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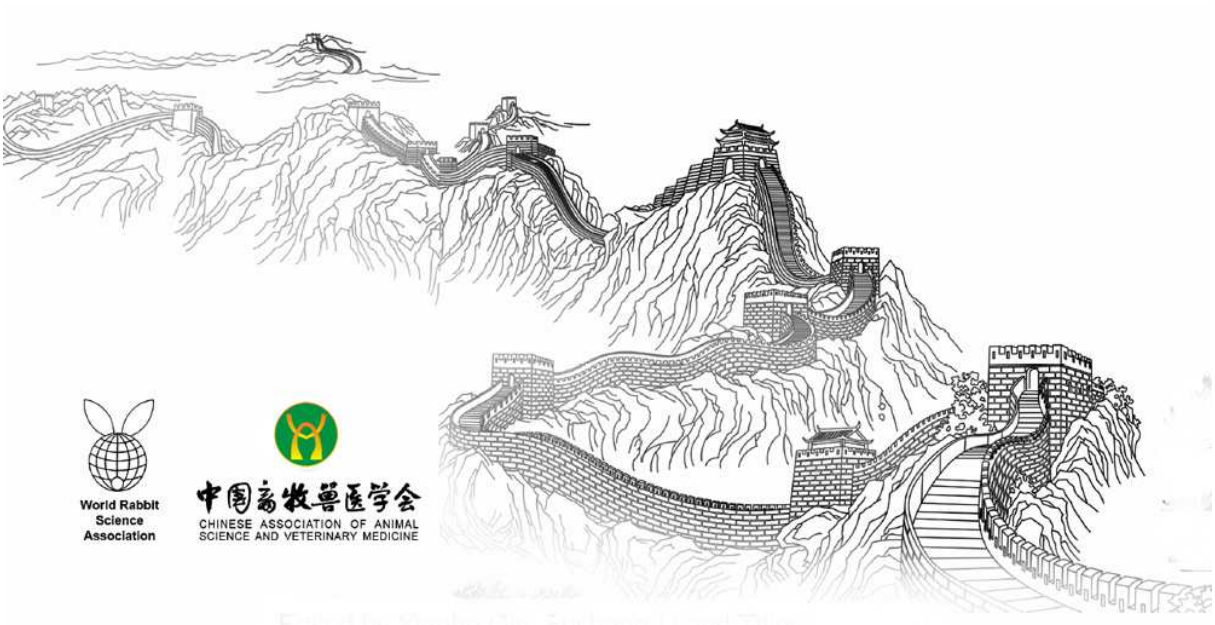

PREIMPLANTATIONAL STUDY IN RABBIT DOES SUPPLEMENTED WITH n-3 POLYUNSATURATED FATTY ACIDS

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ABSTRACT

The aim of this work was to study the effect of a long term enriched diet with polyunsaturated n-3 fatty acids (PUFA) on endocrine and embryo development of rabbit females at preimplantation time. Multiparous rabbits does (n = 28) with two previous parturitions were fed *ad libitum* during all their productive life with two supplemented diets with different fat sources: 30 g/kg mixed fat for the control diet (group C; n = 14) or 60 g/kg of a salmon oil supplement containing a 50% ether extract and 35% of total fatty acids as PUFA n-3 (group P; n = 14). After 84 h from artificial insemination, all does were euthanized to determine the embryo development and a blood sample was taken to determine plasma progesterone concentration. Neither embryo development nor plasma progesterone was affected by diet. In conclusion, a long-time of PUFA supplementation as a dietary intervention had no effect on reproductive and hormonal responses at early pregnancy in rabbit does.

Key words: Reproduction, Progesterone, Embryo, Development, Fertility.

INTRODUCTION

Concentration of PUFA in vegetable ingredients is minimal and animals have to produce these fatty acids from alpha-linolenic acid (ALA; C18:3 n-3) and linoleic acid (LA; C18:2 n-6), through a complex mechanism of elongation and desaturation, with several competitive limiting steps. This frequently leads to suboptimal concentration of EPA (eicosapentaenoic acid; C20:5n-3) and DHA (docosahexaenoic acid; C22:6n-3) in cell membranes, which may negatively affect physiological regulation, including reproductive response and newborn survival (Wathes *et al*., 2007). Therefore, to increase concentrations of EPA and DHA in plasma of females, these two n-3 PUFA have to be directly introduced into the body via the diet. The EPA and DHA are found in fish oil, as well as in microalgae oil.

The diet administered to females around the fertilization period and before implantation, affects the oviductal ambient (Ashworth *et al*., 2009) having unknown effects on process as fertilization and early embryo development. One mechanism that could enhance fertility is that the n-3 diet decreases the concentration of prostaglandin F2 alpha (PGF2α) in the uterus, thus potentially facilitating embryo implantation and reducing embryo mortality (Mattos *et al*., 2004). Paramio *et al*. (2015) speculate that a higher n-3 fatty acids profile in the follicular fluid of goat ovaries could increase oocyte quality and the possibility to reach blastocyst stage. In dairy cows, Elis *et al*. (2016) have observed that a n-3 PUFA diet tends to increase the number of large follicles and decreases the non-fertilization and/or early embryo mortality rate associating these effects with the supplementation period used (first 2 months postpartum). In this line, we have observed that long time supplementation of rabbit females with n-3 PUFA diets affected the composition of ovarian adipose tissue, and progesterone concentrations on day 5 post artificial insemination were higher in pregnant females fed with the supplemented diet (Rebollar *et al*., 2014). Our hypothesis is that this nutritional strategy potentially could affect to ovarian structures (oocytes quality,

follicle rupture or corpora lutea development) and, consequently, to oviductal environment where the first phases of embryo development take place.

This study intends to determine if a long term supplementation of PUFA n-3 in rabbit does diet, throughout their productive life, enhances embryo quality and affects progesterone level in the preimplantation period.

MATERIALS AND METHODS

Animals and experimental design

A total of 28 New Zealand x California sexually receptive multiparous rabbit does (diagnosed by vulva colour) with two previous parturitions weighting 4.4 ± 0.17 kg were used. Animals were randomly allocated in two groups and fed *ad libitum* from rearing and during all their productive life with two diets. The composition of both experimental diets was balanced for energy and protein contents and only varied in the type of added fat: 30 g/kg of mixed fat (n=14 does) for control diet (C group) and 60 g/kg of salmon oil supplement containing a 50% of ether extract and 35% of PUFA n-3 (n=14 does) for PUFA n-3 diet (P group) [13% DHA (C22:6 n-3), 3% docosapentaenoic acid (DPA) (C22:5 n-3), 7% EPA (C20:5 n-3), 7% stearidonic acid (SDA) (C18:4 n-3) and 3% linoleic (C18:3 n-3)].

All females were artificially inseminated (AI) at 32 days post-partum to avoid any lactation effect. Seminal doses with at least 20 million spermatozoa in 0.5 ml of diluent (Magapor S.L., Zaragoza, Spain) were prepared using a pool of fresh heterospermic semen from a group of bucks selected for high growth performance. To induce ovulation, 20µg i.m. gonadorelin (Inducel-GnRH, Lab. Ovejero, León, Spain) were used.

To determine serum progesterone concentration, 84h after AI, blood samples were taken by collecting in tubes containing EDTA. Plasma was obtained after centrifugation at 1200 × g for 10 min at 4 ºC and stored at −20 ºC until further analysed.

Ovulation rate and embryo study

Immediately after blood sampling, animals were euthanized according to the Spanish Royal Decree 53/2013, with an overdose of barbiturates (Dolethal, Vetoquinol, España). Ovaries and reproductive tract were collected in PBS at 37 ºC. Ovulations were confirmed observing early corpora lutea in the ovarian surface and ovulation rate was determined by counting the total number of corpora lutea per doe. Subsequently, embryos were recovered by flushing the reproductive tract from the end of the uterus horn to the infundibulum with PBS + 0.1% BSA at 38.5 ºC. The flushing fluid was deposited in a Petri dish on a warm plate and embryos were searched and evaluated with a stereoscopic microscope (Nikon SMZ-800, Tokyo, Japan). At the time of collection, the embryos were classified into the following categories: morula, blastocyst and retarded embryos. The different embryonic stages observed were defined on the basis of conventional morphological criteria and according to their stage of development following the guidelines of the International Embryo Transfer Society. Fertility rate (number of pregnant does divided by total inseminated does per group x 100) at 84h post AI was determined considering as pregnant those females having corpora lutea and some embryo structure recovered from her reproductive tract.

Chemical Analyses

Plasma progesterone concentrations were analysed using a commercial kit (Progesterone ELISA, Demeditec Diagnostics GmbH, Germany) based on the principle of competitive binding. Previously, plasma samples were extracted with petroleum ether at a 5:1 (v/v) ether:sample ratio (extraction efficiency was 85%). Sensitivity was 0.045 ng/ml. The intra- and inter-assay coefficients of variation were 5.5% and 6.9%, respectively.

Absorbance was measured in a Bio-Tek automatic plate reader (EpochTM Microplate Spectrophotometer, Bio-Tek Instruments, Winooski, Vermont, USA) at 450 and 630 nm, and hormone concentrations calculated by means of a software developed for these techniques (Gen5TM ELISA, Bio-Tek Instruments).

Statistical Analysis

Data were analysed with Statistical Analysis System software (SAS, 1990) considering the feeding regime as the main source of variation. Fertility rate was analysed with a χ^2 (CATMOD procedure). Ovulation rate, progesterone concentrations and *in vitro* determinations were analysed as a completely randomized design by using the GLM procedure. All means were compared using a protected t-test. Differences were considered significant at P<0.05 and a trend when P<0.10. Results are presented as least squared mean (lsmeans).

RESULTS AND DISCUSSION

The results related to the study are shown in Table 1. All does ovulated and no significant difference in ovulation rate was observed (P>0.05). A total of 89 and 102 embryonic structures were recovered from C and P group, respectively. Although the presence of embryo structures in the oviductal fluid determined a fertility rate at 84h post AI a 14% higher in C than in P does, no statistical diet effect on this parameter was observed. The development of recovered structures was similar to that usually obtained on 3.5 days after AI previously described by Arias-Álvarez *et al*. (2010). Recovery rate was higher in does supplemented with PUFA n-3 than in C does (P<0.05), however the structures recovered from C does tended to show a more advanced state of development (P<0.09).

Table 1: Ovarian and embryo parameters of rabbit does fed control (C) and PUFA n-3 supplemented (P) diets.

	C	P	SE	P>f
No of does	14	14		
Ovulated does	14	14		
Ovulation rate (n)[1]	13.07	11.86	1.243	0.4959
Fertility (%)[2]	78.6 (11/14)	64.3 (9/14)		0.4216
Recovery rate (%)	55.9	83.8	9.038	0.0410
Morula (%)	66.0	85.6	10.688	0.2107
Blastocyst (n)	18.6	2.7	6.318	0.0905
Retarded embryos (n)	15.4	11.7	10.283	0.8054

[1]: number of corpora lutea per ovulated doe. [2]: number of does with some embryo structure recovered/total of does x100

Related to progesterone response at 84 h post-AI, there were no differences between diets with a mean of 3.4±0.31 ng/ml (P>0.05). However, Rebollar *et al*. (2014) observed later, on day 5 post-partum, a higher progesterone concentration in does supplemented with a similar supplement compared with control does. Although concentrations of n-3 PUFA in female rat plasma and tissues are positively associated with circulating concentrations of progesterone (Child *et al*., 2008a), in cows fed with fish oils, the increase on corpora lutea on day 7 post-oestrus no determined changes in this hormone Child *et al*. (2008b). More studies are necessary to determine if n-3 PUFA could affect corpora lutea functionality and progesterone concentrations in more advanced stages of embryo development and implantation.

CONCLUSIONS

Long term dietary supplementation of 60 g/kg of a supplement that contained 50% ether extract and more than 30% unsaturated fatty acids as a fat source in multiparous rabbit does neither affect early progesterone response at 84 h post-AI nor embryo development at this preimplantational time.

ACKNOWLEDGEMENTS

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REFERENCES

Arias-Álvarez M., García-García R.M., Rebollar P.G., Nicodemus N., Millán P., Revuelta L., Lorenzo P.L. 2010. Follicular, oocyte and embryo features related to metabolic status in primiparous lactating does fed with high-fibre rearing diets. *Reprod. Domestic. Anim., 45, e91-e100.*

Ashworth C.J., Toma L.M., Hunter M.G. 2009. Nutritional effects on oocyte and embryo development in mammals: implications for reproductive efficiency and environmental sustainability. *Phil. Trans. R. Soc. B., 364, 3351–3361*.

Childs C.E., Romeu-Nadal M., Burdge G.C., Calder P.C. 2008a. Gender differences in the n-3 fatty acid content of tissues. *P. Nutr. Soc., 67, 19-27.*

Childs S., Hennessy A.A., Sreenan J.M., Wathes D.C., Cheng Z., Stanton C., Diskin M.G., Kenny D.A. 2008b. Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology, 70, 595-611*.

Elis S., Freret S., Desmarchais A., Maillard V., Cognié J., Briant E., Touzé J.L., Dupont M., Faverdin P., Chajès V., Uzbekov S. 2016. Effect of a long chain n-3 PUFA-enriched diet on production and reproduction variables in Holstein dairy cows. *Anim. Reprod. Sci., 164, 121-132*.

Mattos R., Staples C.R., Arteche A., Wiltbank M.C., Diaz F.J., Jenkins T.C., Thatcher W.W. 2004. The effects of feeding fish oil on uterine secretion of PGF2alpha, milk composition, and metabolic status of periparturient Holstein cows. *J. Dairy Sci., 87, 921–932*.

Paramio M.T., Roura M., Hammami S., Izquierdo D., Catalá M.G. 2015. Fatty Acid composition in follicular fluid of prepubertal goat ovaries in winter and autumn. Reprod. Fertil. Dev., 27, 181.

Rebollar P.G., García-García R.M., Arias-Álvarez M., Millán P., Rey A.I., Rodríguez M., Formoso-Rafferty N., De la Riva S., Masdeu M., Lorenzo P.L. 2014. Reproductive long-term effects, endocrine response and fatty acid profile of rabbit does fed diets supplemented with n-3 fatty acids. *Anim. Reprod. Sci., 146, 202-209.*

SAS Institute, 1990. SAS/STAT® User's Guide (Release 8.2). *SAS Inst. Inc.,Cary NC, USA.*

Wathes D.C., Abayasekara D.R.E., Aitken R.J. 2007. Polyunsaturated fatty acids in male and female reproduction. *Biol. Reprod., 77,190–201*.

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1 THE MESSAGE

n-3 polyunsaturated fatty acids (PUFA) are related with multitude of beneficial processes to health, however, there are a few studies of the effect of this fatty acids on reproductive activity in rabbit does.

2 INTRODUCTION

This nutritional strategy potentially could affect to ovarian structures and, consequently, to oviductal environment where the first phases of embryo development take place.

The aim of this work was to study the effect of a long term enriched diet with n-3 PUFA on endocrine and embryo development of rabbit females at preimplantation time.

3 MATERIALS & METHODS

28 multiparous rabbit does
(from rearing to 3rd artificial insemination)
2400 kcal ED/kg, 36% FND y 16% PB

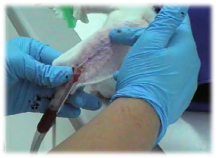
84 h (3.5 days of gestation)

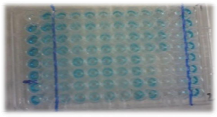
14 does/Control diet — **3% Mixed fat (lard and tallow)**

14 does/PUFA diet — **6% Supplement 50% EE rich in n-3 PUFA**
13% DHA (C22:6 n-3)
7% EPA (C20:5 n-3)

Artificial Insemination (AI)

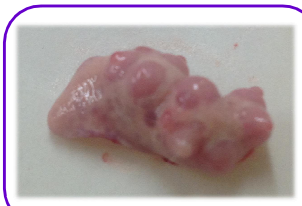
Blood sampling Euthanasia





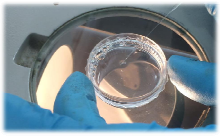
Progesterone EIA Commercial Kit ELISA

Counting of corpora lutea (*)



Ovulation rate

84 h post AI → flushing the reproductive tract with warm PBS



Morphological evaluation of the embryos

Morula Blastocyst

Recovery rate

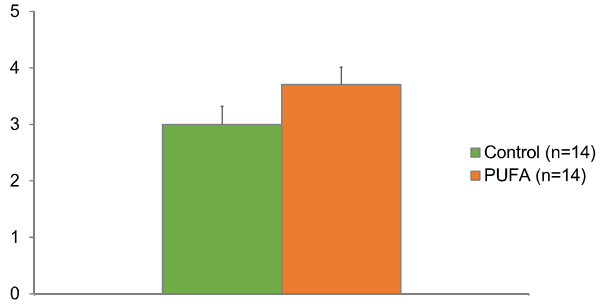
4 RESULTS

Ovarian and embryo parameters of rabbit does fed control (n=14) or n-3 PUFA supplemented (n=14) diets.

	CONTROL	PUFA	SE	P>f
Ovulated does (%)	100 (14/14)	100 (14/14)		
Ovulation rate (n)[1]	13.1	11.9	1.24	0.4959
Fertility (%)[2]	78.6 (11/14)	64.3 (9/14)		0.4216
Recovery rate (%)[3]	55.9	83.8	9.04	0.0410
Morula (%)	66.0	85.6	10.69	0.2107
Blastocyst (%)	18.6	2.7	6.32	0.0905
Retarded embryos (%)	15.4	11.7	10.28	0.8054

[1]: number of corpora lutea per ovulated doe.
[2]: number of does with some embryo structure recovered / total of does x100.
[3]: number of recovered structures / total of corpora lutea x100

Plasma progesterone concentration (ng/ml) at 84 h post-AI



5 CONCLUSIONS

Long term dietary supplementation with n-3 polyunsaturated fatty acids in multiparous rabbit does did not affect early progesterone response at 84 h post-AI nor embryo development at this preimplantational time.

ACKNOWLEDGMENTS
This research was supported by AGL2011-23822 and S2013/ABI-2913 projects.
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