β-carotene supplementation to non-lactating dairy cows can restore β-carotene availability in the follicular environment under negative energy balance conditions

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Implications Daily β-carotene supplementation in dairy cows in negative energy balance can lower oxidative stress to levels similar to not supplemented healthy cows and can significantly increase the availability of β-carotene in plasma and follicular fluid, the micro-environment of the growing and maturing oocyte.

Introduction High energy demands during lactation result in a negative energy balance (NEB), which is typically associated with elevated serum non-esterified fatty acid (NEFA) concentrations. These elevated NEFA concentrations are reflected in the micro-environment of the maturing oocyte, the follicular fluid, and have been associated with increased oxidative stress (OS). We previously showed that bovine oocytes, exposed to elevated NEFA concentrations, have a reduced developmental competence leading to a deviating embryo physiology. Gene expression and functional assays pointed to pathways related to oxidative metabolism, REDOX status and OS in NEFA exposed oocytes, cumulus cells and subsequent blastocysts (Van Hoeck *et al.*, 2013). Therefore, strategic antioxidant supplementation such as β-carotene (bC) can be a promising solution. However, very little is known about the availability of bC in the follicular fluid (FF) and how oral supplementation may affect this. Furthermore, it can be hypothesized that a NEB status may negatively affect this availability in the follicular fluid due to a higher systemic use. To investigate this we aimed to 1) determine the effect of the NEB on bC concentrations in serum and follicular fluid, and 2) how this effect could be altered by dietary bC supplementation. In this first study, a NEB was induced in non-lactating dairy cows by a reduction in dry matter intake.

Material and methods After 6 weeks of acclimatisation, all 6 non-lactating Holstein Friesian cows were subjected to the same order of 4 consecutive dietary treatments, 28 days each: 1) 1,2x maintenance (M) (= positive EB, PEB-bC), 2) 1,2xM with daily 2000mg bC similar to the level of bC intake at grazing (Rovimix 10% bC, DSM) (=PEB+bC), 3) 0,6xM with 2000mg bC (=NEB+bC) followed by a 6 week acclimatisation period and 4) 0,6xM (=NEB-bC). Rations consisted of hay, straw and concentrates. Weight was monitored weekly. In the second half of each dietary period, cows were synchronised by means of a progesterone releasing intravaginal device (PRID, 1,55g P_4, CEVA) for 7 days and a PG F2α injection on day 6 after PRID insertion. Together with blood sampling, FF of the dominant follicle was collected by transvaginal follicle aspiration 2 days after PRID removal. Blood and FF samples were analysed for bC, NEFAs, estradiol (E_2) and progesterone (P_4). Additionally, serum total antioxidant status (TAS) was determined as well as intra-erythrocyte glutathione (GSH) concentrations. Data were statistically analysed using a paired samples T-test, analysing the effect of NEB or bC supplementation (IBM SPSS Statistics 20). Data are presented as means ± standard deviation.

Results All cows on average lost 11.44 ± 1.80% of their body weight during both energy restriction (0,6xM) periods. Fasting resulted in a significant increase in serum NEFA concentrations (0,21 ± 0.11mM vs. 0.36 ± 0.18mM). All follicles punctured displayed a E_2/P_4 ratio > 1 (18,20 ± 10,95 on average). Overall, bC concentrations in FF correlated well with serum concentrations (R=0.645; P=0.001). NEB significantly reduced bC in serum (1.02 ± 0.91µg/ml vs. 0.44 ± 0.18µg/ml; P=0.046) and FF (0.21 ± 0.12µg/ml vs. 0.05 ± 0.02µg/ml; P=0.02). However, bC supplementation drastically increased bC availability in serum and in FF in NEB (X8 in serum P<0.001 and X10 in FF P=0.001) as well as in PEB (X3 in serum P<0.001 and X2 in FF P=0.034). Remarkably, in bC supplemented animals (PEB+bC vs. NEB+bC), no negative effect of fasting (NEB) in bC levels in serum and FF could be detected (P>0.05). Fasting significantly reduced GSH content (657.21 ± 121.11µg/ml vs. 466.64 ± 122.26µg/ml; P=0.003) as well as TAS (1.21 ± 0.09mM vs. 1.14 ± 0.07mM; P=0.027), but bC supplementation to cows in NEB could restore these GSH concentrations in red blood cells (591.47 ± 104.36µg/ml vs. PEB-bC conc; P>0.05). Only in PEB the diameter of the dominant follicle was significantly larger when supplemented with bC (20.40 ± 5.12mm vs. 13.60 ± 2.47mm in PEB-bC; 13.15 ± 1.80mm in NEB+bC and 14.08 ± 1.07mm in NEB-bC; P<0.05), but bC supplementation did not affect serum and FF E_2 concentrations.

Conclusion Fasting associated NEB has a negative effect on bC concentrations in serum and in the oocyte's micro-environment, leading to higher OS levels. bC supplementation was able to significantly increase the bC availability in the FF irrespective of the energy status and could restore the oxidative status as in PEB conditions. Further research will investigate the validity of these intriguing findings in lactating dairy cows early *post partum*.

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Live cell imaging in the female genital tract – new aspects for improving cow fertility

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Implications Live Cell Imaging for the first time allows to visualize physiology and pathology of the oviduct under near in vivo conditions and on a microscopic level thus enabling to create new therapeutic concepts for improving fertility.

Introduction Integrity of the oviduct is essential for oocyte transport, fertilization and early embryonic development. According to own observations up to 60 % of idiopathic fertility problems might be caused by alterations in the oviduct. To date, however, no diagnostic tool is available for examining the uterine tubes under in vivo conditions. Therefore we set out to establish new techniques in bio-imaging of the oviduct and to analyse the effects of morphological changes on functional integrity and on fertility.

Material and methods 45 cows aged 3 to 8 years (breeds: Holstein- Friesian, and Deutsches Fleckvieh) were included in the study. 5 cows were investigated 6-7 hours after ovulation and insemination. In all cows, the oviduct was collected immediately after slaughter. In 17 cows (cycle stage: estrus), the oviducts were co-incubated with spermatozoa (10 µl, 45 Mio/ml).) and/or oocytes gained by follicular aspiration of the ovaries for 15 min at 37 ^{o}C . Pieces of the ampulla and the isthmus with and without co-incubation were transferred to Delta T culture dishes (Bioptechs, Butler, PA), the bottom of which was covered with a thin layer of Sylgard polymer (Dow corning, Wiesbaden, Germany). The dishes filled with warm Hepes buffer submerging the oviduct were transferred to a Delta T stage holder with a constant temperature of 37 ^{o}C. Live cell imaging was done using the TillVision imaging system (Till Photonics, Graefelfing, Germany) based on a BX50 WI fixed-stage upright microscope (Olympus) equipped with an Imago CCD camera with a 1280 x960 pixel CCD chip (Till Photonics). In these experiments numerous movies were acquired elucidating the interaction between cumulus-oocyte complex and oviduct after ovulation under near in vivo conditions. Additionally the sperm-oviduct interaction and the mechanisms of the formation of the sperm reservoir were characterized. These results were correlated to the images and movies obtained by probe–based confocal laser endomicroscopy (pCLE) (Mauna Kea Technologies, France). pCLE has been clinically approved in human medicine as a diagnostic tool in the respiratory tract (Yserbit et al, 2013) and in the gastrointestinal tract (Pittayanon et al., 2012), but has not been used in reproductive medicine yet. The oviducts were investigated using the Cellvizio® confocal microprobe ProFlex™ S1500 (diameter 1.5 mm, resolution 3.3 µm) after incubation in a) 0.01% fluorescein isothiocynate (FITC), or 0.01 % Fluorescein Alcon 10 % (FA). Images and movies were analysed using the IC viewer software (Mauna Kea Technologies, France).

Results After ovulation the cumulus-oocyte complex firmly attaches to the ampullar epithelium initiating a signal cascade leading the sperm to the site of fertilization. Spermatozoa form a sperm reservoir in which they stay motile for 3-4 days. Spermatozoa which lack fertilizing capability are a) either not able to bind to the oviductal epithelium or b) are not able to detach as soon as the oocyte has reached the site of fertilization or c) are not able to find their way to the oocyte. Sperm reservoir formation is inhibited by pathological alterations (i.e. inflammation) of the oviduct. pCLE allows to in vivo examine oviductal cells on a microscopic level and enables to identify pathological alterations in the oviduct which are not recognized macroscopically.

Conclusion The live cell imaging techniques established in the oviduct for the first time allow to identify alterations in the oviduct in vivo and to determine changes in gameto-maternal interaction and fertilization - the integrity of which is crucial for successful pregnancy.

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Correlation study between reproduction and milk production traits in Holstein bulls with genetic evaluation available in Brazil

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Implications In modern dairy farms, in which high milk production is frequently associated with fertility problems, the correlations knowledge between reproductive and productive traits enables us to develop appropriate breeding plans.

Introduction In Brazil, as well as in other countries, milk production per cow has increased frequently because of a combination of factors such as the improved management, better nutrition, and genetic selection. The selection evidence is milk production because the majority of Brazilian dairy industries pay by milk volume, and fewer industries pay by milk quality. Analysing several datasets in previous years, a large number of researchers around the world found great challenge to related the antagonistic relationship between milk production and fertility in dairy cows, and also one of the most important reasons quoted is genetic selection (Hansen, 2000; Lucy, 2001). Great focus on milk production traits is given for selection of dairy cattle. This focus tends to decline fertility and reproductive efficiency in cows. With this information in mind, the objective of this study was to verify the correlations between productive and fertility traits from Holstein bulls with genetic evaluation available for commercialization by semen suppliers companies in Brazil.

Material and methods Records of 385 Holstein bulls in Brazilian companies supplying semen in 2008 were located on the company's websites. Currently, the most of productive dairy cows in Brazil are female calves of the bulls in this study. The genetic evaluation of these bulls to the traits described in the study were located and tabulated from the Dairy Bulls website (http://www.dairybulls.com). Statistical analysis of the correlation between Predicted Transmitting Ability (PTAs) of production traits (milk, fat and protein yield and fat and protein percentage) and reproduction traits (calving ease, productive life, pregnancy rate and stillbirths) were performed by correlation analysis using the software Minitab® (Minitab version 14, 2004, State College, PA) using the tukey test, considering 5% of significance level.

Results In table 01 are shown the correlation coefficients (r) between PTAs for milk production and reproduction traits. It was observed that the milk production has low negative correlation with reproductive traits like calving ease (r = -0.11), productive life (r = -0.05), pregnancy rate (r = -0.37) and stillbirths (r = -0.14). These negative values show that animals with high potential to milk production can have lower efficiency in their reproductive performance, mainly in pregnancy rate.
The same occurs with the association between protein and fat yield with the pregnancy rate. Thus, the selection for yield (milk, protein or fat) can lead to a decrease in herd reproductive performance. Among other reasons, due to the inadequate body condition score, high producing cows may be more affected by metabolic disorders and infectious diseases and these can lead to defective fertility (Weigel, 2006).

Table 1 Correlation coefficients (r) between PTAs of Holstein bulls with genetic evaluations available in Brazil for milk production traits (milk yield, protein and fat, kg and %) and reproduction traits (calving ease, productive life, pregnancy rate and stillbirths).

Traits	Milk yield		Protein (kg)		Fat (kg)		Protein (%)		Fat (%)	
	r	P-Value	r	P-Value	r	P-Value	r	P-Value	r	P-Value
Calving ease	-0.11	0.028	-0.15	0.003	-0.07	0.166	-0.04	0.446	0.04	0.426
Pregnancy rate	-0.37	0.001	-0.36	0.001	-0.27	0.001	0.05	0.372	0.08	0.132
Stillbirths	-0.14	0.005	-0.23	0.001	-0.12	0.024	-0.11	0.027	0.02	0.694
Productive life	-0.05	0.316	-0.08	0.126	0.08	0.131	-0.04	0.447	0.13	0.011

Conclusion Higher milk production, protein and milk fat (kg) are correlated with low pregnancy rates. On the other hand, the selection for milk fat and protein percentage possibly not affect negatively the cow fertility.

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Differential Immunoglobulin G glycosylation in *postpartum* dairy cows with uterine disease: potential for a predictive test

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Implications This study identifies differences in the glycosylation of Immunoglobulin G in cattle who go on to suffer from uterine disease as compared with their healthy herd mates. Differences are significant during the period before calving and within the first week after calving. Therefore, measurement of IgG glycosylation may offer the potential to predict 'at risk' cows, allowing early therapeutic intervention in order to reduce the severity of *postpartum* uterine disease in dairy cattle.

Introduction Uterine disease in dairy cattle is a significant inflammatory condition of the uterus, resulting in reduced fertility. It has been suggested that the immune alterations during pregnancy and the reversion to normality across the peri-partum period are important for post partum uterine health. Immunoglobulin G (IgG) is the predominant Ig in the bovine uterus and is an important immune mediator of pathogen defence. During pregnancy, the immune actions of IgG are modulated by glycosylation and this is reversed after birth and differences in IgG glycosylation are associated with chronic inflammatory disease. Therefore, the aim of this study was to identify whether there are differences in the glycan structure of IgG between healthy cows and cows with uterine disease.

Material and methods Blood samples were collected from 96 dairy cattle approximately 10 days before calving and on days 7, 14 and 21 *postpartum*, and serum was obtained by centrifugation. Uterine health was monitored by vaginal mucus assessment and animals were retrospectively diagnosed based on the definition of Sheldon *et al* (2006). From each serum sample, IgG was purified and the glycan fraction of the immunoglobulin was released and purified. Glycans were then quantified by ultra performance liquid chromatography.

Results Thirty-one glycan peaks, each representing a different glycan structure were identified in bovine IgG. In cows with uterine disease, there was an increase in the percentage of IgG fucosylation as identified by higher quantity of fucosylated glycans. The level of IgG fucosylation in diseased cows was significantly increased on day 10 pre-calving and on days 7, 14 and 21 post-calving ($P<0.001$). Using a data classification and regression training model we were able to correctly predict the disease phenotype based on the IgG fucosylation ratio with an accuracy of 71% on day 10 pre-partum, 82% on day 7 *postpartum* and 90% on day 14 *postpartum* ($P<0.001$).

Conclusion In conclusion, fucosylation of IgG is increased in cows that suffer from *postpartum* uterine disease possibly indicating reduced immune function in these animals. Furthermore, these differences are detectable before calving and in the first week *postpartum*, offering the potential of using IgG fucosylation analysis as a predictive biomarker of disease.

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Optimal insemination time, oestrous characteristics and factors influencing conception rate on Dutch dairy farms equipped with pedometers

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Implications Time of insemination relative to the onset of the increase of activity, type of sperm, milking system, duration of oestrus, uterus tone and dirtiness of the insemination pipette after insemination affected conception rate. Optimal time for insemination was 12 ho
urs after the onset of the increase in activity.

Introduction Pedometers are a reliable tool to detect oestrus in dairy cattle. Based on the onset of the increase in activity advice is given on the optimal time of insemination (Roelofs et al., 2005). The optimal time of insemination based on the study of Roelofs et al. (2005) was 5 to 17 hours after the onset of increase in activity. This research was conducted on one experimental farm. It is known that many factors influence reproductive results and it is possible that optimal insemination time is different for different farms, management strategies, production levels, etc. The aim of this research was to study oestrous characteristics based on pedometers measurements and to validate the advice on time of insemination in the field.

Material and methods Data were collected from 168 dairy farms in the Netherlands which used pedometers (Ovalert system, CRV; pedometers, Nedap Livestock Management) for at least one year. The following data were collected per insemination: cow , parity, body condition score (BCS), insemination date and time, conventional or sexed semen, uterine tone, dirty or clean insemination pipette, onset and end of oestrus based on pedometer measurements and conventional or automatic milking system (AMS). Conception rate (CR) was defined as the number of inseminations that had a positive pregnancy diagnosis divided by the number of inseminations that had a positive pregnancy diagnosis plus the number of inseminations with a new insemination within 25 days. When pregnancy diagnosis was negative, but the interval between inseminations was more than 25 days, the data of the inseminations were not taken into account. The reason for this was that it is impossible to know whether embryonic death had occurred or oestrous detection failed. In total 6,665 inseminations were used in this study. Data were analysed using univariate analysis of variance and Pearson's Chi-Square tests (SPSS Statistics 21). Optimal insemination time was analysed using regression analysis, an individual interval between onset of increase in activity and insemination was taken into account when at least 10 inseminations were performed in that interval.

Results Average duration of oestrus based on pedometer measurements was 10.9±4.22h. Older cows (parity 3 or more), low body condition score (2.5 or less on a scale of 5) and conventional milking (compared to automatic milking systems) resulted in significant shorter duration of oestrus.
Farms with an AMS had better CR than without AMS (59 versus 55%). CR was better with conventional compared to sexed semen (56 versus 47%). Duration of oestrus of less than 10h had worse CR than oestrus periods longer than 16h (52 versus 61%). CR was better when uterine tone was present and the pipette was clean compared with absence of uterine tone and a dirty pipette (57 versus 44%).
Overall CR was 56%, best CR were found in the interval from 6 to 9h after onset of increase in activity (65%). The best predicted CR was 61%. This CR was achieved when insemination was performed 11.9h after onset of increase in activity based on a quadratic regression (R^2=0,70). 63% of the inseminations were performed more than 18h after the onset of the increase in activity.

Conclusion The advice of inseminating 5 to 17h after the onset of increase in activity is sound according to the field data, where best CR are achieved when insemination is performed about 12h after the start. Earlier inseminations resulted in better CR than late inseminations. On many farms (63%), insemination is performed later than the advised interval.

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Phenotypic and genotypic associations between multiple ovulations and milk production, longevity, fertility, and somatic cell count in Irish dairy cows

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Implications This study provides information on phenotypic risk factors for multiple ovulations in Irish dairy cows as well as the implications of current breeding programs on the incidence of multiple ovulations.

Introduction There is limited international evidence to suggest that greater milk production is associated with a greater incidence of multiple ovulations, but, as yet, whether this association is genetic or management driven has not been elucidated. The objective was to quantify the associations between phenotypic milk production as well as genetic merit for milk production, longevity, fertility and somatic cell count with multiple ovulations in a population of early lactation Irish dairy cows.

Material and methods Ultrasound scans of the reproductive tract and individual cow milk test day records (March 2008 to October 2012) were available from the Irish Cattle Breeding Federation database. Multiple ovulations were described by the presence of 2 or more corpora lutea on the ovary. Smoothing splines were fitted separately to individual cow test-day milk, fat and protein yield. Predicted milk, fat and protein yield for the day of the scan were extracted from the fitted splines. Milk yield and composition were standardised within each of the parity groups (i.e. 1, 2, 3, 4 and 5+) and categorised as > 0.5 standard deviation (SD) below the mean (low), ± 0.5 SD from the mean (medium) and >0.5 SD above the mean (high). Predicted transmitting ability (PTA) for milk yield, fat, protein, longevity, fertility and somatic cell count (SCC) for all cows were available and were categorised into 3 groups of equal size (i.e., low, medium, and high). Contemporary group was defined as the herd-year-season of calving and contemporary groups with < 5 animals were removed. Only cows calved between 10 and 70 days when scanned were included for analysis. Following edits, 9,703 records from 8,201 cows in 307 herds remained. Factors associated with the logit of the probability of multiple ovulations were quantified using generalised estimating equations assuming a binomial error distribution. Fixed effects included in the analysis were parity, days in milk, year and month of scan. Both cow and contemporary group were included in the model as random effects. Each phenotypic milk production variable was included individually in the model as a fixed effect to quantify the phenotypic association between multiple ovulations and milk production. Associations between genetic merit for milk, fat, protein, total solids, longevity, fertility and SCC with multiple ovulations were also included individually in the models of analysis. In a separate series of analysis both phenotypic and genetic merit for milk production were included in the multiple regression model; of interest here was the association between phenotypic milk production and likelihood of multiple ovulations after accounting for difference in genetic merit.

Results The likelihood of multiple ovulations was greatest in higher yielding cows and also cows with greater genetic for yield with the exception of genetic merit for fat yield (Table 1). After adjustment for the genetic potential for milk production, cows with higher fat yield, protein yield and total solids had a greater likelihood of multiple ovulations. Cows genetically predisposed to superior reproductive performance, longer longevity and lower SCC had a lower likelihood of multiple ovulations compared to cows genetically predisposed to inferior reproductive performance, shorter longevity and higher SCC, respectively. Milk composition, including fat to protein ratio, were not associated with the likelihood of multiple ovulations when evaluated at either the phenotypic or genetic level.

Table1 Associations **(**Odds ratio and 95% CI in parentheses) between phenotypic and genetic milk production with multiple ovulations[1]

Phenotypic				Genetic Merit		
Yield trait	*P-value*	Medium	High	*P-value*	Medium	High
Milk	<0.05	1.34 (1.07 – 1.68)	1.53 (1.20 – 1.96)	0.001	1.41 (0.13 – 1.77)	1.59 (1.27 - 2.00)
Fat	<0.005	1.29 (1.03– 1.61)	1.81 (1.41 – 2.31)	0.22	1.07 (0.87 – 1.34)	1.21 (0.97 – 1.51)
Protein	<0.05	1.18 (0.94 – 1.48)	1.65 (1.29 – 2.09)	<0.05	1.31 (1.05 – 1.63)	1.39 (1.10 – 1.74)
Total solids	<0.005	1.37 (1.09 – 1.72)	1.69 (1.31 – 2.16)	<0.05	1.20 (0.96 – 1.49)	1.31 (1.05 – 1.64)

[1] Low category was the referent category (i.e. odds ratio =1)

Conclusion This study determines that dairy cows of higher phenotypic and genotypic milk production have an increased likelihood of multiple ovulations.

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Incidence of clinical and subclinical endometritis in pasture managed dairy cows in Argentina and their effects on pregnancy rate at 100 days post partum

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Implications Endometritis in cattle is defined as inflammation of the endometrium and usually occurs without causing any systemic symptoms of infection. The present study reports data from the incidence of clinical and subclinical endometritis in pasture managed dairy farms in Argentina and their negative effect on pregnancy rates to 100 days *postpartum*.

Introduction Endometritis in cattle is defined as inflammation of the endometrium and usually occurs without causing any systemic symptoms of infection (Dubuc et al., 2010). Opportunistic pathogens have the potential to contaminate the uterus either from the vagina or the environment during parturition. In many instances, the cow's own immunological system is capable of clearing the infection within a few days or weeks following parturition; however, in some animals, subclinical or clinical acute or chronic endometritis can develop and persist. *Postpartum* endometritis has a negative effect on reproductive performance, predominantly by increasing the required number of services to conceive, increasing the interval from calving to the first service, and by increasing the interval from calving to conception, all of which combine to decrease the overall pregnancy rate (Kasimanickam et al., 2004). The economical losses due to uterine disease, including endometritis, have been estimated to be US$285 per lactation (reviewed in Gilbert, 2012). The incidence of endometritis has been reported to vary from a low of 7.8% to a high of 61.6% (Gilbert et al., 2012). The objective of this experiment was to determine the incidence of clinical and subclinical endometritis in dairy farms in the area of Villa María, Córdoba, Argentina and to determine its effect of pregnancy rate at 100 days after calving.

Material and methods The experiment was performed over a two-year period in seven different farms that are managed in typical Argentina conditions, which is grazing alfalfa (35% or the diet) and supplemented with a TMR of corn silage and grain (65% of the diet). Cows were producing on average 7550 kg of milk per lactation. The number of cows examined were 296 cows (95 primiparous and 201 multiparous) in year 1 and 244 cows (78 primiparous and 166 multiparous). The gynecological examination was performed between 25 to 35 days *postpartum* (dpp) and consisted of rectal palpation and observation of vaginal discharges using metrichek and endometrial cytology by the cytobrush technique as described by Kasimanickam et al. (2004). The association between endometritis and interval from calving to conception at 100 days *postpartum* (dpp) was evaluated using a proportional regression model of Cox, including dairy farm and status of the cow (primparous vs multiparous) as covariates. Additionally, the validity of the endometrial cytology obtained by cytobrush was evaluated using a ROC (receiver operational) curve. All analyzes were performed using the software SPSS for Windows Version 20.

Results Based on the examinations, 56.5% (305/540) of the cows were diagnosed as "healthy" cows (no purulent discharge and <18% PMN in the cytobrush smear), 20.9% (84/540) of cows had clinical endometritis (i.e. purulent or muco-purulent discharge evaluated by metricheck) and 15.6% (113/540) of the cows had subclinical endometritis. Furthermore there were 7.0% (38/540) of cows with mucopurulent or purulent discharge to the metrichek but without elevated PMN counts by cytobrush, which were defined as "false positives ". Healthy cows were 2.68 (95% CI, 1.53 to 4.68) times more likely to become pregnant than those with clinical or subclinical endometritis. On the other hand, when the effect was analyzed excluding cows with clinical endometritis, healthy cows were 2.07 (95% CI, 1.01 to 4.2) times more likely to become pregnant during the first 100 dpp. Considering the cytology as the diagnostic test and pregnancy at 100 dpp as the event it was found that the validity of it is moderate, with an area under the curve of 0.58 ($P < 0.05$). The discriminating thresholds for cytology used in this study (i.e. >18% PMN) resulted in a sensitivity and specificity of 86 and 31%, respectively.

Conclusion The incidence of clinical and subclinical endometritis (20 and 15%, respectively) in pasture managed dairy cows in Cordoba, Argentina is similar to those reported elsewhere. Furthermore, both conditions negatively affected the probability of cows becoming pregnant by 100 dpp.

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The effect of genetic merit for fertility traits on the transcriptome of the bovine endometrium on day 13 of the oestrous cycle

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Implications
The results improve our understanding of the causes of poor fertility by identifying differentially expressed (DE) genes in the endometrium that are potential mediators of fertility in lactating dairy cows.

Introduction In cattle, elevating progesterone (P4) concentrations has been shown to advance temporal changes in the endometrial transcriptome and to promote embryo elongation (Forde *et al*, 2009). Cummins *et al*. (2012) reported greater P4 concentrations in cows with good genetic merit for fertility (Fert+) compared with cows with poor genetic merit for fertility (Fert-). The objective of this study was to use RNA-seq to (1) compare the endometrial transcriptome in Fert+ cows and Fert- cows on d 13 of the oestrous cycle (a critical period for embryo development) and (2) to determine the concordance between DE genes and the frequency of single nucleotide polymorphisms (SNP) associated with fertility phenotypes.

Material and methods At ~60 d *postpartum*, 14 cows (8 Fert+, 6 Fert-) were enrolled in an ovulation synchronisation protocol. Endometrial biopsies were collected on d 13 of the oestrous cycle. RNA was extracted using a trizol-based method. cDNA libraries were created for each sample and sequenced on the HiSeq 2000 platform to generate 40 million 100 bp paired-end reads. Raw reads were aligned to the Bovine reference genome (UMD 3.1). Data were analysed using a general linear model in the Bioconductor package edgeR with genotype, parity and sample date included as fixed effects. A negative binomial distribution was assumed. Library size was normalised by the Trimmed Mean of M-values. The biological *cv* was calculated from genewise dispersion estimates. Likelihood ratio tests were performed to identify DE genes. A false discovery rate (FDR) of 5% was used to control for multiple testing (Benjamini and Hochberg method). Fertility genome-wide association studies had been performed to determine the association of SNP with calving interval (10,000 Holstein genomes project, 800k SNP; Kemper *et al*, personal communication) and commencement of luteal activity (RobustMilk, 50k SNP; Berry *et al*, 2012). The number of SNP, significant ($P<0.001$) and total, located 500kb either side of DE genes was determined. A FDR of 0.1% was used to determine how many SNP were expected to be significant by chance (Pryce *et al*, 2011).

Results The concordance with RNA-seq was higher than expected by chance for six and two genes using the results of the 10,000 Holstein genomes and RobustMilk projects, respectively (Table 1).

Table 1.		Number of significant SNP		More significant SNP than chance	
Gene ID	Log FC	RobustMilk	10,000 Holsteins	RobustMilk	10,000 Holstein Genomes
Uncharacterized	-1.92	0	13	No	Yes
LOC509034	-1.76	0	2	No	No
GPC3	1.60	0	12	No	Yes
PRKAG3	1.63	1	1	Yes	Yes
SPP1	1.76	1	0	Yes	No
SAA3	1.80	0	2	No	Yes
LOC782922	2.01	0	5	No	Yes
KCNE3	2.30	0	1	No	No
LOC528412	3.09	0	22	No	Yes

DE genes: FDR P-value < 0.05; FC: fold change (positive values indicate up-regulation in Fert- cows)

Conclusion The concordance analysis confirmed that RNA-seq is a powerful method to identify DE gene, implying that these genes are important mediators of fertility.

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The *in vitro* assessment of sex-sorted fresh and frozen bull sperm

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Implications Grass based dairy production systems, such as in Ireland offer the opportunity to use sex-sorted fresh sperm on a commercial scale due to the short breeding season.

Introduction The use of flow cytometry to sort X- and Y- bearing sperm is the only semen sorting technology available that gives a strong and reliable bias in offspring gender. However, previous reports have demonstrated significantly lower conception rates following the use of sex-sorted frozen-thawed sperm when compared to conventional frozen sperm (Healy et al., 2013). The aim of this study was to assess the *in vitro* quality of X- and Y- sorted sperm processed in a fresh or frozen form from both beef and dairy bulls and to compare it to conventional unsorted fresh and frozen sperm.

Material and methods - Experiment 1: Semen was collected from Holstein Friesian bulls (n = 9) at a commercial artificial insemination (AI) centre. Each ejaculate was split across 4 treatments and processed into 0.25 mL straws as follows (i) Unsorted fresh at 3 x 10^6 sperm/straw (Fresh unsorted 3M; control) (ii) X-sorted frozen at 2 x 10^6 sperm/straw (Frozen X-sorted 2M) (iii) X-sorted fresh at 2 x 10^6 sperm/straw (Fresh X-sorted 2M) and (iv) X-sorted fresh at 1 x 10^6 sperm/straw (Fresh X-sorted 1M). A fifth treatment of unsorted frozen at the routine dose of 20 x 10^6 sperm/straw (Frozen unsorted 20M), was commercially sourced from AI centres from previous ejaculates (n=3) of the same bulls. On Day 1, 2 and 3 post-sorting each sample was assessed *in vitro* for progressive linear motility (PLM) of the motile sperm population, agglutination and morphology using microscopy based techniques. In addition, viability and acrosomal status were assessed with a flow cytometer using the fluorescent probes Propidium Iodide (12 μM) and Alexa Fluor 647 (6 μg/mL), respectively, while the proportion of live sperm positive for the superoxide anion was assessed using the fluorescent probe MitoSOX Red (4 μM) and the dead stain SYTOX Green (0.25 μM). **Experiment 2:** Semen from Aberdeen Angus bulls (n=4) was collected and transported as per Experiment 1. Each ejaculate was split across 4 treatments as follows (i) Unsorted fresh 3 x 10^6 sperm/straw (Fresh unsorted 3M; control) (ii) Y-sorted fresh at 1 x 10^6 sperm/straw (Fresh Y-sorted 1M) (iii) Y-sorted Fresh at 2 x 10^6 sperm/straw (Fresh Y-sorted 2M) and (iv) X-sorted fresh at 2 x 10^6 sperm/dose (Fresh X-sorted 2M). As per Experiment 1, unsorted frozen at 20 x 10^6 sperm/straw (Frozen unsorted 20M) was sourced from previously collected ejaculates as a control. *In vitro* analysis was carried out as per Experiment 1. Data were examined for normality, transformed where appropriate and analysed using repeated measures in Statistical Package for the Social Sciences (SPSS; version 20.0). The model included the main effects of day, treatment and day x treatment interactions.

Results - Experiment 1: The proportion of viable sperm was not affected by day of storage but was affected by treatment ($P<0.001$) with an average % alive of 65.3, 89.7, 84.2, 86.4 and 42.3 for the fresh unsorted 3M, frozen X-sorted 2M, fresh X-sorted 2M, fresh X-sorted 1M and frozen unsorted 20M treatments, respectively. The two sex-sorted fresh treatments had higher levels of agglutination in comparison to the other treatments ($P<0$ .001). The fresh unsorted 3M treatment had the highest proportion of sperm with PLM ($P<0.05$). There was no effect of treatment on the proportion of acrosome-reacted sperm or live sperm positive for superoxide production. **Experiment 2:** Trends from Experiment 1 were mirrored in Experiment 2. The three fresh sex-sorted treatments had higher levels of agglutination when compared to the other treatments ($P<0.001$). PLM was highest in the frozen unsorted 20M treatment ($P<0.05$) while, the fresh sex-sorted treatments had the highest % alive ($P<0.001$). Unlike Experiment 1, there was an effect of treatment on the proportion of live sperm positive for superoxide anion ($P<0.05$), represented by greater oxidative stress in the unsorted treatments.

Conclusion Sex-sorting sperm yielded a greater population of viable sperm compared to other treatments. When stored fresh, high levels of agglutination were observed which may have negative effects on subsequent pregnancy rates.

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