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Assisted reproductive technologies in the reproductive management of small ruminants☆

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ABSTRACT

In modern agriculture, assisted reproductive technologies are being used for out of season oestrus induction, enhancement of reproductive performance and genetic improvement. In addition, they can have substantial contribution in preservation of endangered species or breeds, as well as in eradication programs of various diseases. While their applications are widespread in cattle, in small ruminants it is almost restricted to artificial insemination. The main limitations of a wider application in small ruminants are the naturally occurring anoestrus period, the variability of response to superovulatory treatments, the fertilisation failure and the need of surgery for collection and transfer of gametes and embryos. Nonetheless, during the last 30 years, considerable progress has been made in sheep and goat embryo technologies, especially in the fields of oestrus synchronisation, superovulation and *in vitro* embryo production. This paper reviews the status of assisted reproductive technologies in sheep, analysing the prospects offered by recent advances in *in vivo* and *in vitro* embryo production from mature and juvenile lambs.

1. Introduction

Sheep and goats are significant contributors in the global food and fibre production. In the continuously growing world population, small ruminants possess an emphatic role, mainly for the economies of developing countries, and in particular, for those with harsh climatic conditions, or subfertile lands. Seasonal breeding of small ruminants has fostered the adaptation of assisted reproductive technologies (ART), to overcome this intrinsic reproductive restriction and to improve genetic gain in these species.

Traditionally, ART includes artificial insemination, as well as *in vivo* and *in vitro* embryo production. *In vivo* embryo production and transfer offer a powerful technology for genetic improvement in sheep and goats, if they were strategically applied in the form of multiple ovulation and embryo transfer (MOET). However, the high cost of the method has restricted its application to the production of superior males that would be subsequently used as semen donors for artificial insemination. *In vitro* embryo production can replace and overcome cost related problems of MOET, however, by offering cheap embryos from superior genetic merit dams and males. Intense genetic improvement can be achieved by the reduction of generation interval. While classical MOET offers a generation interval of approximately 12 months, using pre-pubertal ewe-lambs as oocyte donors for *in vitro* embryo production, generation interval can be substantially reduced by 5–6 months; this shortened generation interval can theoretically double the genetic gain in the flock/herd. An additional improvement can be achieved if sexed semen is used for production of offspring of pre-determined gender. Although most – if not all – ART have been developed

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and applied in sheep, the commercial uptake of these technologies is less than that with cattle. According to the international embryo transfer society in 2009, the total number of transferred embryos – produced *in vitro* or *in vivo* – for cattle and small ruminant species were 842,376 and 2086, respectively.

Objective of this paper is to describe the improvements and the modification of the traditional ART that could be applied either to support programmes for genetic improvement in flocks/herds with small ruminants or to produce low cost embryos for research purposes.

2. *In vivo* embryo production

2.1. Oestrus control

In sheep, ovarian follicular development occurs in a wave like pattern that is not interrupted during the seasonal anoestrous period or during pregnancy, with the three wave pattern being the most prominent during oestrus (Noel et al., 1993; Souza et al., 1996; Ravindra and Rawlings, 1997; Bartlewski et al., 1998). A transient increase in FSH peripheral concentration stimulates the emergence of a synchronous growth of a cohort of small follicles and, depending on intrinsic factors (breed), one or more follicles acquire dominance and grow to a diameter of >5 mm, preventing further development of the smaller follicles (Ravindra et al., 1994; Seekallu et al., 2010). Ovulation occurs from dominant follicles or these follicles regress and a new follicular wave commences. Smaller follicles (subordinates), though becoming gradually atretic, still retain their capability to respond to exogenous gonadotrophic stimuli and to resume growth.

The most common protocol for oestrous synchronisation in sheep/goats is based on progestagen/progesterone treatment in the form of intravaginal implants (sponges/CIDR) (Abecia et al., 2011). This hormonal manipulation that can be used during the breeding and the seasonal anoestrous period, induces a great negative feedback on LH secretion and, in some instances, may cause spontaneous luteolysis, while after withdrawal of the pre-ovulatory LH surge, ovulation occurs in an almost controlled manner. Treatment with progestagens during the luteal phase accelerates follicular development, but, at the same time, it reduced the number of large follicles, increased follicular atresia rate and supported the persistence of large oestrogenic follicles; treatment during the follicular phase reduced both the number of large follicles and ovulation rate (Noel et al., 1994; Leyva et al., 1998). These observations are likely attributed to the follicular status at the time of progestagen treatment or to the fact that progestagen concentrations gradually decrease after the second day of treatment, which alters the physiologic mode of LH secretion (Robinson, 1965; Scaramuzzi et al., 1988). To overcome this insufficient progestational support, the use of two consecutive sponges inserted in a 7-day interval has been proposed in conjunction with a luteolytic dose of PGF2α at the time of second sponge removal (Gonzalez-Bulnes et al., 2004). Without a clear physiological reasoning, the duration of progesterone treatment traditionally lasts for 11–14 days. Short-term exposure to progesterone can sustain acceptable synchronisation rates and superovulatory response (Menchaca et al., 2009; Martemucci and D'Alessandro, 2011). Beyond the possible alteration in endocrine status, use of progestagens has also increased consumers' concern on the safety of products from treated animals (Galbraith, 2002).

Prostaglandin F2α is the most potent luteolytic agent for small ruminants and it can be used during the breeding season as an alternative to progestagens for oestrous synchronisation. The responsiveness of the corpus luteum to PGF2α is limited between days 3 and 14 of the oestrous cycle and, consequently, the stage of the oestrous cycle when PGF2α is administered, affects timing of preovulatory LH surge and subsequent ovulation (McCracken et al., 1972; Acritopoulou and Haresign, 1980). Acceptable embryo production can be achieved in previously superovulated sheep by a single cloprostenol injection administered during the mid-luteal phase of the oestrous cycle (Mayorga et al., 2011).

During the breeding season, combined administration of a GnRH analogue and PGF2α resulted in desirable oestrous synchronisation rates, as exemplified by the acceptable conception rate (50%) subsequently to fixed time insemination. The protocol consists of two GnRH injections given 7 days apart, while PGF2α is administered on the fifth day. Fixed time laparoscopic intrauterine insemination is performed 12–14 h after the second GnRH injection (Amiridis et al., 2005; Deligiannis et al., 2005). Therefore, this protocol can substitute the use of inserts and allow the accurate prediction of the stage of the oestrous cycle at which subsequent superovulatory treatment should commence.

2.2. Superovulation of the donor animal

Unpredictable variability in the superovulatory response is the most critical step in sheep embryo production programs. This is attributed to a number of endogenous (genetics, nutritional status, follicular status, season of the year) and exogenous (superovulatory treatment, nature and possible 'contamination' of the gonadotrophin) factors. However, the contribution of each factor is almost impossible to assess (Baril et al., 1993; Cognie, 1999; Gonzalez-Bulnes et al., 2004).

The gonadotrophins that have been used for superovulation, include equine chorionic gonadotrophin (eCG), porcine or ovine follicle stimulating hormone (FSH) and human menopausal gonadotrophin (hMG) (Loi et al., 1998). Though the superovulatory response of hMG could be similar to that obtained by eCG, its use was never very extensive, due to its serious disadvantage to induce premature luteal regression (Schiewe et al., 1990).

Since the 1960s, eCG has been used for sheep superovulation alone or in combination with FSH (Newton and Betts, 1968). Early studies have shown that the superovulatory response using eCG is less in comparison to FSH, while time of ovulations is not well synchronised (Walker et al., 1986, 1987; Ryan et al., 1991).

At the ovarian follicle, compartmental pattern of development and steroidogenesis was grossly perturbed in