Energy homeostasis in rabbit does during pregnancy and pseudopregnancy

Laura Menchetti[1], Egon Andoni[2], Olimpia Barbato[1], Claudio Canali[1], Alda Quattrone[1], Daniele Vigo[3], Michela Codini[4], Giulio Curone[3], Gabriele Brecchia[3]*

[1]Department of Veterinary Medicine, University of Perugia, Via San Costanzo, 4, 06126, Perugia, Italy

[2]Faculty of Veterinary Medicine, Agricultural University of Albania, Rr Paisi Vodica, Koder, 1029, Kamez, Albania

[3]Department of Veterinary Medicine, University of Milano, Via dell'Università, 6, 26900 Lodi, Italy

[4]Department of Pharmaceutical Sciences, University of Perugia, Via A. Fabretti 48, 06123, Perugia, Italy

*Corresponding author: Address: Via dell'Università, 6, 26900 Lodi – Italy;

e-mail: gabriele.brecchia@unimi.it; Phone: +39 0250334583

ABSTRACT

This study was conducted to evaluate the changing concentrations of metabolic hormones and metabolites in pregnant (P) and pseudopregnant (PP) rabbit does. Twenty-five New Zealand White rabbit does were submitted to artificial insemination (AI) and then classified as P (n = 15) or PP (n = 10). Blood samples were collected weekly until day 32 post AI. During pregnancy, leptin concentrations were greater on Days 14 and 21 (P<0.05), while insulin was greater on days 21 and 32 post AI (P<0.05) compared to PP does. The triiodothyronine/thyroxine (T3/T4) ratio was greater in the first and last week (P<0.001); whereas, cortisol concentrations were greater in the last week of pregnancy and after parturition (P<0.01) compared with that of PP does. Non-esterified fatty acids (NEFA) concentrations increased from day 7 until day 32 post AI (P<0.05). Glucose concentrations were unchanged throughout pregnancy although concentrations were positively associated with litter size. These results indicate concentrations of hormones and metabolites change during pregnancy to ensure energy requirements are met for both the foetuses and the maternal tissues. Physiological hyperleptinemia, hyperinsulinemia, and changes in cortisol as well as thyroid hormones indicate there is an adaptation of metabolic functions induced by pregnancy. These adaptations could be mediated by gonadal steroids because changes mainly occur in the second half of pregnancy when the profile of the sex hormones differs between P and PP does.

Keywords: Rabbit; Pregnancy; Energy homeostasis; Animal models; Leptin; Insulin

1. Introduction

In the last decades, cyclic production systems and artificial insemination have become routine practices in rabbit breeding. In this context, the doe's capacity to sustain a dynamic pattern of energetic metabolism is important for not only sustainable rabbit farming but also animal welfare (Fortun-Lamothe, 2006; Menchetti et al., 2015b; EFSA Panel on Animal Health and Welfare, 2020).

Ovulation in rabbits is induced either by the injection of GnRH analogues such as Buserelin acetate (Rebollar et al., 2012; Dal Bosco et al., 2014) or by the stimuli associated with coitus which causes the release of GnRH and subsequently of LH (luteinizing hormone) as well as FSH (follicle stimulating hormone). The corpora lutea (CL), which are formed after ovulation, persist for the entire gestation (about 29-32 days) and are responsible for the progesterone concentrations essential to maintain pregnancy until parturition. Progesterone increases from day 3 after mating, reaching maximal concentrations on day 16 of gestation; then, rapidly decreases before parturition (Boiti et al., 1999; Brecchia et al., 2006). If fertilization does not occur after mating and ovulation, the rabbit doe can manifest PP. The CL during pseudopregnancy (PP) does develop similar to what occurs during pregnancy, although after day 13 subsequent to mating the CL start to regress until these structures can no longer be detected and progesterone concentrations return to basal values at day 18 to 20 if there was no pregnancy resulting from the mating with the male (Boiti et al., 2003, 2004; Zerani et al., 2010; Carter et al., 2016).

The concentration and interactions of reproductive hormones are quite well understood both during pregnancy (P) and PP in rabbit does; however during pregnancy, mammalian species have marked changes in energy homeostasis that require adaptations such as increasing food intake and mobilizing body lipid reserves.

The main hormones and metabolites involved in maintaining energy homeostasis are leptin, insulin, triiodothyronine (T3), thyroxine (T4), cortisol, glucose, and non-esterified fatty acids (NEFA) (Brecchia et al., 2006; Fortun-Lamothe, 2006; Menchetti et al., 2015a). Leptin functions as an indicator of body energy stores status, functioning at the central nervous system to modulate food intake (anorexigenic signal) and energy expenditure. During pregnancy, leptin appears to have different functions, from the regulation of placental development to an immunomodulatory function. In some species, however, the typical functions of leptin as a satiety signal seems to fail because increases in leptin concentrations in blood are accompanied by hyperphagia (Ladyman et al., 2010; Pérez-Pérez et al., 2018; Gustafson et al., 2019). In addition, during pregnancy, maternal insulin sensitivity decreases, and the pancreatic endocrine cells undergo structural and functional changes leading to hyperinsulinemia as a compensatory response (Ciampelli et al., 1998; Ladyman et al., 2010; Menchetti et al., 2015a, 2018). Thus, leptin and insulin resistance may be indicative of the maternal physiological adaptations not only to ensure glucose supply for the foetus but also to maintain glucose homeostasis; however, both of these conditions can lead to pathological outcomes (Ladyman et al., 2010; Menchetti et al., 2015a, 2018).

Total T3 and T4 concentrations increase during the first weeks of human pregnancy contributing both to the maintenance of maternal energy homeostasis and to the neuronal development of the foetus (Soldin, 2006; Lazarus, 2011; Micke et al., 2015). Conversely, cortisol serum concentrations increase only in late pregnancy which induces the onset of parturition (Soma-Pillay et al., 2016). Furthermore, glucose and NEFA are important metabolic energy sources which change as a result of a changing energy status of animals (Brecchia et al., 2006; Fortun-Lamothe, 2006; Menchetti et al., 2015a).

The study of the different neuroendocrine functions and the reciprocal relationships in rabbit pregnancy could be useful to help its reproductive management as well as to understand the mechanisms involved in human pregnancy complications such as hypertension, preeclampsia, and gestational diabetes (Ladyman et al., 2010; Pérez-Pérez et al., 2018). These factors have not been previously investigated to a great extent, and to the best of our knowledge, the energy homeostasis of P and PP rabbit does has not been previously compared.

The aims of the study were as follows: i) to evaluate the hormonal and metabolic changes in P and PP rabbit does, and ii) to compare the metabolic physiological adaptations observed in pregnant rabbits with previous research on other animal species.

2. Materials and methods

2.1. Animals and experimental design

The experiment was conducted at the farm of the Agricultural University of Tirana, Faculty of Veterinary Medicine, Albania. The experimental protocol was in accordance with the local regulations for Animal Experimentation of the Agricultural University of Albania. All efforts were made to minimize animal distress and to use only the number of animals necessary to produce reliable results.

Multiparous non-lactating New Zealand White rabbits (n = 25) were individually housed in controlled environmental conditions where the temperature ranged from +15 to +28 °C, with the relative humidity being from 60% to 75%, and the lighting schedule 16 L:8 D. Rabbits were provided 130 g/d of commercial food and water *ad libitum*. The composition of the diet supplied to the does is described in Table 1. All does completely consumed their daily rations.

The does were submitted to artificial insemination (AI) performed with a heterospermic pool of fresh semen (0.6 ml) diluted 1:5 in a commercial extender. Before AI, ovulation was induced by injection of 10 μg of synthetic gonadotropin-releasing hormone (GnRH; Receptal, Hoechst-Roussel Vet, Milan, Italy) (Brecchia et al., 2014). The day of AI was designated as Day 0. Pregnancy diagnoses were performed using the abdominal palpation approach 12 day after AI classifying the rabbits as pregnant (P group; n = 15) or pseudopregnant (PP group; n = 10). At AI and subsequently on Days 7, 14, 21, 28, and 32 all does were subjected to blood sampling. The samples were collected from the marginal ear vein into tubes containing EDTA, and immediately centrifuged at 3000 x g for 15 min; furthermore, plasma was stored frozen until assayed for hormones and metabolites. On the same days, the body weight (BW) of each rabbit doe was determined using an electronic scale (Isolad - Vignoli - Forli, Italy). The mean BW of does (± standard deviation, SD) at the time of AI was 3.97 ± 0.36 kg.

The following productivity indices were calculated: fertility (number of parturitions/number of inseminations x 100), prolificacy (total number of born and stillborn kits per doe), perinatal mortality, and pre-weaning mortality. The perinatal period comprised the first 48 h after parturition. The pre-weaning mortality rate was calculated as the percentage of weaned kits/litter subsequent to the perinatal period (Menchetti et al., 2019).

2.2. Hormone and metabolite assays

Plasma leptin, insulin, triiodothyronine (T3), and cortisol concentrations were determined using RIA procedures that have been previously described (Brecchia et al., 2006). Leptin concentrations were quantified using a double antibody RIA utilizing the multi-species leptin kit (Linco Research Inc., St. Charles, MO, USA). The limit of sensitivity was 1.0 ng/ml and intra-

and inter-assay coefficients of variations were 3.4% and 8.7%, respectively. Plasma insulin was quantified using the double antibody/PEG technique using a porcine insulin RIA kit (Linco Research Inc.). The antiserum was guinea pig anti-porcine insulin, while both labelled antigen and standards that were used were purified recombinant human insulin. The limit of sensitivity was 2 μU/ml and intra- and inter-assay coefficients of variations were 6.8% and 9.2%, respectively. Total T3 and T4 were assayed by RIA using the procedure provided by the manufacturer (Immunotech, Prague, Czech Republic). The sensitivity of the assay was 0.26 nmol/l and 10.63 nmol/l for T3 and T4 kit, respectively. The intra- and inter-assay coefficients of variations were 6.3% and 7.7%, respectively for T3; whereas, they were 3.29% and 7.53%, respectively for T4.

Cortisol concentrations were evaluated by RIA, using the CORT kit (Immunotech, Prague, Czech Republic). The limit of sensitivity was 2.5 nM and intra- and inter-assay coefficients of variations were 5.8% and 9.2%, respectively.

The NEFA and glucose concentrations were analysed according to García-García et al. (2011) and Rommers et al. (2006), respectively. The NEFA concentrations were quantified using a two-reaction enzymatic-based colorimetric assay from Wako (NEFA-C, Wako Chemicals GmbH, Neuss, Germany), based on the capacity of NEFA to acylate coenzyme A in the presence of CoA synthetase. Glucose concentrations were quantified using the glucose oxidase method utilising the Glucose Infinity kit from Sigma (Sigma Diagnostic Inc., St. Louis, MO, USA).

2.3. Evaluation of insulin sensitivity

Insulin sensitivity was determined using the Glucose-to-Insulin Ratio and homeostasis model assessment for insulin resistance (HOMA-IR) utilising the following equation: [insulin

concentration × (glucose concentration/18)]/22.5 (Menchetti et al., 2015a, 2018). The relatively lesser HOMA-IR values indicate a relatively greater insulin sensitivity, whereas the relatively greater HOMA-IR values indicate a relatively lesser insulin sensitivity.

2.4. Statistical analysis

The Linear Mixed model was used to analyze BW, hormone and metabolite concentrations. In these models, animals and days after AI were included as subjects and repeated factors, respectively. The models evaluated the main effects of time (six levels: 0, 7, 14, 21, 28, and 32 days after AI), physiological state (two levels: P and PP groups), and the interaction. Sidak adjustment was used for conducting multiple comparisons. Furthermore, for P group, Linear Mixed models were developed including time as factor and numbers of total born as covariate to assess whether there was an association between values for hormone concentrations and metabolites during pregnancy and with litter size.

Diagnostic graphics were used to evaluate assumptions and outliers, and the Log (insulin and T4 concentrations) or Log(x+1) (T3/T4 ratio and HOMA index) transformations were used (Barbato et al., 2017). Results are expressed as estimated marginal means ± standard error (SE) or back-transformed estimated marginal means ± SE, while raw data were depicted in figures. The effect of the total number of kits born on hormone and metabolite concentrations was reported as an estimated *b*-parameter and the associated standard error.

Statistical analyses were performed using the SPSS Statistics version 23 (IBM, SPSS Inc., Chicago, IL, USA). There were considered to be differences in mean values for variables with there was a $P \leq 0.05$.

3. Results

3.1. Production variables

Fertility rate was 60%, prolificacy was 7.4±2.6 (mean ± SD), and the number of weaned kits was 5.9 ± 2.6 (mean ± SD). Perinatal and preweaning mortality was 11% and 13%, respectively.

3.2. Effect of time and physiological state on body weight

The BW of P does gradually increased from day 7 to 28 post AI and was greater than PP does from day 14 post AI until the last day of observation ($P < 0.05$; Fig. 1SM).

3.3. Effect of time and physiological state on concentrations of hormones and metabolites

There was an effect of physiological state on leptin concentrations ($P < 0.05$). In P does, leptin concentrations were greater than in PP does at days 14 and 21 post AI (mean difference: -0.6 ± 0.3 ng/mL; $P < 0.05$; Fig. 1).

There were differences in the log-insulin concentrations due to the physiological state ($P < 0.01$): the P does had greater insulin concentrations than PP does at Days 7 ($P = 0.05$), 21 ($P < 0.01$), and 32 ($P < 0.05$) post AI (Fig. 2).

The T3 concentration was not affected by any factor although results from pairwise comparisons indicated there was a greater T3 concentration in P than PP does at day 7 post AI ($P =0.05$; Fig. 3). Estimates of marginal mean of log T4 concentrations were greater in PP (38.11 ± 1.75 nmol/l) than P (28.31 ± 1.30 nmol/l; $P < 0.05$) does; however, when the two groups were compared on each day of the study, the means were not different (Fig. 4). In pregnant does, however, T4 values increased during the postpartum period compared to day 28 of pregnancy ($P < 0.05$). The large amount of variability of T4 concentrations could explain these inconsistent

results. Furthermore, the estimated marginal mean of T3/T4 ratio of P does (0.06 ± 0.01) was greater than that of PP does (0.04 ± 0.01; $P < 0.05$), and in particular, there were differences at days 7 and 28 post AI ($P < 0.05$) although there was a large amount of variation in values for P does on day 28 (Fig. 5).

Cortisol concentrations were affected by time ($P < 0.001$), group ($P < 0.001$), and the respective interaction ($P < 0.001$). Estimated marginal means were greater in P does (3.87 ± 0.09 nmol/l) than PP does (3.32 ± 0.12 nmol/l; $P < 0.001$); however, values for pairwise comparisons were different between P and PP does only on days 28 ($P < 0.001$) and 32 ($P < 0.01$) post AI (Fig. 6).

Glucose concentrations were affected by time ($P < 0.05$), there being greater concentrations at day 7 post AI compared with the day of AI (estimated marginal means: 6.7 ± 0.2 and 7.8 ± 0.3 mmol/l at day 0 and 7 post AI, respectively; $P < 0.05$). There, however, was no effect of group (estimated marginal means: 7.3 ± 0.2 mmol/l and 7.2 ± 0.1 mmol/l for PP and P does, respectively; $P > 0.1$; Fig. 7).

The NEFA concentrations were affected by time ($P < 0.001$), group ($P < 0.001$), and the respective interaction ($P < 0.01$). There were no significant changes in NEFA concentration in the PP does during the study ($P > 0.1$), while in P does the NEFA concentrations increased progressively until day 21 post AI ($P < 0.001$). The NEFA concentrations were greater in P than PP does after day 7 post AI ($P < 0.05$) until the last observation ($P < 0.001$; Fig 8).

3.4. Insulin sensitivity

The estimated mean of the HOMA was greater in P (0.11±0.01) than PP does (0.08 ± 0.01; $P < 0.01$). The mean of the HOMA of P does was greater on day 7 compared with the day of AI

($P = 0.05$), and results from pairwise comparisons indicated there were differences between groups at days 7 and 21 post AI ($P < 0.1$; Table 2).

The glucose-to-insulin ratio was affected by time ($P < 0.01$), group ($P < 0.001$), and group × time interaction ($P < 0.01$). In particular, this ratio was greater in P than PP does at days 21 and 32 post AI ($P < 0.01$; Table 2).

3.5. Effect of litter size on hormones and metabolites of pregnant does

The results from models used to evaluate the effect of the total number of kits born on hormones and metabolites indicated there was a trend toward a positive association only with the glucose concentrations ($b = 0.13 \pm 0.08$; $P = 0.092$).

4. Discussion

Pregnancy induces adaptive changes in hormonal and metabolite secretions to result in both the energy requirements for the growth of the foetuses and the storage of body energy reserves for lactation (Fortun-Lamothe, 2006; Ladyman et al., 2010; Menchetti et al., 2015b).

Leptin and insulin are the hormones that have marked functions in maintaining energy homeostasis and for which there are important changes after artificial insemination; these hormones also have important functions in pathological disorders. Plasma leptin concentrations are greater in P compared with PP does, particularly in the second and third week of pregnancy (about +20%). There is also this leptin pattern in women, rodents, cows, and bitches (Kawai et al., 1997; Block et al., 2001; Reitman et al., 2001; Cardinali et al., 2017; Troisi et al., 2020); however, both the biological and physiological functions of leptin appear to be species-specific during pregnancy. The increase observed in the P does appears to be lesser than in other species.

Maternal leptin concentrations increase two to three fold during pregnancy in women and rats (Kawai et al., 1997; Reitman et al., 2001), while there is an increase of six or more times in mice (Gavrilova et al., 1997; Gustafson et al., 2019). In women, compared to the adipose tissue leptin production (Reitman et al., 2001; Pérez-Pérez et al., 2018), placental leptin production contributes to pregnancy hyperleptinemia; on the contrary in rats and mice, the placenta is not a major source of leptin (Kawai et al., 1997; Gustafson et al., 2019). Troisi et al. (2020) have recently reported that leptin concentrations increase proportionally with the number of puppies that are *in utero* in bitches, suggesting a contribution of the feto-placental units to the maternal leptin. The contribution of the placenta and the adipose tissue to circulating leptin concentrations in rabbit does remains to be further investigated, because in the present study, there was no association between the number of rabbit kits born and leptin concentration.

In P does in the present study, the increase in leptin concentrations was not regulated as a result of a negative feedback loop that should, when activated, reduce food intake and body weight. This finding of this dysregulation of leptin functions is typical of the leptin resistance condition. In non-pregnant animals including humans, leptin resistance is generally considered a pathological condition associated with obesity (Ladyman et al., 2010). During pregnancy, however, leptin resistance represents an important adaptation for generating a positive energy balance to meet the increased maternal requirements and to prepare for the subsequent demands of lactation (Ladyman et al., 2010; Menchetti et al., 2015a; Cardinali et al., 2017). Gustafson et al. (2019) have reported that the suppression of leptin transport into the brain contributes to leptin insensitivity in the central nervous system although the specific cause of leptin resistance is still unknown. It is likely the hormonal milieu which characterises pregnancy, such as

increasing progesterone, placental lactogen, prolactin, and decreasing oestrogens, contribute to leptin resistance and hyperphagia (Ladyman et al., 2010; Gustafson et al., 2019).

In the present study, differences in leptin concentrations between the two physiological states occurred during the second half of pregnancy when progesterone concentrations in PP does decrease, whereas these concentrations remain relatively greater in P does. For this reason, a contribution or an interaction between leptin and progesterone can be hypothesised. The experimental protocol used in the present study does not allow for further investigation of leptin resistance because the does were fed a daily ration of consistent content, and progesterone concentrations were not monitored. Because there was a consistent ration fed throughout pregnancy in the present study, this allows for evaluation of the effect of the physiological condition on the endocrine-metabolic changes without the confounding effect of the food intake.

Leptin could also have functions in the metabolic allocation of nutrients during the transition from pregnancy to lactation, when the energetic priority shifts from the building of energetic stores within the body to the release of energy from these stores (Block et al., 2001). Results of the present study indicated leptin concentrations return to concentrations similar to those of PP does during late pregnancy and after birth which also occurs in women, bitches, and cows but not in ewes (Block et al., 2001; McFadin et al., 2002; Pérez-Pérez et al., 2018; Troisi et al., 2020). The lesser leptin concentration in the prepartum period could be mediated by changes of the other hormones and metabolites and/or could be due to the energy deficit that is prevalent during the last days of pregnancy of rabbit does (Block et al., 2001; Menchetti et al., 2015a; Cardinali et al., 2017).

Similar to leptin, the relatively greater concentrations of insulin and HOMA indicate the insulin resistance in P does. These results are consistent with those of previous studies in rabbits

293 (Menchetti et al., 2015a, 2018) and bitches (Cardinali et al., 2017) as well as women (Ciampelli
294 et al., 1998). During pregnancy, insulin resistance is believed to be caused by relatively greater
295 concentrations of leptin, oestrogens, progesterone, placental hormones, cortisol, prolactin, and
296 tumor necrosis factor α (Ladyman et al., 2010; Sonagra, 2014). These hormones and factors
297 decrease insulin sensitivity in the hypothalamus as well as in peripheral tissues such as adipose
298 tissue and skeletal muscle by interfering with insulin receptor signalling (Ladyman et al., 2010;
299 Newbern and Freemark, 2011). In the present study, similar to leptin, insulin concentrations were
300 greater in the second half of pregnancy, when the profile of the sex hormones between P and PP
301 does differs. During late gestation, both insulin resistance and insulin concentrations decreased
302 which is probably due to an increased glucose transfer from the maternal tissues to the foetuses;
303 thus, even in this case, an interaction between insulin and progesterone can be hypothesised.
304 During normal pregnancy, the thyroid is hyper-stimulated, resulting in changes in plasma
305 thyroid hormone concentrations. In the present study, there was an increase in the T3/T4 ratio
306 during the first part of the gestation period, although there was considerable variation in the
307 concentrations. The increase of thyroid hormones concentrations which also occurs throughout
308 gestation in women is due both to an increase of the synthesis of these hormones and to a
309 reduction of hepatic clearance of T4-binding globulin (Soldin, 2006). These changes are
310 mediated by maternal oestrogen and facilitate the transfer of iodine to the foetus (Fisher, 1996).
311 Thus, in addition to the maintenance of energy homeostasis, the increase of the T3/T4 ratio in P
312 does could be related to the neural development of the foetus (Lazarus, 2011; Micke et al., 2015).
313 In the present study, there was an increase of cortisol concentrations during the last period of
314 pregnancy compared to the PP does, and similar results were also reported in several other
315 species including women (Brunton et al., 2008). Cortisol, as well as other glucocorticoids, is

316 essential for the development of foetal organs and the onset of parturition (Soma-Pillay et al.,
317 2016). Interestingly during pregnancy, the activation of the hypothalamic-pituitary-adrenal axis
318 (HPA) seems to be reduced to protect the foetus from excess glucocorticoids that may induce
319 deleterious effects on infant neurodevelopment and the adult cardiovascular and metabolic
320 systems (Brunton et al., 2008).
321 Results of the present study indicate the circulating concentrations of NEFA increased in P
322 as compared with PP does. This increase of plasma concentration of NEFA in P does indicates
323 the mobilization of energy reserves during mid- and late-pregnancy (Fortun-Lamothe, 2006),
324 which is consistent with the leptin reduction and insulin resistance. During both pregnancy and
325 lactation, NEFA concentrations increase because the energy intake is not sufficient to meet the
326 energy requirements, and as a consequence, the animal begins to mobilize body lipid reserves
327 (Brecchia et al., 2006; Fortun-Lamothe, 2006; Menchetti et al., 2015a). Glucose needs of the
328 foetuses increases leading to an imbalance between the maternal capacity to absorb/synthesize
329 glucose and the foetal utilization of energy during the latter days of gestation in rabbit does
330 (Rebollar et al., 2011; Menchetti et al., 2015a). This response is a result of the large increase in
331 rate of foetal growth which doubles in the latter part of the gestation period; however, results of
332 the present study indicate the energy deficit of does begins in the first half of the gestation
333 period. Furthermore, in the present study, NEFA plasma concentrations remained relatively
334 greater than in PP does for several days after parturition. The present study, however, was
335 conducted with multiparous rabbit does, therefore, it is hypothesised that multiple pregnancies
336 made these does sensitive to energetic stress not only earlier but also for a longer period of time.
337 The multiparous status of rabbit does could have been a limitation of the present study because it
338 could have affected the metabolic and hormonal status (Meikle et al., 2004; López-García et al.,

2013); therefore, to clarify this aspect, a comparison of rabbit does with different parity statuses could be important to enhance understanding of the various hormonal interactions that were addressed in the present study. Furthermore, other studies on pregnant rabbits could be useful because relatively greater NEFA concentrations are associated with the negative energy balance that occurs during pregnancy and pregnancy disorders such as preeclampsia, gestational diabetes mellitus, and the smaller than typical birth weights of foetuses (Villa et al., 2009; Martínez-Paredes et al., 2012).

In the present study, glucose concentrations were not affected by the day of gestation and there were no differences in these concentrations between P and PP does. This result indicates that, in well-fed rabbits, the endocrine mechanisms regulating energy homeostasis have the capacity to regulate concentrations of this important metabolite (i.e., glucose) during pregnancy (Menchetti et al., 2015a). In the present study, however, there was a trend toward the increase in circulating glucose concentrations being associated with the number of foetuses; therefore, this confirms that there is a marked foetal demand for glucose and the energy needs of does that are proportional to litter size of the doe. If nutritional requirements are not met, especially during mid- and late-pregnancy when the foetuses need considerable amounts of maternal glucose, the homeostatic regulation of glucose metabolism may not be sufficient for energetic needs of the doe and the foetuses (Fortun-Lamothe, 2006; Menchetti et al., 2015a).

5. Conclusions

Metabolic adaptations during pregnancy involve coordinated changes of metabolic hormones together with carbohydrate and lipid metabolism which preserve maternal homeostasis and allow the transfer of energy to the foetus to optimally support foetal growth and

development. The physiological adaptations of maternal metabolism not only prepare the body for the large demands of foetal growth but also support lactation after parturition. These adaptations could be mediated by gonadal steroids because changes mainly occur when the profile of the sex hormones differs between P and PP does. In the present study, the hormonal and metabolic profiles during rabbit pregnancy indicates there are several analogies with those of women and other animal species, such as hyperinsulinemia and hyperleptinemia. In future studies, it would be important to determine if leptin resistance, leptin placental production and if there are interactions with reproductive hormones in the rabbit during pregnancy.

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Conflicts of Interest

The authors declare no conflict of interest.

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Declarations of interest: none

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Table 1

Formulation and chemical composition of diet

	Unit	C
Ingredients		
Dehydrated alfalfa meal	g/kg	300
Soybean meal 44%	“	150
Barley meal	“	410
Wheat bran	“	52
Soybean oil	“	30
Beet molasses	“	20
Calcium carbonate	“	7
Calcium diphosphate	“	13.5
Salt	“	7
DL-methionine	“	0.5
Vitamin-mineral premix†	“	10
Analytical data		
Crude protein	g/kg	175
Ether extract	“	480
Crude Fiber	“	124
Ash	“	89
Digestible Energy§	MJ/kg f.m.	10.6

. †Per kg diet: vitamin A 11.000 IU; vitamin D3 2000 IU; vitamin B1 2.5 mg; vitamin B2 4 mg; vitamin B6 1.25 mg; vitamin B12 0.01 mg; alpha-tocopheryl acetate 200 mg; biotine 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg

§Maertens et al., 1988

Table 2

Variables indicating insulin sensitivity in pregnant (P; *n* = 15) and pseudo-pregnant (PP; *n* = 10) rabbit does after the artificial insemination (AI) and *P*-values for the effect of Time (days after AI), Physiological state (PP compared with P), and interaction between Time and Physiological state; Values are means ± standard errors

Variable	Day post AI	Group		*P value*		
		PP	P	Time	Physiological state	Time x physiological state
HOMA	0	0.11^{a} ±0.01	0.11^{a} ±0.02	0.099	0.002	0.139
	7	0.10^{a}±0.01	0.24^{b}±0.06			
	14	0.08^{a} ±0.02	0.12^{a} ±0.03			
	21	0.07^{a}±0.01	0.14^{b}±0.03			
	28	0.10^{a} ±0.04	0.11^{a} ±0.02			
	32	0.05^{a} ±0.00	0.12^{a} ±0.02			
Glucose to insulin ratio	0	1.02^{a} ±0.08	1.26^{a}±0.27	0.005	<0.001	0.002
	7	1.49^{a} ±0.14	0.90^{a}±0.14			
	14	1.50^{a} ±0.11	1.26^{a}±0.16			
	21	2.15^{a} ±0.21	0.97^{b}±0.10			
	28	1.73^{a} ±0.62	1.25^{a}±0.21			
	32	2.59^{a} ±0.09	1.37^{b}±0.18			

HOMA was obtained as [insulin concentration × (glucose concentration/18)]/22.5

Data were analyzed after log(x+1) transformation but raw data are presented

Values followed by the same superscript letter in each row do not differ (P≤0.05; multiple comparisons using Sidak correction)

Figure captions

Fig. 1. Leptin concentrations after artificial insemination (AI) in pregnant (P; *n* = 15) and pseudopregnant (PP; *n* = 10) rabbit does; Values are means ± standard errors; Asterisks indicate differences between P and PP does at each time point (**P*<0.05; Sidak correction)

Fig. 2. Insulin concentrations after artificial insemination (AI) in pregnant (P; *n* = 15) and pseudopregnant (PP; *n* = 10) rabbit does; Values are means ± standard errors (raw data); Asterisks indicate differences between P and PP does at each time point (**P*<0.05; ***P*<0.01; log-transformed data and Sidak correction)

Fig. 3. T3 concentrations after artificial insemination (AI) in pregnant (P; *n* = 15) and pseudopregnant (PP; *n* = 10) rabbit does; Values are means ± standard errors; Asterisks indicate differences between P and PP does at each time point (* = 0.05; Sidak correction)

Fig. 4. T4 concentrations after artificial insemination (AI) in pregnant (P; *n* = 15) and pseudopregnant (PP; *n* = 10) rabbit does; Values are means ± standard errors (raw data)

Fig. 5. T3/T4 ratio after artificial insemination (AI) in pregnant (P; *n* = 15) and pseudopregnant (PP; *n* = 10) rabbit does; Values are means ± standard errors (raw data); Asterisks indicate differences between P and PP does at each time (**P*<0.05; log-transformed data and Sidak correction)

Fig. 6. Cortisol concentrations after artificial insemination (AI) in pregnant (P; n = 15) and pseudopregnant (PP; n = 10) rabbit does; Values are means ± standard errors; Asterisks indicate differences between P and PP does at each time point (**P<0.01, ***P<0.001; Sidak correction)

Fig. 7. Glucose concentrations after artificial insemination (AI) in pregnant (P; n = 15) and pseudopregnant (PP; n = 10) rabbit does; Values are means ± standard errors

Fig. 8. NEFA concentrations after artificial insemination (AI) in pregnant (P; n = 15) and pseudopregnant (PP; n = 10) rabbit does; Values are means ± standard errors; Asterisks indicate differences between P and PP does at each time point (*P<0.05, **P<0.01, ***P<0.001; Sidak correction)