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The effect of undernutrition on the establishment of pregnancy in the ewe

J.A. Abecia*, C. Sosa*, F. Forcada* and A. Meikle**

*Grupo de Investigación Biología y Fisiología de la Reproducción,
Instituto de Investigación en Ciencias Ambientales. Universidad de Zaragoza (Spain)
**Laboratorio de Técnicas Nucleares, Facultad de Veterinaria,
Universidad de la República, Montevideo (Uruguay). alf@unizar.es

Abstract. This review will particularly focus on the effects of undernutrition on the potential causes of reproductive failure including abnormalities of the ovum or the embryo, luteal inadequacy and failure of the supply of progesterone to the uterus, or the mechanisms involved in maternal recognition of pregnancy. The level of nutrition and peripheral progesterone concentrations are inversely related, and increased rates of embryo loss, associated with higher progesterone concentrations in ewes with low levels of nutrition have been reported. Undernutrition may act through changes in the distribution of progesterone in the endometrium. Thus, lower endometrial levels on day 5 of the cycle in ewes fed half of their maintenance requirements have been observed, providing a link between the known role of progesterone in embryo survival by the modulation of uterine function and the higher embryo losses found in undernourished ewes. The evidence of an effect of maternal nutrition on IFNτ secretion from the conceptus and of PGF2α production from the uterus is presented. Moreover, undernutrition provokes a reduction in the sensitivity of the endometrium to progesterone that may affect embryo survival. Finally, a state of undernutrition induces changes in the endometrial sensitivity to steroid hormones at early stages of pregnancy that could adversely alter uterine environment to the detriment of embryo survival.

Keywords. Ewes – Pregnancy – Undernutrition – Progesterone – Uterine environment – Oocyte quality – Embryo-maternal signals – IFNτ – PGF2α – Endometrium sensitivity.

Effet de la sous-nutrition sur le déclenchement de la gestation chez la brebis

Résumé. *Cet article examine en particulier les effets de la sous-nutrition sur les causes potentielles de l'échec reproductif, parmi lesquelles les anomalies de l'ovule ou de l'embryon, l'inadaptation lutéale et le manque d'apport de progestérone à l'utérus, ou les mécanismes impliqués dans la reconnaissance maternelle de la gestation. Le niveau de nutrition et les concentrations périphériques de progestérone sont inversement corrélées, et on a observé des taux plus élevés de perte embryonnaire, liés à des concentrations plus fortes de progestérone chez les brebis ayant de faibles niveaux de nutrition. La sous-nutrition peut intervenir par modification de la distribution de progestérone dans l'endomètre. On a ainsi observé des niveaux plus faibles dans l'endomètre au jour 5 du cycle chez des brebis recevant la moitié de leurs besoins d'entretien, ce qui permet de faire le lien entre le rôle connu de la progestérone pour la survie de l'embryon à travers la modulation de la fonction utérine et les pertes embryonnaires accrues chez les brebis sous-alimentées. Les résultats concernant un effet de la nutrition maternelle sur la sécrétion d'IFNτ à partir du conceptus et la production de PGF2α à partir de l'utérus sont présentés. De plus, la sous-nutrition provoque une réduction de la sensibilité de l'endomèter à la progestérone, ce qui peut influencer la survie de l'embryon. Finalement, un état de sous-nutrition modifie la sensibilité de l'endomètre par rapport aux hormones stéroïdes en début de gestation, ce qui pourrait causer un effet adverse sur l'environnement utérin au détriment de la survie embryonnaire.*

Mots-clés. *Brebis – Gestation – Sous-nutrition – Progesterone – Environnement uterine – Qualité de l'oocyte quality – Signaux embrio-maternels – IFNτ – PGF2α – Sensibilité endometriale.*

I – Introduction

The relationship between nutrition and reproduction has been widely reviewed (Rhind, 1992; Robinson, 1996; O'Callaghan and Boland, 1999; Boland *et al.*, 2001; Martin *et al.*, 2004). Whilst early investigations focused on the effects of nutrition on the hypothalamic-pituitary axis, more recent studies have tested the hypothesis that nutritional signals (e.g. metabolic hormones) exert a direct effect at the ovarian level. Less research has been performed on the effect of undernutrition on the uterine environment that plays a major role in determining the success of the embryo in the maintenance of pregnancy. We will focus our attention on the effect of undenutrition on the mechanism of establishment of pregnancy which is essential to the survival of the embryo.

II – Effects on follicular development and oocyte quality

No effect of undernutrition on mean number of oestrogenic follicles (secreting >500 pg oestradiol per follicle per hour) has been observed (Abecia *et al.*, 1995, 1997). Similarly, Rhind and McNeilly (1998) found no treatment difference in the number of large follicles (>2.5 mm). No differences on testosterone and oestradiol follicular secretion in vitro were found in follicles derived from ewes fed 1.5 and 0.5M diets (Abecia *et al.*, 1995, 1997).

Results of studies on the effect of level of food intake on oocyte quality in sheep are contradictory, and most of the work has been done using the superovulated ewe model. Yaakub *et al.* (1997) observed that low dietary intake altered oocyte morphology in both naturally-ovulating and superovulated ewes. In contrast, Boland *et al.* (2001) reported no differences in the morphology of oocytes collected from ewes offered 0.5M compared with 2M diets. However, Lozano *et al.* (2003) reported a lower number of good quality oocytes and embryos per animal treated in superovulated ewes fed an ad libitum diet compared with ewes offered control (1.5M) or low energy (0.5M) diets, concluding that ad libitum diets are highly detrimental for superovulatory programmes. In a study designed to determine if the nutrition of the oocyte donor ewe influenced the success of somatic cell cloning, Merino ewes were fed at either a high- or a low-nutrition level for 3–5 months before superovulation treatments and no differences were detected in the numbers of follicles aspirated, of oocytes recovered, or of oocytes of a quality suitable for cloning (Peura *et al.*, 2003).

III – Effects on luteal function and progesterone supply to the uterus

An inverse relationship between level nutrition and peripheral progesterone concentrations has been observed (Parr *et al.*, 1982; Rhind *et al.*, 1989, Lozano *et al.*, 1998). In vitro secretion of progesterone by corpora lutea from both undernourished and control ewes collected at day 8 (Abecia *et al.*, 1997), 9 (Abecia *et al.*, 1999) and 14 (Abecia *et al.*, 1995) of the oestrous cycle, is similar. Increased rates of embryo loss, associated with higher progesterone concentrations, were reported in undernourished ewes (Brien *et al.*, 1981; Parr *et al.*, 1987). Parr (1992) demonstrated that ewes on high planes of nutrition had lower concentrations of peripheral plasma progesterone because of an increase in the metabolic clearance rate of progesterone, rather than to changes in the secretion rate of the hormone from the corpora lutea. Since ovarian venous progesterone can pass into the ovarian artery, via a counter-current mechanism (Einer-Jensen and McCracken, 1981), and a branch of this artery supplies the oviduct/uterus with blood (Hunter, 1987), the actual progesterone supply to the uterus may be unrelated to the peripheral concentrations of this hormone.

Undernutrition may act through changes in the distribution of progesterone in the endometrium. Abecia *et al.* (1996) observed that pregnant ewes had similar jugular concentrations of progesterone, but higher progesterone concentration in both the ovarian vein and endometrial tissue

than animals experiencing total absence of embryos by day 14 of pregnancy. These results do not prove that the difference in endometrial progesterone content cause pregnancy failure but they are consistent with the hypothesis and suggest that understanding of the effects of nutritional and other treatments on pregnancy and embryonic wastage may be improved by measurement of progesterone concentrations in the ovarian vein/or endometrium.

No differences due to level of nutrition in the delivery of progesterone from the ovary to the endometrium were observed on either day 5 (Lozano *et al.*, 1998), day 8 (Abecia *et al.*, 1997), day 10 or day 15 (Lozano *et al.*, 1998) of pregnancy, although endometrial progesterone levels were lower on day 5 of the cycle in ewes fed half of their maintenance requirements (Lozano *et al.*, 1998). This finding provided a link between the known role of progesterone in embryo survival by the modulation of uterine function and the higher embryo losses found in undernourished ewes.

IV – Effect on embryo quality and development

Abecia *et al.* (1997) observed a delay in development of embryos collected from undernourished ewes 8 days after mating. Significantly less embryos collected on day 15 of pregnancy from restricted ewes reaching the stage of elongated blastocysts were observed by Abecia *et al.* (1999), although no differences in the number of blastocysts were recorded on day 9 of pregnancy. This set of preliminary experiments led to the conclusion that the effects of undernutrition on embryo survival are not necessarily mediated through changes in ovarian function or progesterone delivery to the uterus, but may involve changes in the uterine environment resulting from different patterns of embryo-maternal signals.

V – Effect on uterine gene expression and embryo-mother signalling

In ruminants, establishment and maintenance of pregnancy results from signalling by the conceptus to the maternal system and requires progesterone from the corpus luteum, which is essential for the maintenance of pregnancy. The process through which the regression of the corpus luteum (luteolysis) is blocked in early gestation in ruminants has been termed maternal recognition of pregnancy (Short, 1969; for review see Spencer and Bazer, 2004).

Results presented by Abecia *et al.* (1999) provided the first evidence of an effect of maternal nutrition on IFNτ secretion from the conceptus and of $PGF_{2\alpha}$ production from the uterus. Embryos collected on day 15 of pregnancy from ewes fed 0.5M secreted lower amounts of IFNτ in vitro, and endometrial tissue collected from those ewes secreted higher $PGF_{2\alpha}$ levels than ewes fed 1.5M. This was accompanied by a reduction of embryo survival, as indicated by the ratio of embryos/number of corpora lutea. It was concluded that the lower pregnancy rates observed in underfed ewes could be mediated through altered signals of maternal recognition of pregnancy. It is important to note that before and during the embryo elongation along the uterine horns, the embryo is free-living in the uterine lumen and is completely dependent on uterine secretions for all its metabolic needs. Thus, the limiting factor of early embryonic mortality may be the mother's ability to promote embryo growth (mother-to-embryo signalling). If around the time of maternal recognition of pregnancy in sheep (day 14), the embryo fails to deliver its signal (IFNτ) in an appropriate pattern, luteolysis follows and pregnancy is not maintained.

Because receptor proteins concentrate the specific hormones in the target tissues (Clark *et al.*, 1992), it was hypothesized that the lesser endometrial progesterone content in undernourished ewes was attributable to a reduction in PR expression (Sosa *et al.*, 2004). Thus, undernourished ewes had lesser average intensity of staining for PR (indicating a lesser PR content) in most endometrial cell types on day 5 (Sosa *et al.*, 2004); this may explain the lower endometrial con-

tent of progesterone at day 5 found in these ewes (Lozano *et al.*, 1998). There were no differences in PR immunostaining amongst groups at day 10, an observation that parallels reports of progesterone endometrial content previously found in both groups on this day (Lozano *et al.*, 1998). These results indicate that, in sheep, undernutrition provokes a reduction in the sensitivity of the endometrium to progesterone, i.e. there was a reduction in immunoreactivity of PR and that may affect embryo survival.

Immunoreactivity and mRNA expression of endometrial ER and PR, has been investigated in non pregnant ewes fed to provide either 1.5M or 0.5M and slaughtered at days 5 or 14 of the oestrous cycle (Sosa *et al.*, 2006). On day 5, underfed ewes exhibited reduced uterine ER and PR binding capacities relative to the control group animals. In summary, it is clear that in sheep a state of undernutrition induces changes in the endometrial sensitivity to steroid hormones at early stages of pregnancy (early luteal phase: day 5) that could adversely alter uterine environment to the detriment of embryo survival. This could explain, at least in part, the greater embryo losses and retarded embryo development observed in undernourished animals by other authors (Rhind *et al.*, 1989; Abecia *et al.*, 1997). From our findings (Sosa *et al.*, 2004, 2006) it seems unlikely that changes in endometrial sensitivity to steroids in later stages of gestation (e.g. day 10 or day 14 post-oestrus) contribute significantly to differences in pregnancy rates between undernourished and control ewes.

Undernutrition affects the patterns of gene expression in adipose and hepatic tissues, and the responses differ between pregnant and non-pregnant ewes. In adequately fed ewes, pregnancy up-regulates leptin mRNA expression in adipose tissue, a response that is impaired in underfed ewes (Sosa *et al.*, 2009). The hepatic expression of IGF-I mRNA was increased by pregnancy in underfed animals while no effect was observed in adequately fed ewes. It remains to be determined whether the changes in the endocrine milieu are paralleled by modifications in uterine gene expression that could alter the environment of the embryo during early pregnancy.

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