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Prolificacy in small ruminants

Abstract

Prolificacy is the ability to reproduce abundantly. Litter size, which is highly dependent on ovulation rate, has a high economic value and it is a fundamental reproductive trait, especially in small ruminants. Considering the low heritability of prolificacy, it is possible to improve the progress of the prolificacy trait selection using DNA markers and molecular biology techniques. In this article, an overview of the various factors affecting prolificacy in small ruminants has been emphasized.

Keywords: goat, growth factors, hormones, prolificacy factors, sheep

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Bhuvana Plakkot,[1] Abhina Mohanan,[1] Raji Kanakkaparambil[2]

[1]Former MVSc Scholars, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, India

[2]Assistant Professor, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, India

Correspondence: Bhuvana Plakkot, Former MVSc Scholar, Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala-680651, India, Tel +919940618070, Email bhuvanapakkot@yahoo.com

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Introduction

The production or reproduction in animals mainly depends on the number of young ones born per litter and the number marketed each year. When compared to large ruminants, sheep and goats, which are considered as small ruminants, can produce more than one offspring per pregnancy. Normally, the lambing/kidding interval is around 8 months and they give birth at least three times in 2 years. The gestation period of sheep and goats are around five months (sheep for 146 days and goats for 150 days). There is variation in reproduction between the two species. However, the variation between the various strains of sheep and goats are more when compared between the species. Hence, here we attempted to explore the various factors affecting the prolificacy in small ruminants (sheep and goat). Prolificacy is closely associated with follicular development and ovulation rate. Ovulation rate and litter size are crucial reproduction traits with high economic value.[1] It is controlled by various factors like Single gene mutations, age, season and environmental change, nutrition, hormonal factors, fertilization rate, embryonic and foetal development. Out of these, some of the factors are dealt with in detail here.

a. Hormonal factors
b. Genetic factors
c. Seasonal and Environmental factors
d. Nutrition

Hormonal factors

By manipulation of specific hormonal inputs, follicle recruitment and development that results in ovulation can be increased.[2] Even though, in many studies, the plasma concentrations of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) between sheep and goat breeds differing inprolificacydid not significantly vary[3–13] in one of our recent studies, there was a significant difference observed in the pituitary *LHβ* expression between low prolific Attappady Black and high prolific Malabari goat breeds, with a significantly (P<0.01) high level in Attappady Black.[14] Also, the expression of *Follicle Stimulating Hormone Receptor* (*FSHR)* and *Luteinizing Hormone/ Choriogonadotropin Receptors (LHCGR)*during the periovulatory period and rate of ovulation was observed to be ambiguous in many studies.[3,9,10,15,16] Ovulation rate can be increased in numerous ways, such asby lowering progesterone, injection of gonadotropins-FSH followed by LH, Equine Chorionic Gonadotropin (eCG)- FSH like, Human Chorionic Gonadotropin (hCG)- LH like, Combination of eCG and hCG (P.G. 600), sequential treatments with gonadotropin-releasing hormone. In the process of follicular development, hormones play a significant role in morphological, physiological changes to the follicles. The experiment conducted for oestrus synchronization in Alpine and Saanen goats gave evidence that the treatments with too high progestagen level can decrease fertility but no effect in prolificacy. The fertility tended to be low in goats treated with a whole implant and was significantly lowered in goats, which received a half-implant of norgestomet.[17] Oestrus synchronization and fertility after oestrus synchronization were studied in multiparous Mashona goat and found out that all the 4 treatments with intravaginalprogesterone (P4) sponges, norgestomet ear implants, cloprostenol or a combination of P4 sponges and cloprostenol were effective in synchronizing oestrus and none of the methods affected overall fertility of the does.[18] The studies were conducted using Chronogest CR sponge, which allowed a reduction of the progestagen load from 45 to 20mg with high fertility and prolificacy without detrimental effects on synchronization, fertility, and prolificacy.[19] The insemination effect on does in natural andcloprostenol-synchronized oestrus with frozen semen were studied in Egypt during breeding season of Damascus goats, and it was found that the kidding rates did not vary significantly among does in natural (55.26%) and synchronized (53.85%) oestrus and a higher (P < 0.05) prolificacy was obtained after their insemination in natural (1.81+/-0.16) rather than in synchronized (1.22+/-0.11) oestrus.[20] However, according to Panicker et al.[21] using the progestagen (TRIU-C®) synchronization protocol, the prolific Malabari crossbred goats showed a higher conception rate. Also, a correlation was observed between the oestrus intensity and the conception rate in goats. In another study by Panicker et al.[22] it was observed that the serum progesterone concentrations in prolific Malabari cross-bred goats on the day of insemination showed significant difference (P<0.05) between the goats those conceived and the ones that failed to conceive, with a significantly lower progesterone level in the conceived group.

The immune reaction to eCG negatively influenced the percentage of ovulating females as well as kidding rate but showed no effect of antibodies on prolificacy in the studies conducted in alpine goats.[23] Prolificacy potential in goats was examined by recording the response

to Gonadotropin-Releasing Hormone (GnRH) challenge test and also suggested the single blood sampling at day 63 prior to parturition as the most suitable time for discriminating kidding size using plasma progesterone as a marker.[24] The studies conducted with the hypothesis that increased Insulin-like Growth Factor- 1 (IGF-1) concentrations during preovulatory follicular and early embryonic development will improve prolificacy and lambing rate in sheep is a practical tool for the improvement of prolificacy in sheep.[25] It also concluded that a single dose of bovine Somatotropin, 5 days before progestin withdrawal, increases the lambing rate.

Gonadotropins in supra-physiological quantities resulted in different outcomes on the rate of ovulation and also the oocyte quality.[26] The adjustment of threshold levels of gonadotropins defines differentiation between mono-ovulatory and poly-ovulatory species, and thereby, these hormones play a significant role in prolificacy. *Follicle Stimulating Hormone Receptor (FSHR)and Luteinizing Hormone Receptor* (*LHR)* expression induction and modulation of responsiveness to Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are observed to be influenced by numerous intra-ovarian growth regulators.[27–29] Growth Differentiation Factor 9 (GDF9), Bone Morphogenetic Proteins (BMPs), inhibins, Anti-Mullerian Hormone (AMH), activins and activin/BMP binding proteins have an in vivo effect directly or indirectly.[30–33] The granulosa cells at an early stage acquire FSHR and LHR and mediate dominant follicle development in the declining phase of FSH levels during the follicular phase of the oestrus cycle.[33,34] The selection of follicles occurs as a result of the reduction in pituitary *FSH* and only those follicles with the newly attained LHR can retain oestrogen production during the pre-ovulatory phase.[36] The expression of *FSHR, LHR* and *aromatase* and synthesis of inhibin subunits (PA) in granulosa cells can be induced by FSH Jia et al.[37] Adams et al.[4] observed that plasma concentrations of FSH during the pre-ovulatory period were significantly elevated in the ewes of low-prolific Galway breed. The concentration of LH at the height of surge was significantly reduced in the prolific Finnish Landrace line compared to the low prolific Galway breed. There was no observable variation in plasma concentration of FSH and LH[10,38–40] between breeds that differed in ovulation rates even if slightly greater plasma concentrations[8,11,41,42] and expression of *FSH* mRNA Abdennebi et al.[3] had been observed in few prolific breeds. Similar to previous study in Boer goats which are prolific compared with low-prolific Yunling black goats Cui et al.,[9] Zi et al.[16] reported that *FSHβ* and *LHβ* mRNA expression levels were significantly greater ($p<0.01$) in pituitary of highly prolific Lezhi Black (LB) goats compared to low-prolific Tibetan (TB) goats. Their data provided evidence that a greater gonadotropin expression during the follicular phase was responsible for a higher ovulation rate in the LB goat compared to the TB goat.

It was presumed that at an earlier size of the follicle, the follicles became more sensitive to FSH and this may be the reason for the increase in ovulation rate.[43,44] In Small Tail Han sheep and Booroola sheep, an elevated FSH and LH concentrations in plasma was observed.[45,46] Variation in *FSHR* and *LHR* mRNA levels could determine the response of follicles to gonadotropins and this might induce varied ovulation response and ovulation in mature follicles.[47] The follicles with a greater gonadotropin receptor density are considered to be more responsive to the gonadotropins and continue to increase in their size during a natural cycle.[34,48,49] Various reports suggest that prolific sheep[50,51] and goats[9] have greater *FSHR* expression in ovaries and the *FSHR* mRNA levels in growing follicular cells is higher in prolific breeds than in low-prolific breeds,[3,9] indicating that a higher gonadotropin responsiveness during the early follicular phase may be responsible for a higher ovulation rate in these breeds. However, Zi et al.[16] reported that *LHR* and *FSHR* mRNA expression levels in the follicle of low-prolific TB goat were 7.3-fold and 5.1-fold ($p<0.05$) greater than those in high-prolific LB goats respectively. The reason for this variation is still unknown.

In granulosa cells of small and large follicles of Romanov (ROM) sheep (multi-ovulatory species), the number of FSHR was higher than those in follicles of Ile-de-France (IF) sheep (mono-ovulatory). In theca cells and granulosa cells of small follicles, *LHR* mRNA levels were also greater in ROM than in IF ewes, which increased with an increase in the size of the follicles.[3] Downregulation of *FSHR* expression in granulosa cells was observed at the cyclic dominant follicle selection stage, happening between ~8-10 mm in the human and ~1-1.7 mm in the Merino sheep.[15,52] In human and animal models, down regulation of *FSHR* and *LHR* expression was observed at the time of follicle maturation which was associated with a change from oestrogen to progesterone production in the ovulatory follicles and a decrease in proliferation.[15,52–54] The FSHR and LHR density were elevated significantly in the developing antral follicle granulosa cells of prolific sheep breed compared to the non-prolific sheep breed. The pre-ovulatory follicle from both breeds had low receptor density compared to the subordinate follicles, which indicates a necessary prerequisite down-regulation prior to ovulation.[15]

Significantly fewer granulosa cells were observed in Booroola sheep follicles than the normal wild-type.[43,55] Greater cAMP, oestrogen, and androstenedione were produced from the large antral follicle with the same number of cells when the granulosa cells were stimulated in vitro by FSH or LH.[55] Here, an increased cellular capacity plays a major role to synthesize oestrogen compensating for the smaller number of granulosa cells. Hence, due to the attenuated BMPR1B signal, multiple follicles are produced by Booroola sheep because of greater density FSHR and LHR.[15] Expression of *FSHR* and *LHR* on mature surface granulosa cells was significantly increased in the Booroola compared to the young wild-type Merino sheep.[15] The plasma LH, P4, and E2 concentrations in the prolific breed was lower than the nonprolific breed, whereas mRNA expression levels in ovaries of these genes did not vary between the two breeds. Also, variations in the amino acid sequences of *FSHB, LHCGR* and *Beta-1, 4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2)*were observed.[56]

Driancourt et al.[57] suggested that oestradiol production is determined by the number of granulosa cells and follicle size. However, they observed higher oestradiol output per granulosa cell in prolific ewes compared to low-prolific ones. Abhina[58] observed that the serum oestradiol concentration (blood collected in follicular phase) was significantly higher in prolific Malabari breed when compared to the Attappady Black breed even though granulosa cell thickness was lesser in secondary and antral follicles of Malabari breed. Also, in prolific crossbred Malabari goats, the oestradiol level in the follicular fluid increased significantly as the size of the follicle increased.[59] The concentration of FSH and oestradiol in the serum were higher in prolific Finn ewes than non-prolific western white-faced ewes.[5] Ruoss et al.[60] using Radioimmunoassay (RIA) estimated serum progesterone. The concentration of progesterone between older (around five years) Merino and Booroola ewes showed a significant difference and it was higher in Booroola ewes. However, this pattern of steroidogenesis was not observed in the early ages in this breed.

Genetic factors

In order to improve reproductive efficiency, marker-assisted selection (MAS) and molecular genetics have great importance.[61]

Studies in sheep revealed the presence of three major fecundity genes namely *Bone Morphogenetic Protein Receptor type- 1B (BMPR1B)* also known as *FecB* on chromosome 6,[62] *Bone Morphogenetic Protein 15 (BMP15)* known as *FecX* on chromosome and *Growth Differentiation Factor-9 (GDF9)* known as *FecG* on chromosome 5.[63] These genes are found to be involved in controlling fertility in sheep. The findings from the mutation studies in Inverdale sheep established that *BMP15* is essential for female fertility and the natural mutations in an ovary-derived factor can cause both increased ovulation rate in heterozygotes and infertility in homozygotes.[64] A naturally occurring point mutation on the *BMPR1B* gene in Booroola Merino sheep resulted in an increased ovulation rate.[47,62,66,65] Polymorphism studies of fecundity genes were performed in Indian prolific Black Bengal goat in which *BMPR1B* was found to be polymorphic and influencing prolificacy in this breed.[67] Pramod et al.[68] have studied differential ovarian morphometry and expression of prolificacy genes in relation to prolificacy in Black Bengal and Sirohi goats. Reduced concentrations of active *BMP15* and *GDF9* in the antral follicles decrease granulosa cell proliferation leading to a decrease in steroid and inhibin production. Consequently, an additional number of follicles are selected, and more ovulation occurs in prolific sheep breeds.[69–72] Differential ovarian morphometry and expression of prolificacy genes serve as important indicators for prolificacy in Black Bengal and Sirohi goats.[68]

In China, the *Growth Hormone* (*GH*) gene polymorphism on goat production was first studied in Matou (a high prolificacy breed) and Boer (low prolificacy breed). The two polymorphisms of the goat *GH* gene at the loci A 781 G and A 1575 G were detected and the results showed that the two loci of *GH* gene are highly associated with abundant prolificacy and super ovulation response in goat breeds.[73] Alpine, Damascus and Murciano-Granadina goat breeds were imported and used as paternal genotypes by crossing with the local population in southern Tunisia and reported that kid's mortality and reproductive performances are largely related to the genotype adaptative potentialities.[74] The polymorphism studies by PCR-SSCP, PCR-RFLP and sequencing established that*Inhibin*, *alpha(INHA)* may be a major gene controlling the prolificacy of goat and allele G is positively correlated with litter size.[75] The studies were undertaken to find the association between *FecB* and high prolificacy in Raighar goats could not detect the affinity of the *FecB* gene for greater prolificacy.[76]

The single nucleotide polymorphisms in exon 1 and exon 2 of the *BMP15* gene in both high fecundity breed (Jining Grey goats) and low fecundity breeds were detected and the *BMP15* gene was identifiedas a major gene that influenced the prolificacy of Jining Grey goats.[77] The polymorphism of *Bone Morphogenetic Protein Receptor 1B (BMPR-1B)* gene was studied as a candidate gene for the prolificacy of goats in high prolificacy breed (Jining Grey goat) and low prolificacy breeds (Wendeng Dairy and Inner Mongolia Cashmere goats) using polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) method. These results preliminarily identified that the detected loci of the *BMPR-1B* gene had no significant effect on the prolificacy of Jining Grey goats.[78] The polymorphism of *Growth differentiation factor 9 (GDF9)* was detected by PCR-SSCP in five goat breeds which differ in prolificacy and the findings suggested that prolificacy in Jining Grey goats may be due to the allele A.[79] *BMP4* is one of the most important genes in prolificacy due to its major part in growth and differentiation of the follicles, cumulus expansion and ovulation and the first report of a mutation in the coding region of the caprine *BMP4* gene in India was given by a study in nine different goat breeds (Barbari, Beetal, Black Bengal, Malabari, Jakhrana (Twinning>40%), Osmanabadi, Sangamneri (Twinning 20-30%), Sirohi and Ganjam (Twinning<10%)) differing in prolificacy by Sharma et al.[80]. The genetic basis of caprine prolificacy was explored by screening indigenous goats for prolificacy associated markers of sheep *BMPR1B, GDF9* and *BMP15* genes, performing extraction of DNA and PCR amplification was done using primers designed. It resulted in the identification of three non-synonymous SNPs (C818T, A959C and G1189A).[81] In a recent study, ectopic expression of the *beta-1, 4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2)* gene was observed within the ovary in the highly prolific Lacaune sheep.[82] Transgenic technology is a method to rapidly introduce "new" genes into livestock without crossbreeding. Enhanced prolificacy, reproductive performance, feed utilization and growth rate, improved carcass composition, improved milk production and/or composition and increased disease resistance are some of the practical applications of transgenesis in livestock production.[83]

Seasonal and environmental factors

Thermoregulation studies on British Anglo-Nubian and Saanen goats reared in an intensive system in Trinidad concluded that Anglo Nubian is more suitable for the tropical environment with good prolificacy and kidding interval (Lallo et al., 2012).[84] Johansson and Hansson (1943),[85] studied the seasonal variation in prolificacy and seasonal distribution of births in Europe, in the Swedish strains of Shropshire and Cheviots and established that the average number of births per ewe increases until the middle of the mating season and then decreases. Hammond,[86] studied in a small flock of ewes and found that the number of lambs per fertile service peaked in the autumn season and then declined steadily. Roberts[87] also found that the frequency of multiple births was highest in the second to the fourth month of the lambing season, but other authors[88–91] concluded that greater multiple births occurred early in the season.

Kumar et al.[92] studied the factors affecting the reproductive traits in Sirohi goats and the results indicated that the season of kidding had no significant effect. In contrast, weight at kidding and service has a significant role in reproduction and also suggested that the improvement in management and grazing can contribute to better production. Notter[93] studied the effects of ewe age and season of lambing on prolificacy in three US sheep breeds: Targhee, Suffolk and Polypay. He could observe the difference in Prolificacy ($P<0.001$) among ewe age groups in all breeds. Nevertheless, the prolificacy of young Suffolk ewes was higher in relation to that of adult ewes than that observed in Targhee and Polypay and this can be due to the high levels of management and nutrition commonly observed in purebred Suffolk. As per the study, the seasonal effects on prolificacy were substantial, but differences within the main winter and spring lambing seasons were minor since the animals lambing in different seasons would normally be placed in different contemporary groups. In the experiment conducted in Majorera goat, the kidding rate and prolificacy were significantly higher in multiparous than in nulliparous goats. They confirmed the efficiency of the Ultra-Low Freezing (ULF) technique for freezing and storage of goat semen.[94] The practice of sterile service reduces the duration of oestrus and increases fertility in artificially inseminated dairy goats.[95]

Nutrition or flushing

In most cases, a positive effect of nutrition on reproduction is reported and there is a direct relationship between nutrition and reproduction. Supplementation during the mating period (shortly before the mating period and afterwards) could increase the ova shed and improve embryo survival. This practice is called flushing. The

manipulation of reproduction in relation with the nutrition is a vital tool to be considered in management practice which has a direct effect on ovulation rate and litter size particularly with a low cost when compared with the marker-assisted selection done after polymorphism studies and gene expression in prolific breeds of sheep and goat[96] conducted a study on the nutritional effect on reproduction and the treatment group of animals was supplemented with lotus corniculatus for 12 days prior to ovulation and the control provided the normal feed. Both the groups attained oestrus on the ninth day, but the supplemented group had an increase in ovulation rate and more twins were born (p=0.09). Also, the supplementation with corn grain and soybean meal for seven days prior to ovulation increased the ovulation by 14% in supplement animals. The effects of improved energy and protein diet upon reproductive outcomes of adult goats were studied under marginal rangeland grazing conditions and the animals were exposed to the male effect during the anoestrous-dry season. Results suggested that nutritional supplementation and the male effect were able to strongly invoke neurophysiological pathways to cause a rise in ovarian activity and to promote a uterine environment prone to the establishment of pregnancy during the anoestrus season.[97]

Conclusion

The factors affecting sheep and goat prolificacy when studied efficiently could improve their reproduction mechanisms and also provides a piece of prospective information for selective breeding. This review article has highlighted some of the major research concerning the possible prolificacy mechanisms in sheep and goats. Most of the studies associated with prolificacy are concentrated on identifying mutations in growth factors and their differential expressions. Recent studies are targeting genome, transcriptome and proteome analysis to gather more information on molecular mechanisms controlling prolificacy.

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Conflicts of interest

Author declares that there are no conflicts of interest.

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