereas mean TAG tended to be higher at day 35 after calving (2.29 vs. 2.54%, fresh basis, P = 0.06) and indicated moderately fatty liver. At day 35 postpartum, both plasma cholesterol and TAG tended to have a positive correlation with oocyte quality (%), at r = 0.44 (P = 0.08) and 0.40 (P = 0.05), respectively, but no associations between glucose or PUN and oocyte recovery traits were found at any time. The percentage of oocytes with high quality was found higher (86.7 vs. 52%, P = 0.04) in those cows with more than 4 oocytes recovered. In conclusion, there was a positive relationship among hepatic TAG, plasma cholesterol, and quality of oocytes in Carora breed lactating cows.

Key words: Oocytes, triacylglycerol, postpartum, cow, cholesterol

1. Introduction

After calving, dairy cows of high genetic merit, depending upon the severity of the negative energy balance, develop a lipid mobilization syndrome characterized by a dramatic elevation in plasma nonesterified fatty acid (NEFA) concentrations. As suggested by some studies, increased levels of plasma NEFAs consequently result in higher NEFA concentrations in follicular fluid, which may have a toxic effect on oocytes that reduce their quality (1,2). In contrast, a recent in vivo study found a positive association between number of cleaved oocytes and NEFA concentrations (3). Moreover, the composition and balance of saturated and unsaturated NEFAs in follicular fluid may distinctly modify these potential toxic effects of NEFAs on oocyte developmental competence. As recently demonstrated both in vivo and in vitro, exposure of maturing oocytes to elevated NEFAs can affect either β-oxidation or lipogenesis, crucial mechanisms for postfertilization developmental competence (4,5).

Generally, high plasma NEFA concentrations trigger hepatic triacylglycerol (TAG) infiltration and the development of fatty liver. Although the relationship between severe liver damage and poor oocyte quality and development has recently been demonstrated in vitro (6–8), the association between hepatic TAG and oocyte quality, either in medium- or high-yielding dairy cows, need to be elucidated.

Fatty liver in dairy cows may be accompanied with either hypoglycemia or hyperglycemia, as well as insulin resistance or hyperinsulinemia (9,10). All of these metabolic features of fatty liver may also directly affect oocyte quality and compromise oocyte developmental capacity (11,12). Additionally, liver TAG accumulation may compromise the ability of liver to detoxify ammonia, contributing to elevated plasma urea nitrogen (PUN). PUN is highly correlated with both ammonia and urea concentrations in follicular fluid during early lactation (13,14), suggesting that the oocyte is susceptible to damage

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by high PUN concentrations. Recently, a high percentage of abnormal oocytes was associated with an elevated (5.2 mmol/L) mean PUN concentration in repeat breeder cows (15).

The Carora breed is a synthetic Venezuelan breed that has a white or light brown coat with pigmented skin and short hair. This breed originated from a region named Carora, but it is raised in different places in Venezuela and other South American countries. Although it has moderate genetic dairy merit (16), the actual milk performance of Carora cows is usually compromised because of feeding management based on poor quality roughages along with scanty nutritional monitoring during both pre- and postpartum periods, especially in herds located in the semiarid region of Carora. Metabolic unbalances in the early postpartum Carora cow may otherwise preclude the viability of the transvaginal ultrasound-guided oocyte aspiration (OPU) technique as an alternative for multiplying its small population size through the collection of immature oocytes, a hypothesis that needs to be tested.

Therefore, the objective of this research was to evaluate the relationships among liver TAG, plasma glucose, plasma cholesterol, and PUN concentrations and the morphological quality of oocytes obtained from Carora cows in the early postpartum period.

2. Materials and methods

2.1. Management of cows

The present study was conducted in a commercial dairy farm that is located at 400 m a.s.l. in Carora, Lara State, Venezuela. This is a dry area with an average temperature of 28.0 °C and an average annual rainfall of less than 700.0 mm. Relative humidity is very variable, but lower than 70%. Twenty multiparous lactating Carora cows (2 to 7 lactations) were selected on the basis of body condition score (BCS) at calving.

The pre- and early postpartum feeding management was conducted under conditions of total confinement (loosely housed) and collective grouping. A month before calving, the ration was composed (dry basis) of 75% chopped elephant grass [*Pennisetum purpureum* 'morado': 7% crude protein (CP), 1.06 Mcal of net energy for lactation (NE_L)/kg DM, 77% neutral detergent fiber (NDF), and 6.5% nonfiber carbohydrate (NFC)], 25% commercial supplement for dry cows (17% CP, 1.53 Mcal NE_L/kg DM, 35% NDF, and 30% NFC), and 80 g of prepartum mineral mix per cow per day. In the postpartum period, the ration supplied was divided into 2 portions (0800 and 1400 hours) and consisted (dry basis) of 60% elephant grass, 8% citrus pulp, 30% commercial supplement for lactating cows (19% CP, 1.62 Mcal NE_L/kg DM, 30% NDF, and 35% NFC), and 2% molasses, as well as 80 g of Optigen and 80 g of a mineral postpartum mix per cow per day.

Milk yield was recorded at 35 (33.7 ± 0.6) days after calving.

2.2. Follicular aspiration

At both 20 and 35 days after calving, oocyte recovery was performed by the technique of OPU as described by Pieterse et al. (17). Before each aspiration, epidural anesthesia was performed using 4 mL of lidocaine hydrochloride 2% (Cifarcaina, Behrens, Venezuela) between the first and second coccygeal vertebrae. After being visualized by ultrasound equipment (Aloka SSD-500, Japan), follicles with a diameter of ≥2 mm were aspirated with an 18-gauge needle to a pressure of 50 mmHg using a vacuum pump (Cook, Australia) to remove the follicular fluid, which was deposited in 50-mL conical tubes that contained Ringer lactate solution (Behrens) with 1% fetal bovine serum and 0.1% heparin (Sanderson, Venezuela).

2.3. Oocyte classification

Immediately after aspiration of each cow, the characteristics of the cumulus oocyte complexes (COCs) were evaluated using a stereomicroscope (MOTIC, China) with 7.5× objective and 10× eyepiece, recording the total number of oocytes collected. COCs were classified into 5 morphological categories according to the method of Oropeza et al. (18) as follows: grade 1 were those with at least 3 layers of compact cumulus cells (CCs); grade 2 were oocytes with homogeneous cytoplasm and with less than 3 layers of CCs; grade 3 were oocytes that had a single layer of CCs and cytoplasm of irregular appearance with dark areas; grade 4 were oocytes completely naked, and grade 5 were those oocytes with expanded cumulus. The percentage of good-quality oocytes (grades 1, 2, and 3) was expressed as the sum of oocytes of grades 1, 2, and 3 (viable oocytes) divided by total number of oocytes collected multiplied by 100. Low- high-quality oocyte resultss were those with ≤50% and >50% viable oocytes, respectively.

2.4. Sampling and chemical analyses

BCS was measured at calving and at the time of follicular aspiration using a scale of 1–5 points with a range of 0.25 units (19). Delta BCS or loss of BCS (ΔBCS) was estimated as the difference between BCS at calving and at either 20 or 35 days postpartum. The weight of each cow was determined at calving and at day 20 and day 35 from calving using a mechanical scale (Roman Fairbanks Morse, USA). At days 20 and 35 after calving, liver biopsy samples were collected through the 11th right intercostal space using a cannula and trocar. The liver samples (250–500 mg of tissue) were placed on filter paper to remove excess blood before placing them into cryogenic tubes (Corning Tubes, Fisher Scientific, UK), and were immediately frozen in liquid nitrogen. Subsequently samples were placed on dry ice for about 3 h, then stored at –20 °C until analyzed. Liver samples were hydrolyzed in a mixture of ethanol

and potassium hydroxide, and then TAG concentrations were determined in the supernatant by using an enzyme kit (Qualitest, Qualitest Industries, Venezuela). Liver TAG content was expressed as percentage of fresh liver TAG. Plasma glucose was analyzed in a drop of whole blood obtained by tail vein puncture, which was processed directly and immediately with the automated Accu-Chek method (Roche, Germany). The test was performed in duplicate for each cow. The plasma cholesterol (Bioscience) and blood urea nitrogen (Qualitest) assays were performed using enzymatic methods following the protocols of commercial kits in Venezuela. Plasma metabolite concentrations were expressed as mmol/L.

2.5. Statistical analysis

Statistical analyses were performed using SAS 8.2 (SAS Institute Inc., USA). All data were examined for normality and homogeneity of variance using the Proc UNIVARIATE procedure. Data were log-transformed when normality distribution was not detected (Anderson–Darling test, $P < 0.05$), and hypothesis tests were conducted on the transformed data. Only ΔBCS, number of oocytes, oocyte quality percentage, number of aspirated follicles, and oocyte recovery rate variables were transformed. Analysis of the nontransformed data was used to obtain parameter estimates (least-squares means).

A simple analysis of variance was conducted to determine the effect of period, (25 vs. 35 days) on body weight (BW), BCS, hepatic TAG, blood metabolites, number of oocytes, oocyte quality percentage, number of aspirated follicles, and oocyte recovery rate using the General Linear Model procedure of SAS. BW at calving was included as covariable but was removed because it was not significant. Associations among blood metabolites, hepatic TAG, and the quality of oocytes were examined by Pearson correlation using the CORR procedure of SAS. Fisher's exact chi-square test was used to assess independence between oocyte quality (low vs. high quality) and total number of oocytes (≤4 vs. >4). Statistically significant differences were declared at the 95% level, but a tendency towards significance was considered at $P = 0.05$ to $P = 0.10$.

3. Results

3.1. Metabolite distribution at 20 and 35 days postpartum

The descriptive statistics of BW, ΔBCS, hepatic TAG, and plasma metabolites for both 20 and 35 days postpartum are presented in Tables 1 and 2, respectively. A total of 6, 6, and 8 selected Carora cows had a BCS of ≥3.5, 3.25, and ≤3.0 at the day of calving, respectively. ΔBCS between calving and day 20 postpartum differed significantly from ΔBCS between calving and day 35 postpartum (Table 3). Mean

Table 1. Descriptive statistics of lactating Carora cows for liver TGA, blood metabolites, and oocyte recovery at 20 days postpartum.

Variable	Mean	Median	Minimum	Maximum	SE
Body weight, kg	523.0	524.0	374.0	651.0	14.8
ΔBCS	0.35	0.25	0	0.75	0.04
TAG, % fresh basis	2.29	2.29	1.88	2.78	0.60
Glucose, mmol/L	3.60	3.71	2.61	4.33	0.10
Cholesterol, mmol/L	3.59	3.58	2.49	4.80	0.15
PUN, mmol/L	3.32	3.54	0.96	7.54	0.37
Total number of oocytes/cow	4.0	2.5	0	18.0	0.9
Number of oocytes grade 1/cow	0.3	0	0	3.0	0.2
Number of oocytes grade 2/cow	1.3	1.0	0	5.0	0.3
Number of oocytes grade 3/cow	1.2	0	0	9.0	0.5
Number of oocytes grade 4/cow	1.0	0	0	6.0	0.3
Number of oocytes grade 5/cow	0.3	0	0	1.0	0.1
Good-quality oocytes, %[1]	57.5	64.6	0	100.0	8.6
Number of aspirated follicles/cow	4.8	3.5	1.0	22.0	1.0
Oocyte recovery rate, %	85.2	69.0	0	333.3	17.7

[1]Calculated as the sum of oocytes of grades 1, 2, and 3, divided by total number of oocytes × 100.

Table 2. Descriptive statistics of lactating Carora cows for liver TGA, blood metabolites, and oocyte recovery at 35 days postpartum.

Variable	Mean	Median	Minimum	Maximum	SE
Body weight, kg	512.0	503.5	374.0	651.0	14.5
Milk yield, kg/day	17.0	17.4	3.5	24.4	1.2
ΔBCS	0.44	0.50	0	1.00	0.07
TAG, % fresh basis	2.54	2.43	1.70	3.71	0.60
Glucose, mmol/L	3.74	3.79	3.08	4.33	0.08
Cholesterol, mmol/L	4.35	4.31	2.54	6.35	0.21
PUN, mmol/L	3.75	3.89	1.0	6.82	0.43
Total number of oocytes/cow	3.2	2.0	0	14.0	0.8
Number of oocytes grade 1/cow	0.5	0	0	4.0	0.2
Number of oocytes grade 2/cow	0.5	0	0	2.0	0.2
Number of oocytes grade 3/cow	1.3	1.0	0	5.0	0.3
Number of oocytes grade 4/cow	0.7	0	0	5.0	0.3
Number of oocytes grade 5/cow	0.2	0	0	3.0	0.2
Good-quality oocytes, %[1]	64.9	70.8	0	100.0	8.6
Number of aspired follicles/cow	5.2	3.5	1.0	23.0	1.2
Oocyte recovery rate, %	60.6	60.0	0	166.7	8.9

[1]Calculated as the sum of oocytes of grades 1, 2, and 3, divided by total number of oocytes collected × 100.

Table 3. Effect of postpartum period on body weight, BCS, liver TAG, and blood metabolites of Carora cows.

Variable	Period		SEM	P-value
	20 days	35 days		
BW, kg	522.5	511.6	14.7	0.60
ΔBCS	0.35	0.44	0.06	0.02
TAG, % fresh basis	2.29	2.54	0.87	0.06
Glucose, mmol/L	3.60	3.74	0.09	0.32
Cholesterol, mmol/L	3.59	4.35	0.18	0.01
PUN, mmol/L	3.32	3.75	0.40	0.45

plasma concentration of cholesterol differed significantly between the 2 periods, whereas mean liver TAG tended to be higher at day 35 after calving. BW, plasma glucose, and PUN concentrations did not differ between periods (Table 3).

3.2. Oocyte recovery and quality at 20 and 35 days postpartum

The means, medians, minimums, maximums, and standard errors of number of aspirated follicles (>2 mm), number of oocytes recovered per cow, oocyte quality, and recovery rate is provided in Tables 1 and 2 for days 20 and 35 postpartum, respectively. Those variables had high coefficients of variation because some cows were found with just a few or no oocytes, while others had up to 18 oocytes. Therefore, in some cases oocyte quality percentage was 0%, but it was 100% in others. Oocyte recovery and quality traits were not significantly different between periods (Table 4).

3.3. Association among milk yield, BW, ΔBCS, hepatic TAG, blood metabolites, and oocyte quality

Pearson correlation coefficients among milk yield, BW, ΔBCS, hepatic TAG, plasma metabolites, oocyte recovery, and oocyte quality percentage at 20 and 35 days postpartum are shown in Tables 5 and 6, respectively. At day 20 after calving, there was a trend for a positive correlation between plasma cholesterol and oocyte recovery rate ($r = 0.41$, $P = 0.07$), but not at day 35 after calving. Plasma cholesterol and hepatic TAG had trends for positive correlations with

Table 4. Effect of postpartum period on number of follicles aspirated, recovered oocyte rates, and good-quality oocyte percentages of Carora cows.

Variable	Period		SEM	P-value
	20 days	35 days		
Total number of oocytes/cow	4.0	3.2	0.87	0.31
Good-quality oocytes, %[1]	57.5	64.9	8.6	0.66
Number of aspirated follicles/cow	4.8	5.2	1.1	0.85
Oocyte recovery rate, %	85.2	60.6	14.0	0.68

[1]Calculated as the sum of oocytes of grades 1, 2 and 3, divided by total number of oocytes collected × 100.

oocyte quality percentage, at r = 0.44 and 0.40, respectively, at day 35 postpartum. No associations between glucose or PUN and oocyte recovery-quality traits were found at any time (Tables 5 and 6).

Although some cows evaluated in the present study reached high levels of PUN of 7.5 and 6.8 mmol/L on days 20 and 35 postpartum, respectively, significant correlations among PUN and oocyte recovery variables were not observed.

Results of the Fisher exact test revealed a statistically significant (P = 0.04) positive association between number of oocytes per cow and oocyte quality percentage (Figure), showing that the group of cows with a low number of oocytes (<4.0) had a similar proportion of cows with low and high oocyte quality, at 48% and 52%, respectively, while cows with a high number of oocytes had up to 86.7% high-quality oocytes. Therefore, there was a higher proportion of viable oocytes as the number of oocytes recovered per cow increased.

4. Discussion

The present research aimed to evaluate the relationships among liver TAG, some plasma metabolites, and the morphological quality of oocytes obtained from Carora cows in the early postpartum period. In the present study, 85% of the cows had moderate (2% to 5% hepatic TAG, fresh basis) fatty liver according to the criterion of Kalaitzakis et al. (20), reaching mean liver TAG concentrations of 2.78%

Table 5. Pearson correlation coefficients among body weight, BCS, and blood metabolites and oocyte recovery traits in lactating Carora cows at 20 days postpartum. Statistical significance for values in bold: $^{t}P < 0.10 > 0.05$.

Variable	MY	BW	**ΔBCS**	TAG	GLU	COL	PUN
Number of follicles aspirated	–0.13	–0.06	–0.14	0.01	0.26	0.23	0.21
Number of oocytes	–0.19	–0.01	–0.12	–0.07	0.15	0.21	0.12
Good quality oocyte percentage	–0.02	–0.02	–0.12	**0.40**^{t}	–0.14	**0.44**^{t}	0.04
Oocyte recovery rate	–0.06	–0.02	–0.17	0.32	–0.34	0.31	–0.18

Table 6. Pearson correlation coefficients among body weight, BCS, and blood metabolites and oocyte recovery traits in lactating Carora cows at 35 days postpartum. Statistical significance for values in bold: $^{t}P < 0.10 > 0.05$.

Variable	BW	ΔBCS	TAG	GLU	COL	PUN
Number of follicles aspirated	0.37	0.08	0.08	0.07	–0.06	0.10
Number of oocytes	0.09	0.08	0.19	–0.14	–0.17	–0.01
Oocyte quality	0.01	0.10	0.19	0.10	–0.01	0.06
Oocyte recovery rate	–0.01	–0.02	**0.41**^{t}	–0.10	–0.06	–0.16

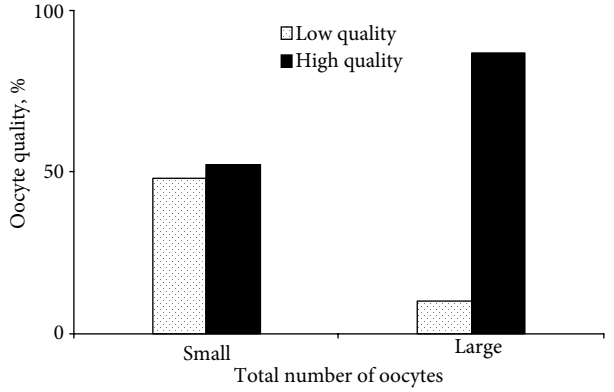

Figure. Association between oocyte quality and total number of oocytes in early postpartum Carora breed cows. Small (≤4) and large (>4) numbers of oocytes were recovered. Low (≤50% viability) and high (>50% viability) qualities of oocytes were recovered. Fisher's P = 0.040.

and 3.71% (fresh basis) at days 20 and 35 postpartum, respectively. These means concentrations of hepatic TAG were greater than those reported for higher milk-yielding Holstein cows at similar sampling times (21), most likely because of greater postpartum loss of BCS. As reported by other researchers (22,23), a linear increase in plasma cholesterol concentration occurred after calving and up to 60 days postpartum, at which time these values (4.3–5.6 mmol/L) were stabilized. The observed changes in the concentrations of cholesterol and TAG between days 20 and 35 postpartum may be explained as a result of the growing energy demand that usually occurs towards the peak of lactation, triggering a light mobilization of adipose tissue.

However, the absence of severe (>5% hepatic TAG, fresh basis) fatty liver in this study is concomitant with the relatively low milk output (Table 2), and it is likely due to low energy and protein intakes, during both pre- and postpartum periods. Based on typical chemical composition of ingredients and calculated postpartum dry matter intake (12 kg DM cow^{-1} day^{-1}), the levels of energy and metabolizable protein in the postpartum simulated diet were enough to support a milk yield equivalent to only 10 kg cow^{-1} day^{-1}. Notably, such unbalanced diets are the result of using poor-quality forages.

Mean plasma glucose levels in the present study (Table 3) are considered normal (>3.0 mmol/L) for similar times after calving (24). Mean PUN concentrations (Table 3) were lower than in other studies (21), more likely due to decreased ruminal protein degradation.

To the knowledge of the authors, this is the first study where ultrasound-guided oocyte aspiration has been used in the early postpartum period in the Carora breed cow. At similar days in milk, only 1.5 oocytes were retrieved from nontreated lactating Holstein cows in the study of Perez (25). The present results from postpartum Carora cows are similar to the results obtained by Caamaño (26) in dairy cows at 30 days postpartum (4 oocytes/cow and 54% recovery rate). More recently, other researchers reported an average of 7 oocytes recovered per cow in nontreated dairy cows with twice-weekly aspirations from 30 to 100 days postpartum (3).

A trend towards a positive association between hepatic TAG and quality oocytes was very interesting because the current literature highlights an opposite association. Thus, severe liver damage in cows has been linked to both a reduced number of secondary follicles and a lower ability to develop preantral follicles. However, comparative studies between healthy and damaged livers were conducted at a slaughterhouse and signs of damage included severe bleeding, telangiectasia, cholangitis, and abscess, but no fatty accumulation (6). Moreover, abnormal liver, but mainly hepatitis along with elevated levels of beta-hydroxybutyric acid (BHBA) in follicular fluid, has been negatively correlated with the proportion of good-quality oocytes and their developmental potential to the blastocyst stage following in vitro fertilization (7). Although there is in vivo evidence for changes in TAG accumulation in CCs due to elevated NEFA in follicular fluid of fasted Holstein heifers (5), the present study is the first to quantify the relationship between hepatic TAG content and quality of bovine oocytes.

In the present study, mean concentrations of TAG at days 20 and 35 showed that there were moderate rather than severe cases of fatty liver, which limited the maximum TAG values observed to less than 4%. However, the quantitative correlation between either TAG or cholesterol and oocyte quality showed a trend (Table 4) that supports the hypothesis of Mutoba et al. (3) that NEFAs may have a favorable effect on oocyte viability. Thus, it is likely that a low lipomobilization (low influx of NEFAs to the liver) led to a slight increase in the concentration of liver TAG not exceeding 4% (fresh liver), and, at the same time, improved the development of CCs and the morphological qualities of COCs.

As suggested by in vivo and in vitro experiments, CCs may have a high capacity for storing fatty acids as neutral lipids (both TAG and diacylglycerols) and protecting the surrounded oocyte against elevated levels of NEFA in follicular fluid (5,27) in early postcalving, during the period of negative energy balance. Furthermore, higher total lipid levels, and in particular TAG presenting acylation with oleic and palmitic acids, are observed in cumulus-enclosed than in denuded oocytes (5). Stored neutral lipids may be very important energy sources to be used through β-oxidation as well as precursors for de novo membrane synthesis during embryo development (28).

Recent evidence suggests that lipogenic activities are higher in CCs in order to provide needed lipids to the oocyte for use as energy sources by lipolysis (29). As was recently demonstrated, a higher expression of the fatty acid synthase gene is observed in CCs, whereas hormone-sensitive phospholipase protein is less abundant in CCs than in oocytes (29). Furthermore, the expression of genes related to fatty acid β-oxidation was downregulated in NEFA-exposed oocytes and CCs (4).

Extremely low plasma glucose levels (<2.5 mmol/L) compatible with subclinical ketosis were not found in the present study. Subclinical ketosis may impair the development of oocytes following in vitro maturation (30). Additionally, elevated levels of BHBA in follicular fluid have been negatively correlated with the proportion of good-quality oocytes and their developmental potential to the blastocyst stage following in vitro fertilization (7). Interestingly, those negative effects of high BHBA concentrations combined with low glucose levels may appear throughout the development of fatty liver syndrome at levels of liver TAG of greater than 4%, because blood BHBA usually increases after a moderate to severe fatty infiltration of the liver has developed.

In conclusion, there was a positive relationship among hepatic TAG, plasma cholesterol, and quality of oocytes in Carora breed cows in the early postpartum period. Results also suggest that the development of a moderate fatty liver may favor oocyte viability through a concomitant increased accumulation of neutral lipids in the CCs. Hepatic TAG and plasma cholesterol concentrations have the potential to be used as an index to predict oocyte competence. Nutritional management pre- and postpartum must be improved to ensure the viability of the OPU program undertaken in herds from Carora, Venezuela.

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