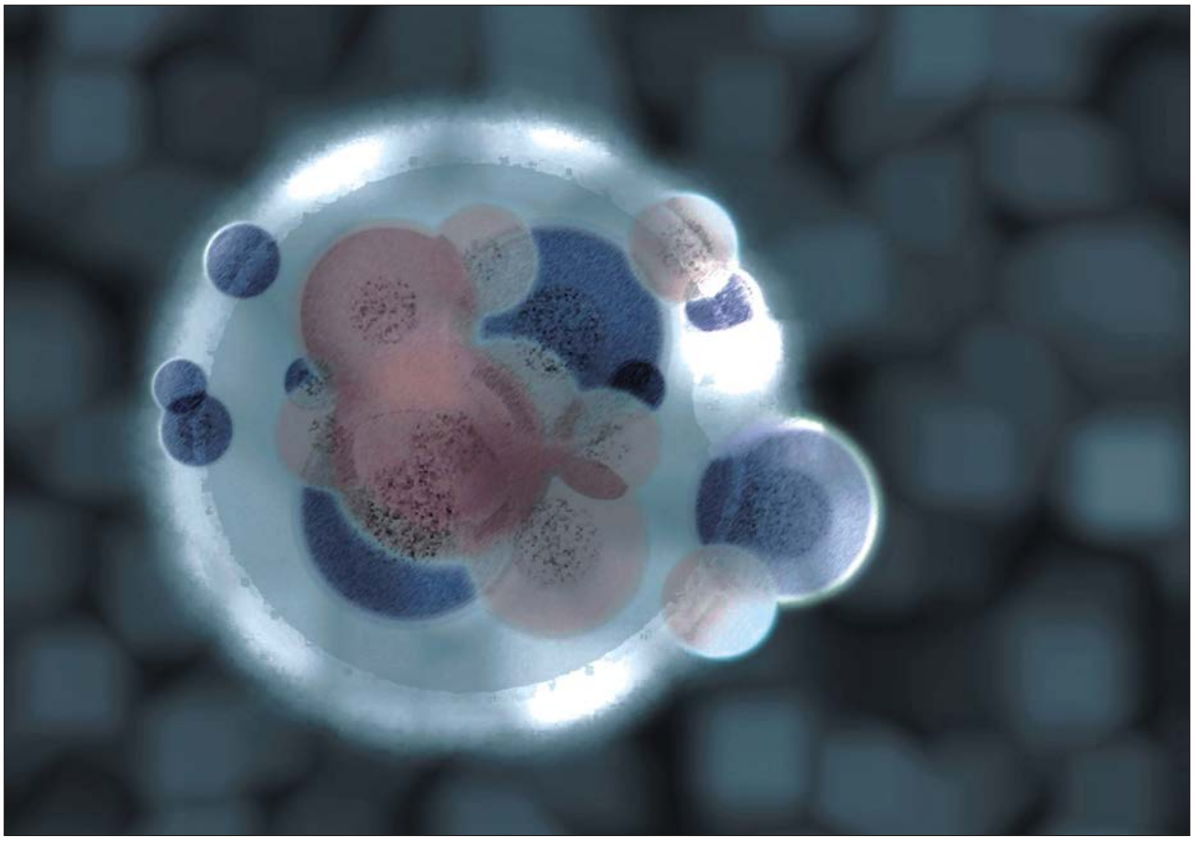

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Estrus Synchronization and Superovulation in Goats: A Review

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Abstract: The study was intended to review the recent developments and advances of estrus synchronization and superovulation protocols in goats with a view to improve oocytes/embryo recovery for *In Vitro* Production (IVP) efficiencies. Although a number of estrus synchronizing protocols has been developed in goat, the most widely used one is the treatment of progesterone for 9-11 days followed by a luteolytic dose of prostaglandin administered in the period 48 h prior to removal of intravaginal sponge. Until now, Controlled Internal Drug Release (CIDR) device and subcutaneous implants are more preferable than sponges. Ovulation in goat can be synchronized more precisely by administering Gonadotrophin Releasing Hormone (GnRH) around the time of estrus that improves the success of fixed-time Artificial Insemination (AI) and oocytes or embryos collection at a controlled stage. Superovulation is the hormonal treatment for increasing a large number of oocytes that ultimately accelerate genetic improvement in any species. Generally an exogenous follicle-stimulating gonadotrophin is administered that mimics the effect of Follicle Stimulating Hormone (FSH) near the end of the luteal phase of the cycle (9-11 days) or around 48 h before the end of the synchronizing treatments. The major commercial products applied are equine Chorionic Gonadotrophin (eCG) or Pregnant Mare Serum Gonadotrophin (PMSG) and FSH. This review paper describes estrus synchronization, ovarian superovulation as well as the normal physiology of estrous cycle and ovulation in goats.

Key words: Estrous cycle, follicular wave, ovulation, FSH, eCG, oocytes, goat

INTRODUCTION

In this modern era Assisted Reproductive Technologies (ARTs) plays a major role in animal reproduction and production. The estrus synchronization and ovarian superovulation already become one of the popular ARTs in goat industry. Estrus synchronization plays a major role in fixed time breeding, Artificial Insemination (AI), Multiple Ovulation-Embryo Transfer (MOET), Laparoscopic Ovum Pick-up (LOPU) for oocyte or embryo collection and Embryo Transfer (ET). The value of estrus synchronization is vital in goats as the duration of both estrous cycle and estrus is variable and estrus detection cannot be accomplished safely without a buck (Jainudeen *et al*., 2000; Rahman *et al*., 2008). On the other hand, superovulation is the hormonal treatment for harvesting increased number of oocytes from the ovary than normal. This will ultimately accelerate genetic improvement in goat like any other livestock. It is a means to induce maturation, ovulation and increase the number of follicles available for oocyte recovery.

A complete and scientifically detailed understanding of any physiological process is vital for its successful manipulation. The basic premises of any estrus synchronization and superovulation protocol pivots around the predictable control of events associated with the animal's reproductive physiology. Control of reproductive events can be achieved through pharmacological administration of biologically active agents. Usually the agents used in estrus synchronization and superovulation protocols are either based on or are the hormones that occur in the female goat or doe at various times during her cycle. Therefore, works related to estrus synchronization and superovulation treatments of the doe have been reviewed briefly in this paper following a short description of estrous cycle.

This study was conducted in Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia from 2004 to 2008.

ESTROUS CYCLE OF THE DOE

The signs of estrus; length and duration of different phases of estrous cycle; hormonal changes during estrous cycle; follicular dynamics, waves and dominant follicles and ovulation in the doe are described following:

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Signs of estrus: Signs of estrus are important indicators of onset of estrus or heat and, therefore, very important for estrus detection. These signs are mainly two types, primary and secondary signs. The primary signs are the most reliable and well accepted for indication of estrus behavior in most female animals. The best method of estrus detection is the observation of primary signs exhibited by the doe in response to the buck. However, the doe seldom mount as often as the cow, but demonstrate some behavior when they are in heat such as seeking out bucks, waggling of the tail when being exposed to the buck and also bleating, more frequently if the buck is absent. Apart from the primary signs, physical signs, for example, redness and swelling of the vulva and a clear mucous discharge from the vulva also indicative of estrus. Restlessness, frequent urination, isolation from others, general loss of appetite and constant vocalizations are the secondary signs of estrus. However, secondary signs are less reliable as they vary in length and may be confused with the symptoms of any minor illness. Although it is possible to detect estrus signs in the doe as described above, but a doe in heat may not exhibit all the signs at the same time. These signs appear and disappear progressively with the onset and termination of estrus behavior. Therefore, estrus detection is dependant upon the careful observation and close attention of such phenomena, including the primary and physical signs of the doe experiencing estrus. Therefore, a routine check-up of heat twice daily (morning and evening) is indispensable to obtain optimal results. Estrus detection is a valuable and useful tool for natural mating, AI, predicting parturition date as well as prediction of ovulation time for LOPU.

Length and duration of different phases of estrous cycle: Like ewes, does are also seasonally polyestrus as their reproductive cycle responds to changes in day length (Attwood, 2007). Sexual activity is usually greatest during autumn and winter. Estrus activity of doe is greater in the tropics than in temperate climates. The average length of estrous cycle in the doe is 21 days, but can vary from 18-22 days depending on the breed differences, stage of breeding season and environmental stress (Jainudeen *et al*., 2000). The abnormally short cycles that are observed in the doe early in the breeding season may be associated with premature regression of the corpus luteum. The estrus lasts for 24-48 h in the doe and the duration of estrus can be influenced by breed, age, season and presence of the buck (Jainudeen *et al*., 2000). Angora does have a shorter duration of estrus (22 h) than the dairy breeds. Estrus is of shorter duration at the end of breeding season and in the first breeding season of young does. The characteristic events of estrous cycle in doe are shown in Table 1. The complete estrous cycle

Table 1: Duration of characteristics events of estrous cycle in doe

Characteristic events	Average time
Duration of estrous cycle	18-21 days
Duration of estrus	24-48 h
Time of ovulation	21-36 h after estrus

Source: Modified from Devendra and McLeroy (1982) and Jainudeen *et al*. (2000)

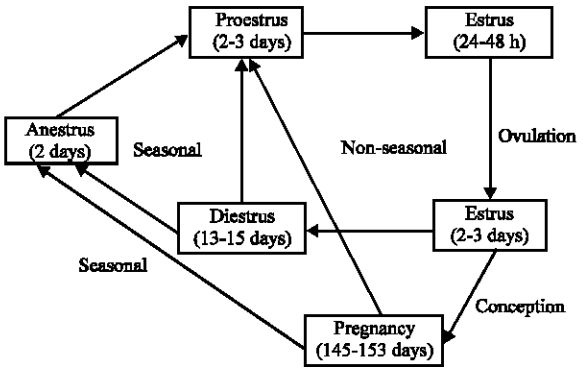

Fig. 1: The estrous and reproductive cycles of the female goat. Illustration is based on Pineda (1989) using data cited in Jainudeen *et al*. (2000), Massita (2003) and Attwood (2007)

in doe is divided into 4 well marked phases, namely proestrus, estrus, metestrus and diestrus. In prepubertal and aged does anoestrus (stage of sexual quiescence characterized by lack of estrus behavior) normally occurs. This may be due to the complete suppression of ovarian activity or silent ovulatory cycles without behavioral signs of estrus. In all domestic animals including the doe, anoestrus also may occur as a pathological condition (Pineda, 2003). The duration of each event associated with the estrus cycle and pregnancy in the doe is illustrated in Fig. 1.

Hormonal changes during estrous cycle in doe: Like other domestic farm animals, improvement in the efficiency of estrus synchronization in the doe depends on a better knowledge of the endocrine patterns that occur during induced and natural estrus (Chemineau *et al*., 1981). Cyclic changes in the circulating levels of the ovarian hormones, progesterone and estrogen have direct effects on the growth and metabolism of cells in the reproductive tissues. Unlike the ewe, data about the endocrinology of the estrous cycle in the doe is sparse. The main events of the estrous cycle are related to the periods of growth of the ovarian follicles and the corpus luteum. The sequence of hormonal events during the estrous cycle is similar in both doe and ewe, but the doe has a longer progesterone phase than the ewe (Jainudeen *et al*., 2000). During the 21 days cycle, progesterone dominates for about 15 days and estrogen dominates for 5-6 days.

D0 (day 0) of the cycle is generally designated as the first day of behavioral estrus, which is the result of increasing estradiol-17β levels produced by the developing pre-ovulatory follicle. The rise in estradiol-17β levels and the maximum values before natural estrus in the doe are similar to that of the ewe (Chemineau *et al*., 1981). The rise of estradiol-17β is followed in less than 12 h by simultaneous peaks of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and prolactin as reported for the ewe (Pant *et al*., 1977; Herriman *et al*., 1979; Cahill *et al*., 1981). These peaks occur 8.5 h after the onset of natural estrus (Chemineau *et al*., 1981). Within 48 h of the first peak, a second FSH peak occurs (Chemineau *et al*., 1981) which is similar in the ewe (Pant *et al*., 1977). High estradiol-17β levels are believed to cause a surge of Gonadotrophin Releasing Hormone (GnRH) and consequently an LH peak at estrus resulting in spontaneous ovulation towards the end of estrus. Following ovulation the ruptured follicle becomes a functional corpus luteum, which is the main source of progesterone in the cycling doe. Blood levels of progesterone are low at estrus (less than 1.0 ng mL^{-1}) through to 2 days of diestrus and then rapidly increase to maximal levels at 7 days and remain elevated until 13-15 days in natural estrus (Fig. 1). Regression of the corpus luteum (luteolysis), induced by prostaglanding $F_{2\alpha}$ ($PGF_{2\alpha}$), occurs if an embryo is not present in the uterus, resulting in a rapid drop in plasma progesterone (reviewed in Rahman, 2006). Ovarian oxytocin stimulates endometrial secretion and release of prostaglandins. The onset of the follicular phase of the next cycle is characterized by low progesterone levels and increasing GnRH and LH levels, while the FSH levels present at the onset of this phase are progressively decreased. These events are controlled by estrogen and inhibin, which are produced in increasing amounts by the developing follicles (Rahman, 2006).

Follicular dynamics, waves and dominant follicles: The mature ovary of farm animals contains varying numbers of follicles in different stages of development. Unlike other farm animals and ruminants, reports of caprine follicular waves and their association with hormones during estrous cycle are limited. The term follicular wave is defined as one or more antral follicles growing from 3 mm to ≥5 mm in diameter before regression (Ginther *et al*., 1995; de Castro *et al*., 1999; Bartlewski *et al*., 2000). Individual follicle emerging within a maximum of 48 h can be regarded as a single follicular wave (Medan *et al*., 2003). However, distinct differences exist in the follicular dynamics in the ovary among different farm animals. In cow and mares, distinct groups of follicles develop during the estrous cycle. Although 1 or 2 waves occur during the estrous cycle in mare by Pierson and Ginther (1987a), in cow 2 or 3 waves were detected (Pierson and Ginther, 1987b). The phenomenon called follicle dominance is generally present in each wave of follicular development, both in mare and in cow (Pierson and Ginther, 1987a, b, 1988; Savio *et al*., 1988; Sirois and Fortune, 1988). Dynamics of follicular development in ewe is a bit different and characterized by an initial wave of follicular activity at the beginning and another at the end of the estrous cycle (Bobes *et al*., 2003). However, the growth of ovarian follicles in the ovary of doe is characterized by the presence of 4 or more waves of follicle growth in the same cycle and it is in the final wave where the dominant follicle ovulates (Ginther and Kot, 1994; de Castro *et al*., 1999; Medan *et al*., 2005). Unlike other farm animals, the subsequent follicular wave begins even though the dominant follicle of the previous wave is still in its peak of development. This behavior strongly suggests that follicular dominance is less apparent in the ovary of doe (Ginther and Kot, 1994). Each follicular wave is preceded by an increase in FSH secretion (Medan *et al*., 2005). It is found that progesterone treatment affects follicular dynamics in dairy does (Menchaca and Rubianes, 2002).

Ovulation: Ovulation can be defined as the rupture of the mature ovarian follicle on the surface of the ovary and the release of its contents, including the maturing oocyte (Pineda, 2003) with adhering corona radiata cells, Cumulus Cells (CCs) and Follicular Fluid (FF). Ovulation is the most significant event of estrus. The point of ovulation can be seen in the resulting corpus luteum on the ovary days after ovulation. Ovulation is controlled by gonadotrophins: FSH is predominant during the phase of follicular growth and LH is generally regarded as ovulation inducer and also responsible for the formation of corpus luteum (Perry, 1971). In the doe, LH is released from the pituitary in a surge (50 ng mL^{-1}) (Bono *et al*., 1983), which induces final preparation of the follicle 24 h prior to ovulation. At ovulation, LH level in the peripheral blood circulation of the doe reduces rapidly and FSH level begins to increase (Bono *et al*., 1983). Following rupture, external part of the follicle collapses and the follicular cavity becomes filled with clotted blood or serous fluid. The ruptured follicle reduces in size, the granulosa and theca internal cells begin to proliferate exuberantly under the influence of LH and form the corpus luteum (Sanga *et al*., 2002). Ovulation in the doe is spontaneous and most goat breed ovulates between 24-36 h after onset of estrus, the Nubian goat ovulates later, which is

possibly due to a longer estrous cycle in this breed (Jainudeen *et al*., 2000). The average ovulation rate in the doe is 1-3 oocytes, but can vary from 1-5 depending upon the breeds and management conditions (Pineda, 2003).

ESTRUS SYNCHRONIZATION

Estrus synchronization is a key element of all the ART-protocols in livestock animals and has a major influence to increase the overall efficiencies of these programs (Baldassarre and Karatzas, 2004). Estrus synchronization plays a major role in fixed time breeding, AI, LOPU for oocyte or embryo collection and Embryo Transfer (ET). The value of estrus synchronization is vital in does as the duration of both estrous cycle and estrus is variable and estrus detection cannot be accomplished safely without a buck (Jainudeen *et al*., 2000). This technique has been developed in the early 1960s and since then a number of synchronizing methods has been developed for goats. Approaches towards synchronizing estrus in livestock have to focus on either the manipulation of the luteal or the follicular phase of the estrous cycle. In the doe, the window of opportunity is generally greater during the luteal phase, which is of longer duration and more responsive to manipulation. Different approaches have been concerned with either extending the luteal phase by supplying exogenous progesterone or with shortening this phase through regression of the corpus luteum. Successful techniques must not only establish synchrony, but also provide a reasonable level of fertility in the synchronized cycle.

A number of synchronization methods for goats have been evaluated under research conditions. The most widely used method in the doe is the treatment of progesterone or progestagen for 9-11 days followed by a luteolytic dose of prostaglandin (or an analogue) administered in the period 36-48 h prior to removal of intravaginal sponge (Baldassarre and Karatzas, 2004). The progesterone or progestagen treatment can be delivered through an intravaginal sponge, a Controlled Internal Drug Release (CIDR) device or a subcutaneous implant (Evans and Maxwell, 1987; Ritar *et al*., 1989; Freitas *et al*., 1997). Although sponges are widely used either in conjunction with Pregnant Mare Serum Gonadotrophin (PMSG), FSH or prostaglandin to more tightly synchronize and/or induce a superovulatory response, but sponges are not preferred as these frequently cause discomfort and may adhere to the vaginal wall causing problems with removal (Holtz, 2005). An alternative means of supplying continuous, exogenous progesterone has been the CIDR developed for goats in New Zealand. The CIDR device is constructed from natural progesterone impregnated medical silicone elastomer molded over a nylon core. Currently the CIDR and subcutaneous implants are preferable than sponges because these are easy to use (Holtz, 2005). Ovulation in the does can be synchronized more precisely by administering GnRH around the time of estrus (Pierson *et al*., 2003), which improves the success of fixed-time AI and the collection of oocytes or embryos at a controlled stage of development for specific applications such as oocytes for *In Vitro* Production (IVP), Intracytoplasmic Sperm Injection (ICSI) or Somatic Cell Nuclear Transfer (SCNT) and zygotes for pronuclear microinjection (Baldassarre and Karatzas, 2004). Although, in the past, a considerable attention was focused in estrus synchronization, however, there is an urgent need for additional research conducted in a well-organized and systematic fashion to help establish guidelines for efficient breeding, AI, oocyte or embryo recovery and ET programs. Timeline of significant finding in estrus synchronization of does has been depicted in Table 2.

OVARIAN SUPEROVULATION

As multiple litter bearing animals, ovulation rate and litter size have a major impact on the reproductive efficiency of goat. Ovulation rate is influenced by the stage of breeding season, nutrition, genotype and parity. However, it can also be manipulated by pharmacological means which is known as superovulation. Superovulation is the hormonal treatment for increasing a large number of ova released by the ovary, which ultimately accelerate genetic improvement in any species. It is a means to induce maturation, ovulation and increase the number of ovulating or antral follicles (>2-3 mm) on the surface of the ovary available for oocyte recovery. Principles of inducing superovulation in doe are the same as in cow and ewe. An exogenous follicle-stimulating gonadotrophin is administered that mimics the effect of FSH near the end of the luteal phase of the cycle (9-11 days) or around 48 h before the end of the synchronizing treatments. There are a number of ways to superovulate doe, each of which has its advantages and disadvantages. However, superovulation in goats is frequently restricted by the cost of gonadotrophin or the handling requirements. The major commercial products applied are equine Chorionic Gonadotrophin (eCG) or PMSG and FSH used in higher (pharmacological) doses to elicit a superovulatory response; commercial preparations are partly purified from mare's serum and porcine pituitary gland, respectively. PMSG is used to stimulate ovarian activity during seasonal anoestrus (Gordon, 1997) and usually used concurrently following estrus synchronization. PMSG is available in most countries and tended to be a practical hormone to administer for superovulation (Amoah and Gelaye, 1990). PMSG is preferred due to its lower cost and easy availability; it can

Table 2: Timeline of significant finding in estrus synchronization and superovulation in the goats

Significant findings	References
Two injections of cloprostenol synthetic analogue at the rate of 62.5, 125.0 and 250.0 μg, administered 14 days apart, were effective in synchronizing doe during breeding season.	Greyling and Van Niekerk (1986)
Higher numbers of oocytes were recovered with FSH-treated (9.4) than PMSG-treated (5.7) does.	Tsunada and Sugie (1989)
Superovulation with PMSG are prone to premature regression of the induced CL, results in short cycles and have the potential risk of embryo expulsion in goat.	Amoah and Gelaye (1990)
No difference was observed in estrus response and timing in dairy does treated with cloprostenol.	Nuti *et al.* (1987)
Good superovulation was achieved in Angora doe with porcine FSH (22 mg) divided in 4 decreasing dosage, injected twice daily, commenced 1 days before sponge removal after 17 days of progestagen treatment.	Mani *et al.* (1994)
Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and GnRH is an effective supplement used with FSH superovulation regimens in dairy does which enhance early embryo collection for DNA microinjection studies.	Krisher *et al.* (1994)
During anoestrus, fluorogestone acetate (FGA) intravaginal sponges for 11 days in conjunction with PMSG (750 IU) and cloprostenol (50 μg) 48 h before sponge removal resulted in 87.5 and 93.8% estrus response and fertility, respectively in Alpine does.	Freitas *et al.* (1996)
Does that have been synchronized with CIDR for 17 days together with PMSG 2 days before CIDR removal provided 100% OR and 41.2% fertilization rate.	Samsul (1997)
Estrus synchronization in Sudanese Nubian does was achieved with double dose of cloprostenol (125 μg) together with intravaginal sponges impregnated with progesterone inserted for 16 days.	Muna *et al.* (1998)
Two intramuscular injections of cloprostenol (125 μg) administered 10 days apart in Mashana does were as effective as the progestagen treatments tested.	Kusian *et al.* (2000)
Estrus synchronization using progesterone releasing intravaginal device (PRID) for 12 days and $PGF_{2\alpha}$ for 24 h before PRID removal delayed the onset of oestrus by 2-5 days.	Noakes *et al.* (2001)
Estrus synchronization using CIDR for 9 days combined with eCG (100 IU) and cloprostenol at CIDR removal results a 100% estrus in Saanen does within 24 h.	Oliveira *et al.* (2001)
Use of CIDR led to a shorter interval to onset of estrus than 6-methyl-17-acetoxy-progesterone (MAP) and FGA.	Motlomelo *et al.* (2002)
The ovulatory response to commercial FSH preparations is related to the number of small follicles (2-3 mm) present in the ovaries of does at the start of the superovulatory treatruent.	Cognié *et al.* (2003)
GnRH antagonist reduces plasma FSH and LH levels with suppression of the growth of large dominant follicles and a 2-fold increase in number of smaller follicles in Spanish doe. This confirmed that GnRH antagonist treatruent can be used in does to control gonadotrophin secretion and ovarian follicle growth in superovulatory regimens.	Gonzalez-Bulnes *et al.* (2004)
The use of FGA and MAP sponges and CIDRs with $PGF_{2\alpha}$ administration at the time of pessary removal induced an efficient estrus response and acceptable fertility in Nubian doe. The use of CIDR can be considered a worthy alternative to replace intravaginal sponges.	Romano (2004)
Estrus of mixed breeds of does was synchronized with CIDR for 10, 14, 17 or 21 days plus a single dose of 125 μg cloprostenol 36 h before CIDR removal and superovulated with 70 mg FSH (Ovagen™) plus 1000 IU hCG (Profasi) 36 h before LOPU resulted in recovery of 3.7-6.7 oocytes per doe.	Rahman *et al.* (2007a, b), Rahman *et al.* (2008)
Estrus of does were synchronized with CIDR-G® and superovulated with six injections of pFSH (Ovagen) every 12 h starting after 48 h of CIDR-G® insertion resulted in recovery of 21.9 oocytes per doe.	Cox and Alfaro (2007)
Estrus of aged Saanen and Toggenburg does was synchronized with MAP sponges for 10 days plus a single dose of 125 μg cloprostenol 36 h before sponge removal and superovulated with 80 mg NIH-FSH-P1 (Follotrophin-V) plus 300 IU eCG 36 h before LOPU resulted in recovery of 15.7 oocyte per doe.	Baldassarre *et al.* (2007)
Estrus of does were synchronized with MAP sponges plus 50 μg $PGF_{2\alpha}$ and superovulated with 60 mg NIH-FSH-P1 (Follotrophin-V) plus 300 IU eCG 24 h before LOPU resulted in recovery of 5.6-8.0 oocytes per doe.	Gibbons *et al.* (2007)
Repeated superovulation and estrus synchronization in Saanen, Alpine and Toggenburg does with norgestomet implant or CIDR-G® and non-surgical embryo retrieval, coupled with surgical embryo transfer, expedited the production of progeny from transgenic founder does.	Melican and Gavin (2008)
Prolonging the time interval from 36 to 60 and 72 h between the estrus synchronization plus ovarian superstimulation and LOPU increased OR rate in Malaysian does.	Abdullah *et al.* (2008)

be more easily administered than FSH, usually as a single injection of up to 1500-2000 IU, but the superovulatory response to PMSG can be quite variable and is usually lower than in a FSH-induced superovulation (Amoah and Gelaye, 1990). Problems associated with PMSG-induced superovulation are a high number of non-ovulated follicles, early regression of CL, short or irregular estrous cycles and potential risk of embryo expulsion (Amoah and Gelaye, 1990). A combination of eCG and human Chorionic Gonadotrphin (hCG) has also been widely used to superovulate does (Medan *et al*., 2003). FSH is a better choice of hormone for superovulating does as it provides more oocytes than PMSG. FSH is usually administered in decreasing doses of 1-5 mg, injected in 12 h intervals over a period of 3-5 days around the time of termination of the progestagen treatment. Like in cows and ewes, a number of experiments have been performed to compare the superovulatory response between FSH and PMSG, the evidence favors the use of FSH than PMSG (Tsunada and Sugie, 1989; Pendelton *et al*., 1992). In their study, Tsunada and Sugie (1989) reported that average number of oocyte recovery (OR) was significantly higher in FSH-treated does (9.4) than that in PMSG-treated ones (5.7). Studies in our laboratory also support of this finding. Earlier in our laboratory, PMSG alone or in combination with hCG was used to superovulate does. However, due to higher variability of stimulation and lower OR rate, a combination of recombinant ovine FSH (Ovagen™; ICPbio

Limited, New Zealand) and hCG (Ovidrel; Laboratories Serono, Switzerland) as single doses was later introduced in our laboratory (Rahman *et al*., 2007a; Abdullah *et al*., 2008).

Most superovulatory treatments are cumbersome and expensive and, over and above, accompanied by endocrine repercussions that take one or more subsequent cycles to subside (Holtz, 2005). Therefore, several attempts have been made to devise less labor-intensive treatment regimes without compromising oocyte or embryo yield. By applying a one shot-treatment regimen consisting of a single dose of FSH combined with a moderate dose of eCG (e.g., 60-80 mg FSH and 300 IU eCG), Batt *et al.* (1993), Baldassarre *et al.* (2002, 2003), Baldassarre *et al.* (2007) and Gibbons *et al.* (2007) almost equaled the oocyte or embryo yield obtained with the traditional multiple injection regimen. The simplicity of this treatment is appealing. Of all the superovulation protocols in use to date, not a single one fulfils all expectations concerning predictability and reliability of the response. The variability in number of ovulations and yield of viable oocytes or embryos remains the main drawback (Holtz, 2005). Both intrinsic and extrinsic factors are responsible for the variability. Among the intrinsic factors, genetic (Nuti *et al*., 1987), age (Mahmood *et al*., 1991) and stage of the cycle at which the treatment applied (Wani *et al*., 1990) are important. A host of environmental factors such as season, nutrition, health state, AI (Holtz, 2005) and type of gonadotrophin administered (Gordon, 1997) are known to contribute to that variability. Therefore, vigorous research efforts are directed for the establishment of suitable superovulation regimes to augment the develoment of LOPU, ET and associated technologies based on them.

Time interval between the estrus synchronization plus superovulation treatment and LOPU may also influence the umber of oocytes recovered per doe (Abdullah *et al*., 2008). While other LOPU-IVP research groups (Baldassarre *et al*., 2002; Baldassarre *et al*., 2007) obtained optimum OR rates (13.4-15.7 oocytes per doe) after performing LOPU at 36 h of FSH plus hCG treatment, previous OR rates in our laboratory was always less than 7 oocytes per doe (Rahman *et al*., 2007b). In their experiment, Gibbons *et al.* (2007) used a lower time interval of 24 h between FSH plus eCG and LOPU and recovered lower OR rates (5.6-8.0 oocytes per doe). Therefore, we speculated that 36 h time interval between FSH plus hCG treatment and LOPU might not be optimum for proper stimulation of the ovarian follicles with our current protocol and, thus oocytes derived from these follicles had not acquired full meiotic competence and ooplasmic maturation. Keeping this in mind, we increased the time interval between FSH plus hCG treatment and the onset of LOPU from 36 h to 60 and 72 h and obtained 8.6 and 16.1 per doe, respectively, at 60 and 72 h interval (Abdullah *et al*., 2008). With slight modification of superstimulation protocol (decreasing hCG dose rate from 500 IU to 250 IU per doe), later we retrieved 14.9-17.6 oocytes per doe when LOPU performed 60 h post-FSH-hCG treatment (Rahman, 2008). These recovery rates are in agreement with Baldassarre *et al.* (2002), Baldassarre *et al.* (2003) and Baldassarre *et al.* (2007), who used slightly different protocol consisting of a single dose of FSH combined with a moderate dose of eCG (e.g., 80 mg FSH and 300 IU eCG). Using 60 mg FSH and 300 IU eCG in their superovulation protocol, (Gibbons *et al*., 2007) reported lower OR rate (5.6-8.0 oocytes per doe and 5.5-8.8 oocytes per ewe).

Dose rates of hormones for inducing superovulation might also have significant importance. Previous superovulation protocol in our laboratory was consisted of 70 mg FSH (Ovagen™; ICPbio Limited, New Zealand) and 1000 IU hCG (Ovidrel; Laboratories Serono, Switzerland) per doe (Rahman *et al*., 2007b). Later we have lowered the hormone doses from 70 to 35 mg FSH (Ovagen™) and 500 IU hCG (Ovidrel) per doe due to a shortage of hormones in the laboratory at that time (Abdullah *et al*., 2008). However, even with lower doses of hormones similar OR rate at 36 h and significantly higher OR rates at 60 and 72 h time intervals were obtained. Currently we further reduced the dose rate of hCG (Ovidrel) to 250 IU for 60 h time interval and OR rates increased to double (Rahman, 2008). It is not clear whether this increment of OR rates was the effect of time interval or hormonal dose rates or combined effects of time interval and hormonal dose rates. However, decreasing hormonal dose rates alone might not be responsible for increasing OR rates as both higher and lower dose rates of hormones at 36 h time interval provided similar OR rates in the previous studies in our laboratory. Timeline of significant finding in superovulation of does has been shown in Table 2.

CONCLUSIONS

The value of estrus synchronization is vital in goats as the duration of both estrous cycle and estrus is variable and estrus detection cannot be accomplished safely without a buck. Until now a number of studies have been performed to synchronize the estrous cycle of goats as well as to superovulate to increase the reproductive efficiency and genetic gain through the use of ARTs like Artificial Insemination (AI), Laproscopic Ovum Pick-Up (LOPU), *In Vitro* Production (IVP), Intracytoplasmic

Sperm Injection (ICSI) or Somatic Cell Nuclear Transfer (SCNT). Through the application of findings from these studies reproductive performances of goat increased in some extent. Recently, estrus synchronization and superovulation received tremendous attention because of the rapid development of IVP and NT technologies. However, of all the estrus synchronization and superovulation protocols in use to date, not a single one fulfills all expectations concerning predictability and reliability of the response. The variability in number of ovulations and yield of viable oocytes or embryos remains the main drawback. Therefore, more studies are required to solve these problems.

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