SELECTION FOR OVULATION RATE IN RABBITS: OOCYTE CONCENTRATIONS OF GLUTATHIONE AND ATP

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ABSTRACT

The present study was designed to determine the concentrations of glutathione (GSH)and ATP in rabbit oocytes in a line of rabbits selected for ovulation rate (line OR) for ten generations, its unselected control line and a line selected for ovulation rate and litter size (line OR_LS) for ten generations. The relationship of GSH and ATP concentrations with ovulation rate was studied. GSH and ATP concentrations were used to assess oocyte quality. Atotal of 50does of the line OR, 41 does of the control line and 45 does of the line OR_LS were used. Ovulation was inducedby an intramuscular injection of 1*mg* of buserelin acetate and animals were slaughtered 16 hours thereafter. Oocytes were collected by flushing the oviducts.GSH concentration was determined by HPLC, while ATP concentration was determined by bioluminiscency. Ovulation rate was classified into three levels: low (10-13 corpora haemorragica), medium (14-18corpora haemorragica) and high (19-24 corpora haemorragica). Oocyte glutathione concentration was significantly lower in oocytes from females with high (9.1±0.3 pmol) and medium (9.6±0.1 pmol) ovulation rates than in oocytes from females with low ovulation rates (10.2±0.3 pmol). No significant differences were found for ATP concentration. No differences in the concentrations of GSH and ATP between the line selected for ovulation rate and the control line were found, but the line OR_LS had a higher concentration of GSH (around 0.7 pmol/oocyte more). It seems that high ovulation rates could be associated with poorer oocyte quality in comparison with low ovulation rates.

Key words: Rabbits, selection, ovulation rate, ATP, glutathione, oocyte quality.

INTRODUCTION

Selection for ovulation rate was proposed as an indirect way to improve litter size. In pigs, mice and rabbits, ovulation rate responded to selection, but it did not lead to a correlated response in litter size, showing a decrease in prenatal survival (*i.e.* Laborda *et al.*, 2011a in rabbits; Rosendo *et al.*, 2007 in pigs). It has been suggested, based on a chromosomal analysis, that immaturity of ova may account for a substantial proportion of prenatal mortalityin a line of pigs selected for high ovulation rate (Koenig *et al.*, 1986).

There are many methods to assess oocyte maturation. Several authors have proposed the measurement of glutathione (GSH) and ATP inside the cell (reviewed by Krisher, 2004). Both GSH and ATP have been suggested to be indicators of the developmental potential of oocytes (reviewed by Krisher, 2004). In a companion paper, it is shown that mature oocytes have higher levels of GSH and ATP than immature ones (Laborda *et al.*, 2012), in agreement with other studies in different species (*i.e.* Brevini *et al.*, 2005 in pigs).

The aim of this work was to study the oocyte maturation measured as the oocyte concentration of GSH and ATP in a line selected for ovulation rate, its control line and a line selected for ovulation rate and litter size.

MATERIALS AND METHODS

Animals

All experimental procedures involving animals were approved by the Research Ethics Committee of the Universitat Politècnica de València. Animals came from an experiment of selection for ovulation rate (OR) described by Laborda et al. (2011a) and from a cryopreserved control population, detailed in Laborda (2011b). The line OR was selected for 10 generations. A contemporary line derived from the animals of the sixth generation of the line OR, and two-step selection was performed for ovulation rate and litter sizefor 4 generations (line OR_LS, described by Ziadi *et al.,* 2012). A total of 43 multiparous females from generation 10 of the line OR, 34 multiparous females from the control line and 45 multiparous females from the generation10 of the line OR_LS were used to measure GSH and ATP. Additionally, 7 nulliparous females from the line OR and 7 nulliparous females from the control line were used to measure ATP. Ovulation was induced by an intramuscular injection of 1 mg of buserelin acetate (Hoechst, Marion Roussel, Madrid, Spain). Animals were slaughtered 16 hours thereafter and the reproductive tracts were removed. Animals of all 3 lines were housed at the experimental farm of the Universitat Politècnica de València.

Oocytes

Cumulus-oocyte complexes (COCs) were recovered by flushing each oviduct with 5 ml of DPBS supplemented with 2 mg/ml of BSA at room temperature.

From each female, 12 COCs were randomly selected to measure GSH. They were denuded by washing them first in 0.1% hyaluronidase and then in DPBS-BSA, and by stripping off their cumulus cells, using a binocular stereoscopic microscope, Leica MZ75-200x. After that, oocytes were washed three times in stock buffer solution and were transferred to a microfuge tube in 5 µl. Then, 5 µl of phosphoric acid 1.25 M was added, and it was immediately frozen at -80ºC and stored until used.

The remaining COCs from each female and the oocytes from the nulliparous females were used to measure ATP. They were similarly denuded, without removing the corona radiate in order to limit excessive oocytes manipulation which can provoke stress-induced ATP release by the cells. After washing them 3 times in DPBS, 1-4 oocytes from each female were transferred to a straw in 50 µl of DPBS. Several straws belonging to each female were stored in liquid nitrogen until they were used.

GSH Assay

Concentrations of GSH were determined by HPLC according to the method described by Nolin *et al.* (2007) with some modifications. Details about the equipment, the chromatographic conditions and the standard and the sample preparations for the GSH assay are given in a companion communication (Laborda *et al.,* 2012). Samples were analysed in 7 sessions.

ATP Assay

Concentrations of ATP were determined using the bioluminescent somatic cell assay kit FL-ASC (Stojkovic *et al.,* 2001). The ATP assay is detailed by Laborda *et al.* (2012) in a companion communication. Samples were analysed in a single session.

Traits

The following traits were recorded: the oocyte ATP concentration and the oocyte GSH concentration expressed in pmol/oocyte were calculated dividing the concentration of ATP or GSH in each sample by the number of oocytes in the sample; ovulation rate (OR), countedas the number of corpora haemorragica in both ovaries; recovery rate (RR), calculated as the ratio between recovered oocytes and ovulation rate.

Statistical analyses

The effect of OR on the mean oocyte concentrations of GSH (Model 1) and ATP (Model 2) was analysed fittingthe following models:

$y_{ij} = \mu + OR_i + S_j + e_{ij}$ (Model 1)

$y_{ijkl} = \mu + OR_i + P_j + p_k + e_{ijkl}$ (Model 2)

where y is the mean oocyte concentration of GSH (Model 1) and ATP (Model 2), ORis the effect of ovulation rate, with 3 levels (Level $_{OR}$ 1: low ovulation rate (10-13 corpora haemorragica); Level $_{OR}$ 2: medium ovulation rate (14-18 corpora haemorragica); Level $_{OR}$ 3: high ovulation rate (19-24 corpora haemorragica); S is the effect of session, with 7 levels; P is the effect of parity, with 2 levels (nulliparous and multiparous females); p is the permanent environmental effect of the female; and e is the residual of the model. The levels of OR were considered as the mean ± 1 standard deviation.

The model used to analyse the mean oocyte concentrations of GSH (Model 3) and ATP (Model 4) in each line was:

$y_{ij} = \mu + Line_i + S_j + e_{ij}$ (Model 3)

$y_{ijkl} = \mu + Line_i + P_j + p_{ik} + e_{ijkl}$ (Model 4)

Where y is the mean oocyte concentration of GSH (Model 1) and ATP (Model 2); the effect of Line has 3 levels; the other effects are described above.The GLM procedure of SAS (SAS, 1998) was used for both analyses.

RESULTS AND DISCUSION

Raw means, standard deviations and maximum and minimum values of ovulation rate, recovery rate, oocyte GSH concentration and oocyte ATP concentration are summarized in Table 1. Mean ovulation rate was high compared to other studies in maternal rabbit lines (García and Baselga, 2002; Theau-Clement, 2009). Recovery rate was high and similar to results published by Santacreu et al. (1997) using vasectomized males. In this experiment, the mean concentrationsof GSH in rabbit oocytes was 9.6 ± 1.1pmol, and the mean concentration of ATP per oocyte was 2.0 ± 0.7 pmol (Table 1). These values agree with the values reported in a companion communication (Laborda *et al.*, 2012).

Table 1: Raw means, standard deviations (SD) and maximum and minimum values of ovulation rate (OR), recovery rate (RR), oocyte GSH concentration (GSH_{oocyte}) and oocyte ATP concentration (ATP_{oocyte})

Trait	Mean	SD	Minimum	Maximum
OR (ova)	16	2.5	10.0	24.0
RR (%)	95.6	6.8	70.6	100.0
GSH_{oocyte} (pmol/oocyte)	9.6	1.1	7.2	12.0
ATP_{oocyte} (pmol/oocyte)	2.0	0.7	0.5	3.5

The effect of OR on the mean oocyte concentrations of GSH and ATP

Table 2 presents oocyte concentrations of GSH and ATP at different levels of ovulation rate. Oocytes from females with low OR had higher GSH concentrations than oocytes from females with medium and high OR. Regarding ATPconcentration, no differences between these groups could be found. Differences in GSH and ATP concentrations between immature and mature oocytes have been reported (i.e. Brevini *et al.*, 2005 in pigs; Laborda *et al.*, 2012 in rabbits). While oocyte maturation as a measure of oocyte quality has been related with higher GSH concentration (reviewed by Luberda, 2005), its relationship with higher levels of ATP has not been always positive (Brad *et al.*, 2003; Herrik *et al.*, 2003). No other study reporting the association between the concentrations of GSH and ATP and ovulation rate has been found. The data obtained in this experiment support that females with low ovulation rate might have better oocyte quality than females with high ovulation rate.

Table 2: Least squaremeans and standard errors for oocyte GSH concentration (GSH_{oocyte}) and oocyte ATP concentration (ATP_{oocyte})

	GSH_{oocyte} (pmol/oocyte)	ATP_{oocyte} (pmol/oocyte)
Level $_{OR}$ 1	10.2 ± 0.3 [a]	1.7 ± 0.2 [a]
Level $_{OR}$ 2	9.6 ± 0.1 [b]	2.0 ± 0.2 [a]
Level $_{OR}$ 3	9.1 ± 0.3 [b]	2.1 ± 0.2 [a]

Level $_{OR}$ 1: low ovulation rate (10-13 corpora haemorragica); Level $_{OR}$ 2: medium ovulation rate (14-18 corpora haemorragica); Level $_{OR}$ 3: high ovulation rate (19-24 corpora haemorragica).
[a, b] different letters within column indicate significant differences, P<0.05.

The effect of the line on the mean oocyte concentrations of GSH and ATP

In the experiment of selection for OR, there was a difference of around 2 ova between the line OR and the control line, while there was no correlated increase in litter size (Laborda *et al.*, 2011b). A possible hypothesis to explain this lacking correlated increase in litter size was a higher percentage of immature oocytes in the selected line. In this study, the relevant but not significant difference found in OR between the line OR and the control line (Table 3) was probably due to a sampling effect. Regarding the GSH and ATP oocyte concentrations, no differences between the line OR and the control line have been found. Thus, we cannot support the suggested hypothesis with our data. On the other hand, a difference in the concentration of GSH of 0.7 pmol/oocyte was found between the control line and the line OR_LS. A higher concentration in GSH could indicate a higher number of mature oocytes, leading to a higher prenatal survival, which seems to be the case of the line OR_LS versus the other two lines (Ziadi *et al.*, 2012).

Table 3: Least square means and standard errors for oocyte GSH concentration (GSH_{oocyte}) and oocyte ATP concentration (ATP_{oocyte})

	OR (ova)	GSH_{oocyte} (pmol/oocyte)	ATP_{oocyte} (pmol/oocyte)
Control line	14.8± 0.6 [a]	9.4 ± 0.2 [a]	2.0 ± 0.2 [a]
Line OR	16.0± 0.5[ab]	9.4 ± 0.2 [a]	2.0 ± 0.2 [a]
Line OR_LS	16.7 ± 0.5[b]	10.1 ± 0.2 [b]	1.9 ± 0.2 [a]

Line OR (line selected for ovulation rate); Line OR-LS (line selected for ovulation rate and litter size).
[a, b] different letters within column indicate significant differences, P<0.05.

CONCLUSION

Oocytes from females with high ovulation rate have lower GSH concentration than oocytes from females with low ovulation rate. Given that GSH concentration is a measure of oocyte quality, females with high ovulation rate seem to have less oocyte quality than females with low ovulation rate. However, no differences in GSH and ATP concentrations between the line selected for ovulation rate for 10 generations and the control line have been found.

ACKNOWLEDGEMENTS

This study was supported by the Comisión Interministerial de Ciencia y Tecnología CICYT-AGL2008-05514-C02-01 and by funds from Generalitat Valenciana research programme (Prometeo 2009/125).

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