Studies on Improvement of Reproductive Efficiency in Goats

Thesis presented
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ABSTRACT

This study was carried out to improve reproductive efficiency in goats through induction of estrus outside the breeding season and increasing ovulation rate. According to our results, estrus can be induced in seasonally anestrous Egyptian Baladi goats by using norgestomet and $PGF_{2\alpha}$ and the injection of GnRH synchronized ovulation in a higher percentage of goats. We used transrectal ultrasonography for investigation of the reproductive tract to gain knowledge of follicular and luteal development on the ovaries during the estrous cycle and correlate follicular dynamics with hormonal profile. The results demonstrated that follicular waves occurred in goats and the predominant follicular wave pattern was 4 waves with ovulation from wave 4. These results also suggested that the follicular waves' emergence was closely associated with increased secretion of FSH.

In a trial to make superovulation in goats using new techniques, two experiments were carried out to explore the effects of active and passive immunization against inhibin on circulating gonadotropins, estradiol, progesterone levels and ovulation rate. The results demonstrated that inhibin immunization increased FSH concentrations. In addition, immunization of goats against inhibin resulted in increase in ovulation rate. An approximately four-fold increase in ovulation rate in case of active immunization and two-fold increase in case of passive immunization. These observations confirm a physiological role of inhibin as a regulator of FSH secretion in goats and enhanced ovarian follicular development and ovulation rate by promoting increase in pituitary FSH secretion. Therefore, immunization against inhibin could be used to generate increases in litter size or increased numbers of oocytes and/or embryos for multiple ovulation-embryo transfer programs. The early determination of pregnancy can be used as a useful management tool in improving reproductive efficiency. Pregnancy diagnosis will identify the females which require repeat breeding or insemination and will allow separation of pregnant and open females for differential management. The results demonstrated that ultrasound is a reliable and safe method for pregnancy diagnosis in goats. Gestational sac could be first detected at day 20.2 ± 0.6 of gestation and embryos could be detected at 24.3 ± 0.7 days of gestation.

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INTRODUCTION

The goat is one of the smallest domesticated ruminants, which has served mankind earlier and longer than cattle and sheep. It is managed for the production of milk, meat, mohair, cashmere and leather. In addition, goats can be used as a laboratory animals and pets. Goats can survive on bushes, trees, and desert herbs when sheep and cattle would starve to death (Devendra, 1990; Gordon, 1997).

In Egypt, most farmers keep goats and sheep together but unlike sheep, goats are not considered an economic enterprise. They are clean up animals, kept primarily for the family's benefit. Despite the ability of goats to withstand the environmental conditions in Suez Canal area & Sinai and can be used as a mean to improve the socioeconomic status of the rural communities, little effort has been undertaken to improve their reproductive performance. Also, the human population in Egypt is increasing at dramatic rate and the demand for food will continue to grow. Through the use of assisted reproductive technology, each animal has the ability to produce an increased number of offspring.

Reproductive efficiency is one of major economic importance to producers because it greatly impacts overall production efficiency. A better understanding of reproductive mechanisms will give rise to strategies and technologies to enhance reproductive efficiency. This study has been aimed to overcoming seasonal breeding constraints and increase ovulation rate.

Reproductive efficiency in goats is determined by many different processes, for example, the length of the breeding season, cyclic activity and ovulation rate. The short gestation length (150 days) enables the production of more than one kid crop per doe per year. However, seasonal breeding constraints of goats limit the producers' ability to capitalize on this opportunity. It is possible to use exogenous hormones to induce or control breeding (Amoah and Gelaye, 1989; 1990). Thus, goats can be bred during the anestrous (non-cyclic) periods of the year.

In order to improve reproductive efficiency in goats, it is important to study the reproductive physiology aspects and develop methods that allow female to produce more offsprings in shorter time interval. Studies of ovarian follicular dynamics may lead to methods that increase response to estrus synchronization protocols and pregnancy rates resulting in high production efficiency. Transrectal ultrasonography for investigations of the reproductive tract in goats resulted in a gain of knowledge of follicular and luteal development on the ovaries during the estrous cycle (Ginther and Kot, 1994).

As multiple litter bearing animals, ovulation rate and litter size have a major impact on the reproductive efficiency of goats. Superovulation can be induced in goats using exogenous gonadotropins (FSH and equine chorionic gonadotropin). However, a disadvantage of these protocols is the long half-life of equine chorionic gonadotropin which interferes with normal fertilization and embryo development, and repeated equine chorionic gonadotropin treatments induced anti-equine chorionic gonadotropin antibodies that clearly have negative effects on reproduction in goats (Roy, Maurel, Combes, Vaiman, Cribiu, Lantier, Pobel, Deletang, Combarnous and Guillou, 1999). Thus it is necessary to establish an alternative method for induction of superovulation in goats. Considerable interest has been placed on inhibin which by synergistic action with estradiol is involved in the negative feedback regulation of FSH and thereby controlling ovulation rates.

The early determination of pregnancy can be used as a useful management tool in improving reproductive efficiency. Pregnancy diagnosis will identify the females which require repeat breeding or insemination and will allow separation of pregnant and open females for differential management. When fetal numbers can be determined as a part of pregnancy diagnosis, different feeding regimes can be applied to single and multiple bearing females.

The purposes of this study are:

1-Induction of estrus and ovulation in goats out-of-season.

2-Investigation of the ovarian dynamics using ultrasonography and hormonal profile using RIA & ELISA during estrous cycle in goats.

3-Investigation of passive and active immunization against inhibin as an alternative protocols for superovulation.

4-Determination of pregnancy at early stage using ultrasonography.

REVIEW OF LITERATURE

Reproduction traits in goats

Puberty:

Puberty can be defined as the age of the female at which estrus is first detected and is followed by characteristic cyclic ovarian activity in the non-pregnant animal. The direct cause of sexual maturation at puberty is given as a rise in the output of the pituitary hormones leading to an increase in size and activity of the gonads. (Hunter, 1980; Cupps, 1991).

At the onset of puberty, there is a rise in plasma LH due to an increase in the

pulsatile LH discharges which result in one or more of the follicles developing towards the pre-ovulatory stage and in a steady increase in estradiol production which eventually activates the LH surge mechanism. This is seen as a change in frequency rather than amplitude of pulsatile LH release (Foster and Ryan, 1979).

In the young, body weight is of great significance in the attainment of puberty, as the occurrence of puberty is dependent on the animal attaining a certain critical body weight. Generally breeding in goats should be delayed until the animal has attained 60-75 % of its mature bodyweight (Smith, 1980). The body weight at puberty of Boer goat does in South Africa has been set at 30.6 ± 7.2 kg while in Creole goats 24 kg in Venezuela and 25-30 kg in northern Mexico (Greyling, 1988; Delgadillo and Malpaux, 1996; Papachristoforou, Koumas and Fhotiou, 2000).

Seasonal cyclic activity:

Seasonal fluctuation in daylight length and temperature are important factors affecting the length of the breeding season (Chemineau, 1983). In goats, breeding season usually commences as the days become shorter. There is evidence to indicate that the pineal gland, through its secretion of melatonin is involved in mediating the effects of photoperiod on gonadal function (Hafez, 1974). The interval between two consecutive kiddings is a good index of seasonal reproductive activity. Medan (1998) reported that the kidding interval is 281.7 ± 3.9 and 321.4 ± 9.9 days in Egyptian Baladi and Damascus goats, respectively. Reproductive efficiency in the female is thus greatly determined by this seasonality (length of the breeding season).

Estrous cycle and estrus period:

The length of the normal estrous cycle is will documented in most breeds as ranging from 19 to 24 days (Jarosz, Deans and Dukelow, 1971).

The duration of the estrous cycle in mature Boer doe is 20.7 ± 0.7 days and The mean duration of the natural estrus period is 37.4 ± 8.6 h (Greyling, 1988; 2000). Estrous cycle length in Shiba goats in Japan was 21 days (Sawada, Takahara and Mori, 1995). Moreover, The length of estrous cycle in Egyptian Baladi and Damascus goats was 19.9 ± 0.5 and 19.5 ± 0.6 days, respectively (Medan, 1998).

Serum progesterone is very low on the day of estrus (mean 0.35 ng/ml) and increases to maximum levels (mean 5 ng/ml) on approximately Day 13 of the cycle. The position of the LH peak (indicative of ovulation) varied in the Boer doe, being 8.0 ± 1.5 h following the onset of estrus. The time of ovulation in the goat is reported as occurring towards the end of the estrus period (Van der

Westhuysen, Wentzel and Grobler, 1985; Gonzalez-Valle, Batista and Garcia, 1998). In the Boer goat doe, the time of ovulation is recorded as occurring 36.8 h after the onset of estrus, with the mean time interval between the LH peak and ovulation being 24.7 h (Greyling, 2000).

A high ovulation rate is an important characteristic referring to the number of ova liberated and eventually expressed as the number of kids born per doe kidding. The mean ovulation rate recorded in Boer goat is 1.72 ± 0.9 ovulations per doe. The percentage of singletons, twins, triplets and quadruplets born in Boer goat are 24.5, 59.2, 15.3 and 1 %, respectively (Campbell, 1994) while on Egyptian Baladi goats are 34.9, 50.9, 13.3 and 0.9 % (Medan, 1998).

Gestation period and uterine involution:

The mean gestation period for Boer goat is recorded as being 148.2 ± 3.7 days. Furthermore, the pregnancy period in Egyptian Baladi and Damascus goats is 149.2 ± 0.3 and 149.4 ± 0.4 days, respectively (Medan, 1998). The interval from parturition to a subsequent pregnancy is a factor of major economic importance and hence the involution of the post-partum uterus must be seen as one of the important limitations in achieving the goal of optimal reproductive efficiency. In the Boer goat, macroscopic changes of the post-partum uterus in weight and volume rapidly decline from parturition to approximately Day 12 post-partum. On Day 12 post-partum, the uterus weight is 15% of its weight at parturition. By Day 20, it is 8% of that at parturition and only 27% more than the uterus weight of maiden Boer goats. According to these observations, it would seem that the involution process of the Boer goat uterus is macroscopically complete by approximately 28 days post-partum (Greyling and Van Niekerk, 1991).

The post-partum anestrus period:

The interval between parturition and the first post-partum estrus is an important trait which contributes to the productive efficiency. The mean duration of the post-partum anestrus period is 70 days in the Creole goats (Chemineau, 1983), 55.5 ± 24.9 days in Boer goats (Greyling, 1988) and 124.8 ± 4.1 & 166.4 ± 10.6 days in Egyptian Baladi & Damascus goats, respectively (Medan, 1998). Resumption of cyclic activity in the goat is very susceptible to external factors such as season, suckling and presence of the male (Greyling, 2000).

Improvement of reproductive efficiency in goats

Induction of estrus during the non-breeding season

Seasonal control of ovarian function:

The breeding interval of goats in temperate climates is seasonal and initiated by decreasing photoperiod (Robertson, 1977; BonDurant, Darien, Munro, Stabenfeldt, and Wang, 1981; Papachristoforou, *et al*., 2000). Seasonal regulation of reproductive function is controlled in a variety of vertebrate species by body's response to the duration of day length. In mammals, light entering the eye acts through circuitous neural paths to inhibit pineal conversion of serotonin to melatonin. The pineal gland provides the body with humoral translation of day length information through melatonin secretion. Melatonin further acts to modulate reproductive events in various ways for long- or short-day breeding species (Haibel, 1990[a]).

Administration of exogenous gonadotropins:

sheep are short-day breeding vertebrates that have been studied extensively (Legan and Karsch, 1980; Bittman and Karsch, 1984; Robinson, Wayne and Karsch, 1985). Much of approach to the goat is predicated on the assumption that its reproductive cycles are controlled in much the same manner (Maeda, Mori and Kano, 1986).

Gonadotrophic stimulation of goats for out-of-season breeding has been attempted with a variety of agents. Many protocols for out-of-season breeding in goats have been extensively tested. Vaginal pessaries containing 40 or 45 mg flurogestrone acetate are left in place for 18 to 21 days. Forty-eight hours prior to sponge removal, 400 IU for doelings[*] to 600 IU for adult does of pregnant mare serum gonadotropin are administered i.m. Fertile estrus occurs in a high proportion (greater than 80%) of does in the 24-hour period beginning 12 hours after sponge removal, although continued estrous cyclicity does not ensue. (Corteel, 1975). Treatment protocols using repeated small doses of GnRH have been reported in the ewe (McLeod, Haresign and Lamming, 1982) and goat (Knight, Wilde, McLeod and Haresign, 1988). Administration of 1500 ng of GnRH intravenously every 2 hours for 2 to 3 days in progestin pessary-primed animals resulted in estrus and a physiologic level of ovulation (average of 2.75 ova per doe) in 9 out of 10 lactating does. Pregnancy was successfully carried to term in 5 out of 10 does (Knight *et al*., 1988). Subcutaneous ear implants containing the synthetic progestin norgestomet are available and labeled for the

[*] female goats from one year old to first parturition

synchronization of estrus in beef cattle and dairy heifers. Half of the 6-mg cow implant is an effective luteal substitute in the sheep (Ainsworth and Wolynetz, 1982) and goat (Bretzlaff and Madrid, 1985). Implants may be placed subcutaneously on the dorsum of the pinna, as in cattle, or the ventrum of the tail. Synchronization of estrus in seasonally anestrous dairy goats has been demonstrated using norgestomet implants and PMSG (Bretzlaff and Madrid, 1989). Implants were left in place for 11 days, and a luteolytic dose of prostaglandin and 500 IU of PMSG were given 24 hours prior to implant removal. Estrus occurred within 25 hours of implant removal in over 90% of treated goats. Pregnancy rates ranged from 56% to 67% in two naturally serviced herds, as assessed by real-time ultrasonography at 39 to 53 days post breeding.

Follicle-stimulating hormone (FSH) is the pharmacologic mainstay for superovulation in bovine embryo transfer industry. Haibel (1990[a]) conducted a trial on 12 to 14 month-old Alpine doelings using 3-mg norgestomet implants and FSH. Implants were left in place for 11 days, and declining doses of FSH were given twice daily over 3 days, beginning 2 days prior to implant removal. Doelings in three groups of six received no FSH, 5.25 mg or 10.5 mg total dose. Evaluation of ovulation by measurement of progesterone 8 days post implant removal and for pregnancy at 30 days post breeding by transabdominal real-time ultrasonography revealed none of six, two of six and six of six with elevated progesterone and images consistent with pregnancy.

The sudden introduction of the male to does separated from bucks for at least 3 weeks results in a surge of luteinizing hormone (LH) and a rise in progesterone within 5 to 7 days in a high proportion (greater than 90%). Corpora lutea are short-lived (75%) and are generally followed by ovulation and a normal cycle (Ott, Nelson, and Hixon, 1980; Chemineau, 1983). The physical presence of buck provokes higher levels of ovulation in seasonally anestrous females than does pheromonal stimulation alone, indicating the existence of visual and tactile/behavioral cues (Shelton, 1980; Veliz, Moreno, Duarte, Vielma, Chemineau, Poindron, Malpaux and Delgadillo, 2002).

In another study in sheep, Knight, Baptiste and Lewis (2002) demonstrated that abrupt ram introduction increased LH secretion, stimulated follicular development and induced an LH surge and ovulation in ewe lambs during seasonal anestrous.

Effects of progesterone priming:

Anestrus in ewes and goats is characterized by absence of estrus and ovulation due to decreased LH pulse frequency in response to increased

hypothalamic sensitivity to the negative feedback effect of estradiol (Legan and Karsch, 1980; Goodman, Bittman, Foster and Karsch, 1982). The occurrence of some follicular growth during anestrus with the more frequent incidence of small and medium than large follicles suggests that only partial gonadotropin support is present at this time (Matton, Bherer and Dufour, 1977). During anestrus, ovulation can be induced with exogenous LH or GnRH (McLeod and Haresign, 1984; Hunter, Southee, McLeod, and Haresign, 1986), but these treatments always result in a high proportion of ewes with premature luteal regression. This can be avoided by previous priming with luteal-phase concentrations of progesterone (McLeod and Haresign, 1984; Hunter *et al*., 1986; Southee, Hunter and Haresign, 1988[a]). In anestrous ewes induced to ovulate with GnRH, hysterectomy prevented premature luteal regression (Southee, Hunter, Law and Haresign, 1988[b]; Hu, Nephew, Pope and Day, 1991) implicating the uterus as a requirement for luteal regression. Progesterone priming prior to ovulation reduced the concentration of endometrial oxytocin receptors (Hunter, Ayad, Gilbert, Southee and Wathes, 1989) and uterine responsiveness to oxytocin for release of $PGF_{2\alpha}$ (Vallet, Lamming and Batten, 1990), which allowed the corpus luteum to be maintained. These results explain progesterone priming in anestrous ewes since the reduction of uterine sensitivity to estradiol in early diestrus allows normal luteal function. Nevertheless, there may also be effects of progesterone priming on ovarian follicular dynamics, as observed in cows (Sirois and Fortune, 1990).

Out-of-season breeding in goats:

Attempts to induce estrus in dairy goats during the non-breeding season have primarily involved the use of exogenous progesterone and/or gonadotropins (Ahmed, Phelps, Foote and Foote, 1977; Corteel, 1977; Zarkawi, Al-Merestani and Wardeh, 1999). In addition, photoperiod manipulation (BonDurant *et al*., 1981) and sudden introduction of males (Walkden-Brown, Restall and Henniawati, 1993; Veliz *et al*., 2002) induced estrus during the non-breeding season in goats. Pregnancy rates as high as 40-60 % have been attained out of breeding season (Corteel, 1977). Administration of equine chorionic gonadotropin has been shown to be necessary to get a satisfactory ovulatory response in anestrous goats (Ritar, Maxwell and Salamon, 1984). Knight *et al*. (1988) demonstrated that multiple injections of small doses of GnRH induce ovulation and normal luteal function in seasonally anestrous goats at peak lactation. A single i.m. injection of equine chorionic gonadotropin administered at the end of progestagen treatment advanced the onset of estrus, increased ovulation

rate and induced a tighter synchrony of ovulation (Cognie, 1990). Unfortunately, in dairy goats, repeated treatment with eCG induces the production of anti-eCG antibodies that clearly have negative effect on reproduction (Roy *et al*., 1999).

Estrus synchronization:

Estrus synchronization is a valuable management tool that has been successfully employed in enhancing reproductive efficiency particularly in sheep, cattle and goats (Carlson, Pohl, Mercek, Muser and Wheaton, 1989; Odde, 1990; Kusina, Tarwirei, Hamudikuwanda, Agumba and Mukwena, 2000). One of the advantages of estrus synchronization is that a large number of does are bred over a short period. As a result, management can be concentrated during short periods. Does can be bred at a time that will be suitable for best marketing opportunities. Synchronization would also allow producers to schedule kidding to take advantage of feed supplies, labor. and implementation of breeding. Synchronization in does as in cows and ewes is achieved either by reducing the length of the luteal phase of the estrous cycle with prostaglandin $F_{2\alpha}$ and /or its analogues such as cloprostenol (Estrumate) or extend it artificially with exogenous progesterone or more potent progestagens (Molowuku, Ogunbiyi and Sooriyamoorthy, 1980; Devendra and Burns, 1983; Karatzas, Karagiannidis, Varsakeli and Brikas, 1997; Oliveira, Guido and Lima, 2001).

Prostaglandin $F_{2\alpha}$ or its synthetic analogues induce the lysis of the corpus luteum between the 4^{th} and the 6^{th} days of the estrous cycle (Bosu, Serna and Barker, 1978) and consequently are only effective during the breeding season (Thimonier, 1981). The use of the synthetic analogue of $PGF_{2\alpha}$, cloprostenol, showed to be effective when it was used at 125 µg (Moore and Eppleston, 1979) or 62.5 µg (Romano, 1998).

Administration of 125 to 250 µg cloprostenol l0 days apart induced estrus within 48 h in goats. Administration of 2 doses of $PGF_{2\alpha}$ 10days apart resulted in 84% of Red Sokoto does (Molowuku *et al*., 1980) and 92% of Mashona does (Kusina *et al*., 2000) being synchronized.

Multiple injections, Subcutaneous implants, feed additives, intravaginal sponges and devices have been used to administer progestagens in sheep. The development of the intravaginal sponge by Robinson (1976) provided a practical technique for delivering progesterone with abrupt termination that triggered estrus in most treated ewes. In this case, when left in situ for 12 to 14 days, most ewes exhibited estrus within 3 to 5 days after removal of the sponge. Karatzas *et al*. (1997) reported that 91 to 95% of does were synchronized using intravaginal medroxyprogesterone acetate pessaries. Potent analogues of progesterone, mainly

Cronolone (flurogesterone acetate) have been incorporated into commercial intravaginal sponges and devices because they are effective in small amounts. Despite the wide adoption of progestagen-based systems of synchronization, fertility at the synchronized estrus has been variable in cattle (Brown, Odde, King, Lefever and Neubauer, 1988; Odde, 1990) and sheep (Quipse, Zarco, Valencia and Ortiz, 1994). Reduced fertility reported in some studies has been attributed to compromised sperm motility and fertility in the cervix and uterus in ewes (Hawk and Echternkamp, 1973). In addition, abnormal oocyte development was reported to compromise fertility in cattle (Kinder, Kojima, Bergfeld, Wehrman and Peters, 1996). In goats, the use of progestagens has been found to have no adverse effects or depressed fertility and kidding performance (Robin, Laforest, Lussier and Guilbault, 1994; Kusina *et al*., 2000).

Ultrasound and reproduction

Reproductive performance and efficiency is one of the most important factor that affects profitability. Achieving optimum levels of reproductive performance and subsequently high levels of reproductive efficiency is our top goal. Obtaining profitable levels of performance and efficiency require that producers continually evaluate new technologies and tools that become available. One tool that has recently become available for use is ultrasonography. While this is not a new technology, its application to goats is more recent. Ultrasound technology is a non-invasive, non-destructive, humane tool that can be used on live animals (Zalesky, 1993; Bartlewski, Beard, Cook and Rawlings, 2002).

Basic ultrasound physics:

Detailed information on the principles of ultrasonography has been reviewed by Ginther (1995). Ultrasonography utilizes high frequency sound waves to produce images of tissues and internal organs. The high frequency sound waves are produced by vibrations of specialized crystals (piezoelectric crystals) located in an ultrasound probe or transducer. These waves are emitted from the transducer and are directed toward the tissues or structure of interest. The tissue or structures ability to reflect the sound waves will determine its ultrasonic characteristics. Reflected sound waves are sent back to the transducer, converted to electric current and subsequently appear as an echo image on the viewing screen of the ultrasound machine. The echo image on the screen will appear in varying shades of gray (black to white). The shade of gray is determined by the density of the tissue encountered by the sound waves and subsequently the amount of sound waves reflected back to the transducer. Tissue or structures can be classified as

either echogenic or nonechogenic in their capacity to reflect sound waves. Echogenic structures reflect some or most of the sound waves, depending on their density. The denser the tissue, the more sound waves are reflected and the more white the image will appear on the screen. Since bone is the most dense tissue in the body it will appear as very white on the screen. Fluids do not reflect sound waves and hence are non-echogenic. Such structures (embryonic vesicles, ovarian follicles) will appear as black areas on the screen (Haibel, 1990[b]; Beal, Perry and Corah, 1992; Horder, Barnett and Edwards, 1996).

Imaging of ovarian activity

Non-cycling females during the breeding season is one of the biggest factors that reduces reproductive efficiency in goats. Certainly increasing the number of females cycling or hastening conception would improve reproductive efficiency in a given herd. Determining which females are cycling before or during the breeding season would provide useful information to improve reproductive management. Ultrasound imaging can provide such information.

Ovarian follicles:

Folliculogenesis is the process of forming mature follicles capable of ovulation from the pool of nongrowing primordial follicles in the ovary (Spicer and Echternkamp, 1986). Ovarian follicles are fluid-filled structures surrounded by an inner layer of granulosa cells and an outer layer of thecal cells. The oocyte is suspended within the antrum by a specialized pedicle of granulosa cells called the cumulus oophorous. Because fluid absorbs rather than reflects ultrasound waves, fluid-filled structures such as antral follicles appear as black circular structures surrounded by echogenic ovarian tissue. Most veterinary grade ultrasound scanners can resolve ovarian follicles with a diameter of 2 to 3 mm or greater (Pierson and Ginther, 1988).

Corpora lutea:

The CL is a transient endocrine gland that forms after ovulation from the tissues that previously constituted the ovarian follicle. Thus, the CL can be viewed as the terminal stage of follicular development. Corpora lutea appear as distinctly echogenic areas within the ovarian stroma. Many corpora lutea appear as a solid tissue masses but may also contain fluid-filled cavities. Ultrasonographic attributes of CL including cross-sectional diameter, luteal area, and echogenicity have been correlated to luteal structure and function (Kastelic, Bergfelt and Ginther, 1990[a]; Singh, Pierson and Adams, 1997; Battocchio,

Gabai, Mollo, Veronesi, Soldano, Bono and Cairoli, 1999).

Ovarian cysts, tumors and abscess:

Currently, diagnosis of cysts in cattle most often occurs during routine postpartum rectal examinations conducted by a bovine practitioner. Palpation per rectum of a large fluid-filled structure is commonly used as a clinical indication of a follicular cyst. Differentiation between follicular and luteal cysts via rectal palpation is difficult even for experienced practitioners. Accuracy of diagnosis increases when using transrectal ultrasonography (Farin, Youngquist, Parfet, and Garverick, 1992; Dobson, Ribadu, Noble, Tebble and Ward, 2000; Noble, Tebble, Harvey and Dobson, 2000; Lopez-Gatius, Santolaria, Yaniz, Ruthant and Lopez-Bejar, 2001). In addition, ovarian tumor and abscess can be diagnosed (Zulu, Mwanza, Patel, Makondo and Bhaiyat, 2000). On the other hand, diagnosis of ovarian cysts is impossible using rectal palpation in goats and transrectal ultrasonography resolved this problem.

Ovarian structures as diagnostic aids:

Ultrasonic imaging is a highly accurate and rapid method for assessing ovarian structures. Presence or absence of a corpus luteum aids in diagnosing pregnancy status, especially when conducting pregnancy exams early post-breeding. When present, the size and location (i.e., left vs. right ovary) of the corpus luteum indicates the location of the conceptus within the uterus if the goat is pregnant. The presence of multiple corpora lutea is a diagnostic indicator of multiple pregnancies. Ovarian pathologies such as “static ovaries” and follicular and luteinized cysts can easily be distinguished.

imaging the uterus and conceptus

Early pregnancy diagnosis:

Detection of pregnancy with ultrasonography provides two potential advantages over traditional palpation. The first is earlier detection of the presence of an embryo and secondly, increased speed and accuracy of detecting early pregnancies. Earlier pregnancy detection with ultrasound offers the producer an earlier chance to make management decisions regarding open goats. Such decisions could lead to a reduction in overhead and feed costs by identifying and culling open females sooner than normal.

Early embryonic loss:

Pregnancy loss contributes to reproductive inefficiency and using ultrasonography for early pregnancy diagnosis improves reproductive efficiency. The technique of ultrasound itself has not been implicated as a cause of embryonic death (Baxter and Ward, 1997). Furthermore, ultrasound is a much less invasive technique for early pregnancy diagnosis and may minimize the incidence of palpation-induced abortions using rectal-abdominal palpation. Transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by reducing the period from breeding to pregnancy diagnosis with a high degree of diagnostic accuracy and detection of embryonic loss and fetal death (Bourke, Adam and Kyle, 1992; Bretzlaff, Edwards, Forrest and Nuti, 1993; Garcia, Neary, Kelly and Pierson, 1993; Martinez, Bosch and Bosch, 1998).

Identification of fetal number:

Goats carrying multiple fetuses can be accurately identified using ultrasonography. The presence of two or more CL on the ovaries at the time of pregnancy diagnosis is an excellent indicator of multiple pregnancies (Wiltbank, Fricke, Sangsritavong, Sartori, and Ginther, 2000).

Diagnosis of uterine pathology:

Uterine conditions that can impair fertility as mucometra, hydrometra, pyometra can be diagnosed using ultrasonography (Fissore, Edmondson, Pashen and BonDurant, 1986; Kahn and Leidl, 1989).

Ovarian dynamics and hormonal profile during estrous cycle

Understanding factors regulating the estrous cycle of goats is an essential component of reproductive management and improving reproductive efficiency. Studies of ovarian follicular dynamics may lead to methods that increase the response to estrous synchronization protocols and pregnancy rates resulting in an increase in production efficiency.

Growth and turnover of follicles:

Most early studies characterizing follicular dynamics were limited to the growth phase of the ovulatory follicle. Examination of daily ultrasound scans in cattle demonstrated that this phase could be divided in 2 time periods. During the first period, the number of follicles growing (forming a cohort) was higher than

the number of ovulations. During the second period, the number of growing follicles was equal to the number of ovulations. This was associated with the presence of large follicle(s) and simultaneous regression of the other follicles of the cohort. More specifically, recruitment was defined as the initiation of gonadotropin-dependent folliculogenesis. Wave emergence is also used to describe this process (Ginther, Wiltbank, Fricke, Gibbons and Kot, 1996). During the mid-follicular phase, at the time of selection, the number of cohort follicles becomes adjusted to the number of ovulations. As a consequence a dominant follicle(s) appears, while the other follicles of the cohort regress by atresia. In addition, during the dominance phase, recruitment does not occur (Driancourt, Gougeon, Royere and Thibault, 1993; Driancourt, 2001).

At selection the dominant follicle(s) is chosen and the remaining follicles of the cohort become subordinate follicles and enter atresia. This is usually demonstrated by a block in their growth rate followed by a steady decrease in size. It is generally assumed that the largest follicle(s) of the cohort is likely to be the one(s) selected for ovulation (Ginther *et al*., 1996). In all species, the selected follicle(s) appears to be the first one(s) developing LH receptors on its granulosa cells. Follicles develop LH receptors when they reach 4, 5 to 6, 8 and 25 mm in diameter in sheep, pigs, cattle and horses respectively (Driancourt, 2001). In all species, there is a close association between these sizes and the sizes at which follicles are selected. In contrast, the intensity of selection (measured by the proportion of the cohort follicles surviving) is highly variable among species. It can be very low in some horses which have a small cohort size, while it is very high for cattle (one selected follicle out of 5 cohort follicles) and swine (12 selected follicles out of a cohort size of 50 (Driancourt, 2001).

During follicular dominance, preovulatory follicular growth and maturation occur. The other follicles of the cohort complete regression by atresia. There is a direct relationship between the presence of the dominant follicle and the absence of recruitment, as cauterization or aspiration of the dominant follicle immediately induces recruitment (Ko, Kastelic, Del Campo and Ginther, 1991; Tottei, Shi, Ozawa, Imai, Takahashi, Shimohira, Kojima, Watanabe and Taya, 2001).

Mechanisms controlling recruitment, selection and final maturation of follicles:

FSH is the key hormone inducing recruitment. An association between FSH surge and recruitment has been demonstrated in sheep (Ginther, Kot. and Wiltbank, 1995), cattle (Ginther *et al*., 1996) and goats (Medan, Taya, Watanabe, Shalaby, Sharawy and Sasaki, 2001). The main effect of FSH is to induce aromatase activity within granulosa cells (Saumande, 1990), explaining why

follicles gain the ability to produce estradiol and stimulate the production of inhibin and follistatin (Knight, 1996; Singh and Adams, 1998).

The selection process has been exclusively studied and two theories are generally offered to explain the mechanisms involved. The first, selection is controlled by endocrine mechanisms only (reduction in FSH support to follicles) and the second, there is production of compounds by the largest follicle directly inhibit development of the other follicles of the cohort. It is likely that in species with a loose control of ovulation rate (sheep), only the FSH dependent mechanism operates, while in species with a strict control of ovulation rate (cattle, primates) both mechanisms may be operating (Driancourt, 2001; Mihm, Crowe, Knight and Austin, 2002). The idea that the drop in FSH concentrations, which occur 2 to 3 days after recruitment, is a key mechanism in the selection process is supported by several lines of evidence. First, in most species, there is a close temporal relationship between selection and the time when FSH levels reach their nadir. This drop in FSH support is caused by a combined action of inhibin and estradiol which are produced by follicles > 5 mm in diameter and act by negative feedback on the pituitary (Gibbons, Wiltbank and Ginther, 1997; Mihm, Good, Ireland, Ireland, Knight and Roche, 1997). Second, an injection of exogenous FSH to delay the drop in FSH results in a delay in the selection process in sheep (Driancourt, Webb and Fry, 1991) and cattle (Adams, Kot, Smith and Ginther, 1993[a]; Mihm, *et al.*, 1997).

There is general agreement that LH is the key hormone involved in the final growth of the dominant follicle while other follicles in the cohort complete atresia. Exogenous injection of LH pulses to post-partum beef cows, in which a limited pulsatile pattern of LH secretion has been demonstrated, results in an increased maximum diameter of the dominant follicles and increased duration of dominance (Duffy, Crowe, Boland and Roche, 2000). Furthermore, treatment of cows with a GnRH agonist, which acutely suppresses LH pulses, blocks follicular growth at the size at which follicles become dominant *i.e.*, 8-9 mm (Webb, Campbell, Garverick, Gong, Gutierrez, and Armstrong, 1999).

Synchronization of wave emergence:

The aim of exogenous control regimens is to elicit a desired reproductive status at will, so that diagnostic or interventional procedures can be scheduled to optimize time, labor and results. Past regimens have focused primarily on lengthening or abbreviating the luteal phase through exogenous progesterone or luteolytic agents. However, there is considerable variation in the interval from treatment to estrus and ovulation subsequent to such treatment and much of the

variability has been attributed to the status of the follicular wave at the time of treatment. Studies on the response to a luteolytic dose of prostaglandin $F_2\alpha$ given at different times of the estrous cycle indicate that the extant (viable) dominant follicle(s) will ovulate at the time of luteolysis (Kastelic, Knopf and Ginther, 1990[b]). If luteolysis is induced after the mid-static phase of a dominant follicle(s)(defunct), the dominant follicle(s) of the next wave will grow and become ovulatory follicle(s), resulting in a longer interval from treatment to ovulation. To reduce the variability of present synchronization scheme, a method of controlling follicular wave emergence is needed. Progesterone and estrogen treatment or follicle ablation have been used as means of ablating the effects of the dominant follicle(s) and thereby influencing subsequent wave emergence (Adams, 1994).

Imprecision in the degree of ovarian synchrony and variability in response to superstimulation have continued to be the most confounding factors in implementation of advanced reproductive technology (Armstrong, 1993). As a sequence of our new understanding of ovarian function, afforded by ultrasound technology, endogenous rhythms (*i.e.*, wave emergence, follicle selection and dominance) may be exploited to potentiate treatments designed to control, enhance or suppress follicular development and ovulation.

Follicular dominance:

Waves of follicular growth and a dominant follicle are found in cattle (Fortune, 1994; Ginther *et al*., 1996; Roche, Mihm, Diskin and Ireland, 1998) and despite early controversy, it appears that there are also waves of follicular growth in sheep (Noel, Bister and Paquay, 1993; Ginther *et al*., 1995; Leyva, Buckrell and Walton, 1998; Evans, Duffy, Hynes and Boland, 2000). In cattle, a wave of follicular growth involves the synchronous growth (> 4-8 mm in diameter) of a cohort of follicles from the follicle reserve, followed by the continued development of one follicle (dominant follicle) and atresia of all other follicles from the original cohort. Emergence of a new wave occurs after the dominant follicle from the previous wave undergoes atresia (Ginther *et al*., 1996). A wave-like pattern of follicle growth similar to that in cattle has been described in sheep and goats, however the diameter of follicles is smaller than in cattle: emergence occurs at a follicle diameter of 2 mm and the maximum diameter achieved by ovulatory follicle is 6-7 mm (Ginther and Kot, 1994; Evans *et al*., 2000).

The existence of dominance in sheep and goats is still unresolved. Some authors have reported a weak form of dominance may exist on the basis of observation that the largest follicle may delay or prevent the development of other

follicles (Ravindra, Rawlings, Evans and Adams, 1994; Ginther *et al*., 1995; Rubianes, Ungerfeld, Vinoles, Rivero and Adams, 1997). Evans *et al*. (2000) argued for the occurrence of dominance in sheep on the basis of a hierarchy in terms of diameter and estradiol concentration of the largest follicle, and on relationship between the demise of the largest follicle and the emergence of subsequent wave. The mechanism involved in the selection of the dominant follicle is unclear. In cattle, it appears that the future dominant follicle is capable of switching its dependency from FSH-secretion to LH and, therefore, can survive in an environment of decreasing FSH concentrations (Ginther *et al*., 1996; Webb *et al*., 1999). However, there is also evidence that the dominant follicle inhibits the growth of other follicles. In cattle, removal of the dominant follicle on day 3 of estrous cycle resulted in delayed regression of the largest subordinate follicle and early emergence of the second wave of follicular growth (Ko *et al*., 1991; Adams, Kot, Smith and Ginther, 1993[b]). The emergence of a new wave has been associated with a transient increase in FSH concentrations in cattle (Adams, Matteri, Kastelic, Ko and Ginther, 1992; Evans, Komar, Wandji and Fortune, 1997), sheep (Souza, Campbell and Baird, 1998; Bister, Noel, Perrad, Mandiki, Mbayahaga and Paquay, 1999; Bartlewski, Beard and Rawlings, 2000) and goats (Medan *et al*., 2001). Advancing the timing of FSH increase and emergence of a new wave of follicles by ablation of the dominant follicle and postponement of the FSH increase and emergence of a new wave of follicles by iv treatment with follicular fluid demonstrate that the dominant follicle actively suppresses the growth of other follicles in cattle (Adams *et al*., 1992) and sheep (Evans, Flynn, Duffy, Knight and Boland, 2002).

Evans and Martin (2000) concluded that in mammals species-specific numbers of dominant follicles are selected from a cohort of follicles to ovulate when the endocrine environment permits. The mechanism by which one or more follicles continue development while other follicles regress is not known. However, it is likely that this occurs as a result of the integration of external endocrine signals and locally produced paracrine signals.

Ultrasonographic observation of ovarian dynamics:

Until recently, the techniques used in studying patterns of follicular development involved measurement, counting and histological evaluation of ovaries of animals killed at various times during the estrous cycle or marking of follicles with ink, followed by serial laparoscopy. In contrast, the development of ultrasonic probes that can be used intrarectally to visualize ovaries opened new possibilities for examining the dynamics of follicular growth and regression

(Fortune, Sirois, Turzillo and Lavoir, 1991) and provided a mean for repeated, direct, non-invasive monitoring and measuring of follicles within the ovary (Griffin and Ginther, 1992). Serial laparotomy and ink labeling of large follicles at three different times during the estrous cycle led to the conclusion that three or possibly more phases of follicular growth and atresia occurred during the estrous cycle of ewes (Smeaton and Robertson, 1971).

Complete examination and description of ovarian activity during all stages of the estrous cycle is limited. Early studies using serial laparotomies (Camp, Wildt, Howard, Stuart and Chakraborty, 1983) or ovaries from slaughterhouse (Batista, Gonzalez and Garcia, 1993) could not consistently characterize follicular development during the caprine estrous cycle. A recent study in goats using utltrasonography indicated that ovarian follicles reaching ovulatory size throughout the estrous cycle exhibited a wave-like pattern (Ginther and Kot, 1994; de Castro, Rubianes, Menchaca and Rivero, 1999). Some of the factors found to affect the number of waves per estrous cycle include dietary intake (Murphy, Enright, Crowe, McConnel, Spicer, Poland and Roche, 1991), parity and lactational status (Lucy, Savio, Badinga, Delasota and Thatcher, 1992).

Dynamic changes in plasma concentrations of gonadotropins, inhibin, estradiol-17β and progesterone during estrous cycle in goats:

Endocrine events in peripheral blood during the estrous cycle have been studied in details in goats (Chemineau, Gauthier, Poirier and Saumande, 1982), ewe (Campbell, Mann, McNeilly, and Baird, 1990) and cattle (Sirois and Fortune, 1990). However, the temporal relationships between follicular dynamics and hormonal profiles have not been fully clarified yet throughout the goat estrous cycle. In cattle, a high correlation between ultrasonic assessment of the corpus luteum and peripheral progesterone levels has been found (Kastelic *et al*., 1990[b]; Singh *et al*., 1997). However, a functional demise of the CL precedes physical regression by 1 to 2 days (Kastelic *et al*., 1990 [b]). There is a temporal relationship between elevations in mean daily serum concentrations of FSH and emergence of successive follicular waves (Ginther *et al*., 1995; Bartlewski, Beard, Cook, Chandolia, Honaramooz and Rawlings, 1999). Gibbons, Kot, Thomas, Wiltbank and Ginther (1999) found that surges of FSH were rhythmic and periodic (every 3 or 4 days). The hypothesis of a temporal association between FSH surges and both ovulatory and anovulatory follicular waves was supported by the emergence of follicular waves near the peak of an FSH surge. The role of inhibin in regulating the production and secretion of FSH has been documented in sheep, both *in vivo* (Mann, Campbell, McNeilly and Baird, 1992[a]) and *in vitro* (Clarke, Rao, Fallest and Shupnik, 1993).

inhibin: synthesis and practical uses

Structure of inhibin:

Inhibin is a glycoprotein hormone secreted by granulosa (female) and Sertoli (male) cells in response to FSH, and its major action is the negative feedback control of pituitary FSH secretion (Ying, 1988; Knight, 1996). It is found in blood plasma, although difficult to detect until recently. It is found in great quantities in seminal plasma and follicular fluid. Inhibin is a dimeric protein of great complexity. Inhibin has a molecular weight of 32,000 daltons, and consists of one alpha-chain (approx 18 kDa) and one beta-chain (14 kDa) linked by disulphide bridges. The subunits alone possess no known biological action (Robertson, Foulds, Leversha, Morgan, Hearn, Burger, Wettenhall and de Krester, 1985; Leversha, Robertson, de Vos, Morgan, Hearn, Wettenhall, Findlay, Burger and de Kretser, 1987).

The inhibin family is further complicated by the existence of two separate beta-subunit genes, and thus two distinct proteins. These have been termed the beta-A subunit and the beta-B subunit. Thus there are two types of inhibin, inhibin-A or inhibin-B. While inhibins are dimers of alpha and beta subunits, dimers of beta-B subunits form another hormone, activin. Activin is a general growth-stimulating factor, and appears to have local effects on many cell types. Additionally, activin stimulates FSH secretion, thus antagonizing the main biological action of inhibin (Vale, Hsueh, Rivier and Yu, 1990; Li, Philips and Mather, 1995).

In accordance with the negative feedback relationship between inhibin and FSH, an inverse relationship between the release of pituitary FSH and inhibin was reported in ewes (Findlay, Clarke and Robertson, 1990), heifers (Bleach, Glencross, Feist, Groome and Knight, 2001) and goats (Medan *et al*., 2001).

Uses of inhibin in domestic animals:

As inhibin acts systemically to inhibit FSH release, it follows that a reduction of inhibin secretion would increase FSH concentrations and thus offer potential for increased fertility. Domestic ruminants have been immunized against a variety of inhibin preparations, and increases in ovulation rate have been reported in mare (McCue, Carney, Hughes, Rivier, Vale and Lasley, 1992), sheep (Anderson, Bindon, Hillard and O'Shea, 1998), cattle (Glencross, Bleach, Wood and Knight, 1994) and goats (Dietrich, Hennies, Holtz and Voglmayr, 1995).

Ovarian response and hormonal profile following injection of inhibin antiserum:

Inhibin is a glycoprotein hormone that selectively inhibits secretion of follicle stimulating hormone (FSH) from the pituitary gland (Burger, 1988). A negative relationship between plasma concentrations of FSH and inhibin has been established in several mammalian species (Taya, 1993; Taya and Watanabe, 1999). The number of follicles that develop to an ovulatory size depends on both the amount of FSH and the time of exposure to FSH (Picton, Tsonis and McNeilly, 1990; McNeilly, Picton, Campbell and Baird, 1991). The release of FSH by the pituitary is in turn controlled by synergistic action of two of the major products of ovulatory follicles: inhibin and estradiol (Martin, Price, Thiery and Webb, 1988; Mann *et al.,* 1992[a]). The previous findings (Kaneko, Nakanishi, Akagi, Taya, Watanabe, Sasamoto and Hasegawa, 1995; Kaneko, Taya, Watanabe, Noguchi, Kikuchi, Shimada and Hasegawa, 1997), that immunoneutralization of endogenous inhibin produced a significant elevation of peripheral FSH, offer evidence that inhibin is an important factor in the inhibitory regulation of FSH secretion. On the other hand, ultrasonographic observation of the ovary correlated with hormonal profiles demonstrated that an increase in plasma FSH preceded emergence of each follicular wave (Adams *et al*., 1992) and a decrease in FSH was coincident with functional selection of follicles (Sunderland, Crowe, Boland, Roche and Ireland, 1994), suggesting that the fluctuation in peripheral FSH levels is a trigger for growth, selection and atresia of follicles.

Multiple ovulations have been induced successfully by passive immunization against endogenous inhibin in several species such as rats (Rivier and Vale, 1989), ewes (Wheaton, Carlson and Kusina, 1992; Kusina, Meyer, Carlson and Wheaton, 1995), hamsters (Kishi, Okada, Otsuka, Watanabe Taya and Sasamoto, 1996), cows (Akagi, Kaneko, Nakanishi, Takedomi, Watanabe and Taya, 1997), mares (Nambo, Kaneko, Nagat, Oikawa, Yoshihara, Nagamine, Watanabe and Taya, 1998) and guinea pigs (Shi, Mochida, Suzuki, Mateuda, Ogura, Ozawa, Watanabe, Suzuki and Taya, 2000). Thus, these initial results indicated that immunization of animals against endogenous inhibin to induce superovulation through increased endogenous FSH secretion is an alternative method to the current exogenous gonadotropins protocols.

In most studies, the use of a combination of equine chorionic gonadotrophin and human chorionic gonadotrophin has been the most common method to induce superovulation in goats (Pintado, Gutierrez-Adan and Perez Llano, 1998).

However, a disadvantage of these protocols is the long half-life of equine chorionic gonadotrophin which interferes with normal fertilization and embryo development (McIntosh, Moore and Allen, 1975; Armstrong, Pfitzner, Warnes and Seamark, 1983; Ertzeid, Storeng and Lyberg, 1993) and repeated equine chorionic gonadotrophin treatments induced anti-equine chorionic gonadotrophin antibodies that clearly have negative effects on reproduction of goats (Roy *et al*., 1999). Thus it is necessary to establish an alternative simple method for induction of superovulation in goats to overcome these problems.

The aim of this experiment is to determine the effect of passive immunization against inhibin 48 h prior to luteolysis on FSH secretion.

Effect of active immunization against inhibin on hormonal levels and ovulation rate:

In domestic animals, the induction of multiple ovulations is possible by potentiating the stimulatory effects of the endogenous gonadotropin by administering hormones with FSH-like activity or by removing the inhibitory action of ovarian hormones upon gonadotropin release by hypothalamus-pituitary axis. Considerable interest has been placed on inhibin, which by synergistic action with estradiol, is involved in the negative feedback regulation of FSH at anterior pituitary (Findlay and Clarke, 1987; Martin *et al*., 1988; Findlay, Robertson, Clarke, Klein, Doughton, Xiao, Russel and Shukowski, 1992) thereby controlling ovulation rates.

Purification and partial characterization of inhibin molecule revealed that both inhibin forms (A and B) shared a common α-subunit, but different β-subunits (Ling, Ying, Veno, Esch, Denoroy and Guillemin, 1985). Further characterization showed that there was a marked species divergence in the amino acid sequence of the α-subunits compared with the conservative β-subunits (Mason, Hayflick, Ling, Esch, Veno, Ying, Guillemin, Niall and Seeburg, 1985; Stewart, Milborrow, Ring, Crowther and Forage, 1986). Also, the β-subunits were found to exist in dimeric forms (activins), often with opposite biological activity to inhibin (Ling, Ying, Veno, Shimasaki, Esch, Holta and Guillemin, 1986). These observations suggest that the α-subunit, but not the β-subunit, could be suitable as an immunogen to neutralize the activity of both inhibin forms.

Following characterization of inhibin, several synthetic peptides based on the first 25-32 amino acids of amine terminal of bovine (Wrathall, McLeod, Glencross, Beard and Knight, 1990), porcine (O'Shea, Andrews, Bindon, Hillard, Miyamoto and Sinosich, 1991) and ovine (Meyer, Carlson, Rivier and Wheaton, 1991) inhibin, α-subunits were synthesized and used as immunogens to increase

the ovulation rate in ewes. These peptides were conjugated to protein carrier molecules, which is necessary to enhance their immunogenicity (Meyer *et al*., 1991). Immunization with this peptide conjugates was shown to result in the development of antibodies that bound both iodinated synthetic inhibin peptide (Meyer *et al*., 1991; O'Shea *et al*., 1991) and a labeled native inhibin molecule (Wrathall *et al*., 1990). Antibody binding to the native inhibin molecule was significant, confirming speculation that increased ovulation rate after immunization with synthetic peptides based on the inhibin α subunit was due to immunoneutralization of endogenous inhibin.

Immunoneutralization of endogenous inhibin was thought to result in diminished negative feedback on the anterior pituitary resulting in increased follicle stimulating hormone secretion, greater follicular development and increased ovulation rate.

Various strategies have been used to increase prolificacy in goats and other domesticated species. In addition to selective breeding programs (Bradford, 1985), a number of nongenetic methods have received considerable attention including administration of exogenous gonadotropins such as equine chorionic gonadotropin (Gheraradi and Lindsay, 1980; Riesenberg, Meinecke-Tillman and Meinecke, 2001) and autoimmunization against inhibin (Dietrich *et al*., 1995; Anderson *et al*., 1998; Hennies, Voglmayr, Dietrich, Stollmann, Moeller and Holtz, 2001). As such, it would be anticipated that immunoneutralization of endogenous circulating inhibin would lead to an increase in plasma FSH concentrations which could promote ovarian hyperstimulation and increased ovulation rate.

Early pregnancy diagnosis

Ultrasound technology, which had initially been developed to assist ships in avoiding underwater objects was first adopted for use in human medical diagnostics (Dusik, 1942) and later applied to the diagnosis of pregnancy in sheep (Lindahl, 1966).

During recent years, there has been increasing awareness in the need for early diagnosis of pregnancy in goats. A reliable technique for early detection of pregnancy would allow early culling or rebreeding of barren does. A variety of examination methods have evolved over the years. Ultrasonography, hormone assay, and radiography have emerged as the most useful methods utilized today. Older described methods of laparotomy, abdominal palpation, and rectal-abdominal palpation with a rod (Memon and Ott, 1979) have limited utility.

Early diagnosis of gestation and determination of fetal number are of

considerable value in goat reproductive management. Studies on gestation have been done in horses (Ginther, 1986), cattle (Kastelic, Curran, Pierson and Ginther, 1988), llama (Bourke *et al*., 1992), sheep (Garcia *et al*, 1993), buffalo (Pawshe, Appa Rao and Totey, 1994) and goats (Russel, 1990; Martinez *et al*, 1998). Ultrasonic imaging of the heartbeat is regularly used to detect the embryo and to evaluate embryo viability. Heartbeats were first detected between days 19 and 29 in heifers (Pierson and Ginther, 1984; Kastelic *et al*., 1988), ewes (Schrick and Inskeep, 1993) and goats (Martinez *et al*., 1998).

Dawson, Sahlu, Hart, Detweiler, Gipson, Teh, Henry and Bahr (1994) reported that real-time ultrasonography is a reliable method for early pregnancy diagnosis in goats. The technique also enables accurate separation of does carrying singles, Twins and triplets as early as 7 weeks in gestation. Such information can be useful for improved nutritional management. Furthermore, ultrasonography can be used in wild animals. Roth, O'Brien, McRae, Bellem, Romo, Kroll and Brown (2001) used ultrasonography to monitor ovarian activity in Sumatran rhinoceros. Pregnancy was detected as early as day 14 after mating and the heartbeat was detected by day 26.

MATERIALS AND METHODS

Animals and Management:

This study was conducted on 100 Baladi goats in Egypt and 40 Shiba goats in Japan. All goats were housed under natural daylight and temperature. In Egypt, the goats were fed 300 g/head/day of concentrate ration and grazed 4 hours daily on field grasses while in Japan they were fed 700 g/head/day of hay cubes. Clean water and mineralized salt licks were available.

Experiment1:

Induction of estrus during non-breeding season:

This study was carried out using 100 Egyptian Baladi goats (*Capra hircus*) ranging from 2 to 5 years of age. All animals assigned to treatments had low progesterone concentrations (< 0.5 ng/ml) tested 2 times 10 days apart to confirm anestrous condition. Estrus detection was carried out using 5 mature aproned bucks 6-hours intervals for 30 minutes (male to female ratio is 1:20) and all animals exhibited estrus were allowed to be mated. The time of standing estrus, date of mating, date of kidding and litter size were recorded.

Does were randomly assigned to receive either subcutaneous implants containing 3 mg norgestomet[1] inserted on the back of the ear for 11 days and a single i.m injection of 125 μg $PGF_{2\alpha}$ analogue [2] 24 hours before implant removal (group I; n = 40); subcutaneous implants containing 3 mg norgestomet inserted on the back of the ear for 11 days and a single i.m injection of 125 μg $PGF_{2\alpha}$ analogue 24 hours before implant removal followed by im injection of 2.5 ml receptal[3] (10.5 μg) after implant removal by 24 hours (group II; n = 40) or no treatment (control group; n = 20). Blood samples were collected from jugular vein into vacutainer tubes at day 8 after implant removal. Blood samples were left for 3 hours at room temperature to clot and centrifuged at 3000 r.p.m for 15 minutes; serum was separated and stored at –20 °C until assayed for progesterone. To monitor luteal activity circulating progesterone was measured using Radioimmunoassay by direct solid-phase ^{125}I[4].

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[1] Syncro-Mate-B (1/2 implant), Sanofi Animal Health, Inc. Overland Park, KS, USA.

[2] Estrumate; Coopers animal Health Ltd., Berkhamsted, UK.

[3]Synthetic GnRH, buserelin acetate, Hoechst Roussel Vet GmbH D-65203 Wiesbaden, Germany.

[4]Coat-A- Count; Diagnostic products Corporation, Los Angeles, CA, USA.

Experiment2:

Determination of ovarian dynamics and hormonal profile during estrous cycle:

This experiment was carried out on six Shiba goats (polyestrous throughout the year) during 3 consecutive estrous cycles at Laboratory of Veterinary Physiology, Tokyo Univ. of Agriculture and Technology. Their age ranged from 3 to 5 years and their body weight ranged from 25 to 35 kg. Estrous cycle was synchronized with 2 injections of $PGF_{2\alpha}$ analogue [5] (125 μg for each goat) 11 days apart. Estrous behavior was checked every six hours with an aproned mature buck throughout the experimental period.

Ovarian images were obtained with a B-mode scanner[6] equipped with a 7.5 MHz transducer. A slightly arched plastic rod (30 cm length and 20 mm in diameter) was fastened to the transducer to manipulate the probe externally into the rectum. Goats were restrained in a standing position in a wooden chute.

Blood samples were collected daily into heparinized Vacutainer tubes[7]. In order to determine the preovulatory LH surge, blood samples were collected at 2 h intervals beginning 36 h after $PGF_{2\alpha}$ injection and ending at 72 h. Centrifugation at 3000 r.p.m for 15 minutes and plasma was separated and stored at –20 ^{o}C until assayed for hormones.

Ultrasound evaluation:

Ovarian ultrasonic examinations were carried out daily and every 12 hr around ovulation. The operator scanned these animals for 1 month before starting the actual study. All follicles ≥ 3 mm in diameter were recorded and their diameters were measured. Also, diameter, position and characteristics of the CL were registered. After freezing the image on the screen, the maximum internal diameter of each follicle was measured using the in-built electronic caliper. Each day, ovarian diagrams depicting the relative location of follicles ≥ 3 mm and CL were made to determine patterns of growth and regression of individual follicles and CL. The beginning of regression of corpus luteum (luteolysis) was defined as the first day that luteal diameter progressively decreased as reported for heifers (Kastelic *et al.*, 1990[b]).

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[5]Estrumate; Schering Plough Animal Health, New Jersey, USA.

[6] ECHOBAL ultrasound scanner, Hitachi Ltd, Tokyo, Japan.

[7] Terumo Venoject II, Tokyo, Japan.

Follicle data analysis:

The total number of follicles ≥ 3 mm in diameter was assessed daily. The term wave was defined as one or more antral follicles growing from 3 to ≥ 5 mm in diameter before regression (Ginther *et al*., 1995; de Castro *et al.*, 1999; Bartlewski, Beard and Rawlings, 2001). The day of emergence of follicles was identified as the day on which the follicle was 3 mm in diameter. Individual follicles emerging within a maximum of 48 h were regarded as a single follicular wave. The following characteristics of follicular waves were determined for each animal: (1) the number of follicular waves; (2) the days of wave emergence; (3) the number of follicles growing to ≥ 5 mm in diameter per wave; (4) the maximum diameter attained by the largest follicle of the wave; (5) the number of days between the emergence of sequential follicular waves (interwave intervals); (6) the growth and regression rates of the largest follicle of the wave.

The growing phase of a follicle was defined as the period between its emergence and the day that it appeared to stop its progressive increase in diameter. The regression phase of a follicle was defined as the period between its progressive decrease in diameter and the day that it first reached 3 mm in diameter. The static phase of a follicle was defined as the period between the end of the growing phase and the beginning of the regressing phase. The day of ovulation was identified as the first day on which a large follicle disappeared or collapsed (detected retrospectively by their disappearance) and was followed by the development of a CL at that site on the ovary (Bartlewski *et al.*, 2000). The ovulatory follicle was considered to be in a growing phase from 1st detection to ovulation. Ovaries were monitored until at least 7 days after ovulation to confirm ovulation number by the number of CL.

Experiment 3:

Ovarian response and hormonal profile following injection of inhibin antiserum:

This experiment was carried out at Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, on 12 goats clinically normal with regular estrous cycle. Goats were housed under natural day lighting and fed a maintenance diet of 700 g/animal daily of hay cubes. Clean water and mineralized salt licks were available.

Estrous cycles were synchronized with 2 injections of $PGF_{2\alpha}$ analogue 125 μg for each goat 11 days apart. Estrous behavior was checked every six hours with an

aproned mature buck throughout the experimental period. On day 10 of the estrous cycle animals were allocated into two groups: (1) control, treated with i.v. injection of 10 ml normal goat serum (n = 6), (2) inhibin-immunized, treated with i.v. injection of 10 ml inhibin antiserum (n = 6). The time of injection of serum was defined as 0 hr. At 48 h, all animals were injected with 125 μg $PGF_{2\alpha}$ analogue to induce estrus and ovulation.

Blood samples were collected at 6-h intervals from 24 h before until 120 h after treatment and every 2 h from 48 to 72 h after $PGF_{2\alpha}$ injection into heparinized Vacutainer tubes. Additional blood sample for progesterone determination was collected on day 7 after ovulation. Centrifugation at 3000 r.p.m for 15 minutes and plasma was separated and stored at –20 ^{o}C until assayed for hormones.

Ultrasound Scanning and determination of ovarian response:

The follicle populations of the animals were monitored daily starting 24 h before treatment until the end of experiment and at 12-h intervals around ovulation using a B-mode scanner equipped with a 7.5 MHz transducer. A slightly arched plastic rod (30 cm length and 20 mm in diameter) was fastened to the transducer to manipulate the probe externally into the rectum. All follicles ≥ 3 mm in diameter were measured in two planes and the mean was calculated for each follicle. The occurrence of ovulation was assessed as the disappearance of large antral follicles present at the previous transrectal ultrasonography examination confirmed by detection of corpora lutea as described by Rajamahendran, Robinson, Desbottes and Walton (1989). Ovulation rate was determined by matching the number of large antral follicles that disappeared with the number of corpora lutea detected by ultrasonography (Pierson and Ginther, 1988). Follicles were divided into groups according to their mean diameter (small; < 3.5 mm, medium; 3.5-5 mm and large; > 5 mm).

Antisera:

The antiserum against inhibin used in this study was raised in ovariectomized goats to a synthetic peptide of 1-30 amino acid sequence of the N-terminus of the alpha chain of 32 kDa inhibin conjugated to ovalalbumin. The final titre of this antibody, assessed at 50 % binding of ^{125}I-labeled inhibin was 1:40 000.

Determination of inhibin binding activity:

Changes in inhibin binding activity in plasma of the inhibin-immunized goats were determined by measuring the binding of ^{125}I-labeled inhibin (5000 cpm) as reproted previously (Kaneko, Nakanishi, Taya Kishi, Watanabe, Sasamoto and

Hasegawa, 1993). Plasma samples obtained at various times after injection of the antiserum were diluted 1:10 with PBS containing 5% BSA (bovine serum albumin). PBS (100 μL) was added to each aliquot (100 μL) of diluted plasma and incubated for 24 h at 37 ^{o}C with ^{125}I-labeled bovine 32-kDa inhibin. Bound tracer was then separated by adding 100 μL PBS containing 1% bovine gamma globulin and 500 μL PBS containing 25% polyethylene glycol (Mwt, 6000), mixing for three minutes, centrifugation at 3000 rpm for 30 minutes then the radioactivity in the precipitate was counted. Inhibin-binding activity was expressed as a percentage of the total counts added.

Experiment 4:

effect of active immunization against inhibin on hormone levels and ovulation rate:

Preparation of the immunogen:

The immunogen used (inhibin vaccine) was a synthetic peptide, corresponding to the N-terminal sequence (1-26) of α-subunit of porcine inhibin conjugated with rabbit serum albumin as described previously (Konishi, Aoyagi, Takedomi, Itakura, Itoh, Yazawa, Kishi, Taya, Watanabe and Kanagawa, 1996). For immunization 100 μg synthetic peptide per doe was dissolved in 1ml phosphate-buffered saline (PBS) and emulsified with equal volume of Freund's complete adjuvant.

Animals and treatment:

Ten adult Shiba goats (*Capra hircus*) were used in this study. The animals were housed under natural daylight and fed 700 g/animal of hay cubes daily. Mineral salt blocks and water were provided. Estrous cycles were synchronized with 2 injections of 125 μg of $PGF_{2\alpha}$ analogue 11 days apart. Estrus was detected using an aproned mature buck every 6 h. On day 10 of the estrous cycle, animals were allocated to two groups: the immunized group (n=5) treated with s/c injection of 1ml inhibin vaccine emulsified in 1 ml Freund's complete adjuvant into four different sites followed by three booster injections 4 weeks intervals and the control group (n=5) treated with s/c injection of 1ml saline emulsified in 1 ml Freund's complete adjuvant. After the third booster injection, estrous cycles were synchronized by 2 i.m. injection of 125 μg $PGF_{2\alpha}$ analogue. Two further $PGF_{2\alpha}$ injections were given at 11 days intervals to shorten estrous cycles by inducing luteolysis.

Blood samples were taken every 6 h for 3 days before PG-induced luteolysis, every 2 h from 48 to 72 h after PG injection, then every 6 h until next $PGF_{2\alpha}$ injection. The same regimen of sampling was repeated during the next cycles. Blood samples were collected into heparinized Vacutainer tubes. Centrifugation at 3000 r.p.m for 15 minutes and plasma was separated and stored at –20 ^{o}C until assayed for hormones

Determination of inhibin binding activity:

Plasma samples were tested for their ability to bind ^{125}I-labeled bovine inhibin as reported in the previous experiment.

Experiment 5:

Early pregnancy diagnosis:

Twelve Shiba goats were used in this experiment. Estrus was synchronized with a single i.m injection of 125 μg $PGF_{2\alpha}$ analogue after the detection of at least one corpus luteum by ultrasonography. Estrous behaviour was evaluated every 6 h daily by using a teasing buck. The females in estrus were allowed to be mated using mature fertile buck 2 times during estrus.

Ultrasonography:

Ultrasonographic examinations were performed transrectally using a real-time B-mode scanner equipped with a 7.5 MHz transducer. The transducer was modified by taping a plastic rod along its dorsal border to improve its intrarectal use in goats. Main events observed were recorded by using a thermal-video printer (Hitachi EUZ-VP7, Hitachi Medical Corporation, Tokyo, Japan). The transducer was lubricated with carboxymethylcellulose gel (Aquasonic 100, Ultrasound transmission gel, water soluble, hypoallergenic, Parker, USA or Hijelly, Aqueous coupling agent for ultrasound transmission, Hitachi, Tokyo, Japan). The transducer was inserted into the rectum until the urinary bladder was displayed on the screen. The uterine horns were observed cranial to the bladder and the transducer was rotated laterally 90^{o} clockwise and then 180^{o} counterclockwise to image ovaries (for presence of CL) and all reproductive tract. Elevation of the abdomen by standing the doe over a transversly positioned hay bale pushes the uterus back into the pelvic inlet and facilitates visualization of the fetus after 35 days of pregnancy (Haibel, 1990[b]; Martinez *et al.*, 1998).

Estrus synchronization:

Estrous cycles were synchronized using 125 μg of $PGF_{2\alpha}$ during experiments 2-5 and their data were demonstrated in table 2.

Hormone analysis:

Plasma concentrations of FSH were measured by RIA as described by Araki, Arai, Watanabe and Taya (2000) using antiovine FSH, NIDDK-FSH-I-1 for radioiodination, and NIDDK-oFSH-RP-1 as a reference standard (flow chart 1). Plasma concentrations of LH were measured by radioimmunoassay (RIA) system, as described by Mori and Kano (1984) using antiovine LH (YM No. 18), NIDDK-oLH-I-3 for radioiodination, and NIDDK-oLH-RP-24 as a reference standard (flow chart 1). The intra and interassay coefficient of variation were 9.8 % and 12.6 % for FSH and 5.9 % and 6.5 % for LH, respectively. Plasma concentration of immunoreactive (ir-) inhibin was measured by double-antibody (RIA) as described by Hamada, Watanabe, Kokuho, Taya, Sasamoto, Hasegawa, Miyamoto and Igarashi (1989) using bovine 32-kDa inhibin for radioiodination and antibovine antiserum (TNDH-1) (flow chart 2). The intra and interassay coefficient of variation was 3.8 % and 11.9 %, respectively. Plasma concentrations of estradiol-17β and progesterone were determined by a double antibody RIA system using ^{125}I-labelled radioligands as described previously (Taya, Watanabe and Sasamoto, 1985). Antisera against estradiol-17β (GDN 244) and progesterone (GDN 337) were kindly provided by Dr. G.D. Niswender (Animal Production and Biotechnology, Colorado state University, Fort Collins, Co. USA) (flow chart 3&4). The intra and interassay coefficient of variation were 5.7 % and 7.4 % for estradiol 17β and 8.2 % and 9.2 % for progesterone, respectively. Inhibin A was measured by ELISA described for use in human plasma (Groome, Illingworth, O'Brien, Pai, Mather and McNeilly, 1996) and modified for use in sheep plasma (Knight, Feist, Tannetta, Bleach, Fowler, O'Brien and Groome, 1998). Briefly, the ELISA is based on the use of immobilized monoclonal antibody (E4) against the βA subunit as a capture antibody, a biotinylated monoclonal α C specific antibody (PPG1/14/6) as a detection antibody and Immunopurified 32 KDa bovine inhibin in ovariectomized sheep plasma as standard in the range 15.6 – 1000 pg/ ml. The samples were denaturated by boiling in 6% SDS for 3 minutes before oxidation with hydrogen peroxide.

Statistical analysis:

Mean values (± SEM) were calculated and analysis of variance (ANOVA) was used for detection of significant differences using the SAS computer package (SAS, 1987). The follicles were combined for the 2 ovaries and the analysis of data began on Day – 2 rather than Day 0 (Day of ovulation) and ended 2 days before the end of the interval. The hypothesis that waves of follicles emerged at periodic intervals was tested by analysis of variance for sequential data to evaluate day effects averaged over 18 interovulatory intervals. The number of 3 mm follicles on each day was examined. A significant ($P < 0.05$) day effect was followed by Duncan's multiple-range test to detect significant nadirs and peaks.

The association between emergence of follicular waves and the occurrence of identified FSH peaks was studied by paired t-test to compare the number of waves with the number of peaks per interovulatory interval and the interwave and interpeak intervals. In addition, Wilks' Lambda correlation was made between the number of follicular waves & the number of FSH peaks, between the interwave & interpeak intervals and between progesterone levels & the number of corpora lutea. A peak in hormone concentration was identified by a significant difference in mean values between the peak and each of encompassing nadirs according to Ross, Barnes, Brody, Merriam, Loriaux and Cutler (1984).

RIA of goat FSH and LH

1-100 µL standard preparation in 0.05 M PBS containing 1% BSA or 100 µL sample preparation.
2-100 µL 0.05 M PBS containing 1% BSA.

↓

3- 100 µL anti-ovine FSH (1:30 000) in 0.05 M PBS containing 0.4% normal rabbit serum (NRS) or anti-ovine LH (YM #18; 1: 80 000) in 0.05 M PBS containing 0.4% NRS and 0.05 ethylene diamine-N,N,N,N-tetraacetic acid disodium salt (EDTA).

↓

4- Incubation at 4 ^{o}C for 24 h.

↓

5- 100 µL ^{125}I-ovine FSH or ^{125}I-ovine LH in 0.05 M PBS containing 1% BSA.

↓

6- Incubation at 4 ^{o}C for 24 h.

↓

7- 100 µL anti-rabbit γ-globulin (ARGG #42; 1:200) diluted with 0.05 M PBS containing 5% poly ethylene glycol (PEG).

↓

8- Centrifugation at 3000 rpm for 30 min. at 4 ^{o}C.
9- Decanting supernatant and swabbing extra drops.
10-Counting radioactivity of precipitate with a γ-counter.

Flow chart 1: Procedures of RIA for goats FSH and LH

RIA of goat inhibin

1-100 µL standard preparations in 0.05 M PBS containing 5% BSA or 100 µL sample preparation.
2-100 µL 0.05 M PBS containing 5% BSA.

↓

3- 100 µL antibovine inhibin (TNDH-1; 1:100 000) in 0.05 M PBS containing 0.4% NRS and 0.05 M (EDTA).

↓

4- Incubation at 37 ^{o}C for 24 h.

↓

5- 100 µL ^{125}I-32 kDa bovine inhibin in 0.05 M PBS containing 0.15% Bacitracin, 5 mM methionin and 0.1% 3-[(3-cholamidopropyle)-dimethyle-amino]- 1-propane-sulfonate (CAPS.

↓

6- Incubation at 37 ^{o}C for 24 h.

↓

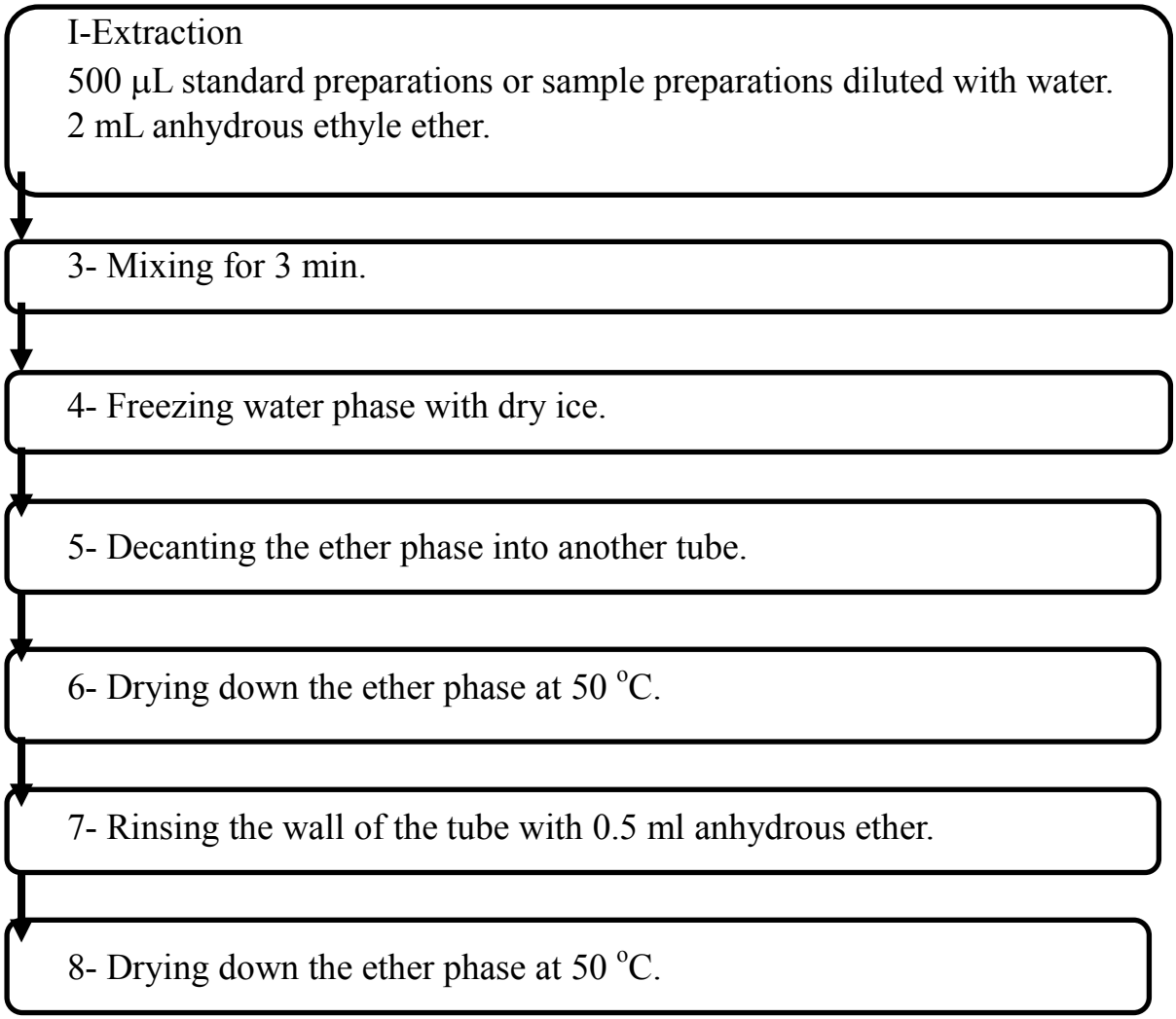
7- 100 µL ARGG #42; 1:200 diluted with 0.05 M PBS containing 5% PEG.

↓

8- Centrifugation at 3000 rpm for 30 min. at 4 ^{o}C.
9- Decanting supernatant and swabbing extra drops.
10-Counting radioactivity of precipitate with a γ-counter.

Flow chart 2: Procedures of RIA for goats inhibin

RIA of steroid hormones-1



Flow chart 3: Extraction procedures of goats steroid hormones

RIA of goats steroid hormones-2

II- Assay procedures after extraction

9- 100 μL 0.05 M PBS containing 1% BSA.

↓

10- 100 μL anti-estradiol (GDN 244; 1:2 000 000) or progesterone (GDN 337; 1: 20 000) in 0.05 M PBS containing 0.25% normal sheep serum (NSS) and 0.05 EDTA.
(Incubation for 48 h at 4 ^{o}C in case of estradiol)

↓

11- 100 μL ^{125}I-labeled each radioligand in 0.05 M PBS containing 1% BSA.

↓

12- Incubation for 24 h at 4 ^{o}C.

↓

13- 100 μL anti-sheep γ-globulin diluted with 0.05 M PBS containing 5% PEG.

↓

14-Incubation at 4 ^{o}C overnight

↓

15- Centrifugation at 3000 rpm for 30 min. at 4 ^{o}C.
16- Decanting supernatant and swabbing extra drops.
17-Counting radioactivity of precipitate with a γ-counter.

Flow chart 4: Procedures of RIA for goats steroid hormones

RESULTS

Experiment 1:

Induction of estrus during non-breeding season:

During the first 72-hour period after removal of norgestomet ear implant, 31 out of 40, 34 out of 40 and 2 out of 20 does in group I, group II and control group, respectively were observed in estrus. The interval from implant removal to the onset of estrus was 46.1 ± 1.5, 34.4 ± 1.5 and 60.0 ± 5.9 h, in group I, group II and control group, respectively. Goats in group II had a significantly shorter period to the onset of estrus than goats in group I ($P < 0.05$) (Table 1). The distribution of goats observed in estrus during the 72-hours after implant removal is shown in Fig. 1.

Most of the blood samples (90 %) collected from the control group 8 days after implant removal showed concentrations of progesterone less than 0.5 ng/ml, while 60% in group I and 80% in group II had high concentrations of progesterone that were more than 1 ng/ml, indicating luteal activity in these goats.

A higher percentage of group II goats gave birth (70 %) compared to group I (57.5 %). On the other hand, there was no significant difference between group I and group II in litter size (Number of offsprings / Number of kiddings) (Table 1).

Estrus synchronization:

The responses of does to different hormonal treatments are shown in Table 2. Estrus was observed in 77.5, 85.0 and 100.0 % in groups treated with norgestomet plus cloprostenol (A); norgestomet, cloprostenol and GnRH (B) or cloprostenol (C), respectively. The mean interval from implant removal to first signs of estrus was 46.1 and 34.4 h in treatment A and B while in treatment C the interval from cloprostenol injection to onset of estrus was 51.2 h. There was a significant ($P < 0.05$) difference between treated groups in the interval from treatment to estrus.

Table 1. Effect of various treatments on induction of estrus and reproductive performance of goats

Item	Treatment		
	Control	Group I	Group II
No. of animals exposed	20	40	40
No (%) of animals exhibited estrus	2 (10%)	31(77.5%)	34 (85%)
Interval from implant removal to estrus (hr)	60.0 ± 5.98 [a] n = 2	46.07 ± 1.52 [b] n = 31	34.41 ± 1.45 [c] n = 34
No. (%) of animals with luteal activity*	2 (10%)	24 (60%)	32 (80%)
No. of animals kidding	2	23	28**
Fertility	10.0%	57.5%	70.0%
Litter size	1.00	1.61 ± 0.14	1.75 ± 0.13
Single birth	2 (100%)	11(47.83%)	11 (39.29%)
Twin birth	-	10 (43.48%)	13 (46.43%)
Triplet birth	-	2 (8.69%)	4 (14.28%)

[abc] Values in the same row with different superscripts differ ($p < 0.05$).

*by using progesterone analysis (higher than 1 ng/ml).

**plus 2 goats aborted.

Animals in group I were treated with norgestomet ear implant for 11 days and Estrumate 24 hours before implant removal.

Animals in group II were treated with norgestomet ear implant for 11 days and Estrumate 24 hours before implant removal and GnRH 24 hour after implant removal.

Control group received no treatment.

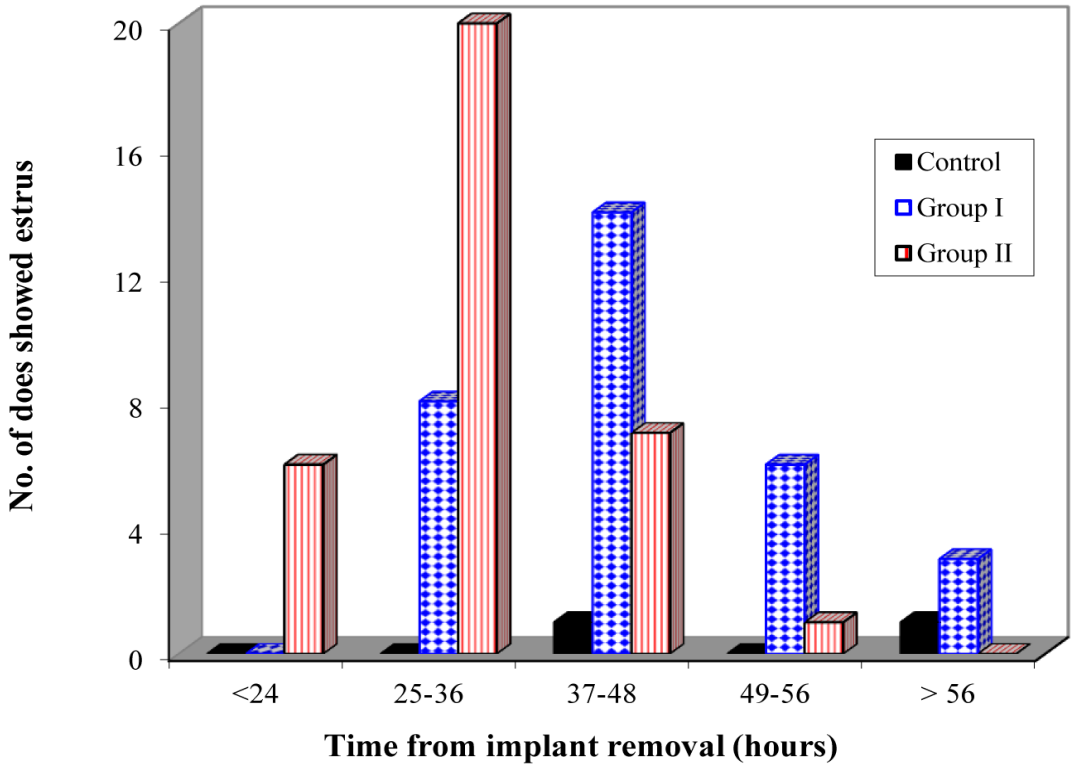


Fig. 1: Time from implant removal to the appearance of estrus in treated groups.

Animals in group I were treated with norgestomet ear implant for 11 days and PGF2α 24 hours before implant removal.

Animals in group II were treated with norgestomet ear implant for 11 days and PGF2α 24 hours before implant removal and GnRH 24 hour after implant removal.

Control group received no treatment.

Table 2. Mean interval from treatment to onset of estrus following synchronization with norgestomet[1] + cloprostenol[2] (A), A + GnRH[3] (B) and cloprostenol (C).

Parameters	Treatments		
	A*	B*	C**
No. of does responding to treatments	40	40	50
Number responded (%)	31 (77.5)	34 (85.5)	50 (100)
Mean interval from treatment to estrus (h)	46.1 ± 1.6^{a}	34.4 ± 1.3^{b}	51.2 ± 1.5^{c}

[abc] Values in the same row with different superscripts differ ($p < 0.05$).

[1] Syncro-Mate-B (1/2 implant), Sanofi Animal Health, Inc. Overland Park, KS, USA.

[2] Estrumate, Coopers animal Health Ltd., Berkhamsted, UK.

[3] Buserelin acetate, Receptal, Hoechst Roussel Vet GmbH D-65203 Wiesbaden, Germany.

*During the non-breeding season.

**The ovary had at least 1 CL (detected by ultrasonography).

Experiment 2:

Ovarian dynamics and hormonal profile during estrous cycle:

Estrus detection and estrous cycle duration:

After injection of $PGF_{2\alpha}$ all animals exhibited estrous behavior. The mean length of estrous cycle was 21.6 ± 0.4 days (n = 18), while the mean interovulatory interval was 21.3 ± 0.5 days (n = 18) and the interval from $PGF_{2\alpha}$ to estrus was 55.7 ± 3 h (n = 18).

Follicular dynamics and characteristics of follicular waves:

The images of ovarian structures recorded in this study are shown in Fig. 2. The follicles were detected as echo-free, rounded, sharp outlined black circles and the corpora lutea were detected as a gray medium echogenic structure with marked boundaries.

The characteristics of follicular waves in those animals, which have 3, or 4 follicular waves are shown in Table 3. Two interovulatory intervals (11.1 %) had 2 follicular waves, five (27.8 %) had 3 waves, 9 (50.0 %) had four waves and two (11.1 %) had five waves. In all animals, the last follicular wave of the interovulatory interval contained ovulatory follicle(s) and the ovulation rate was 1.8. Growth rates for the largest follicles of the successive waves were not different. On the other hand, the maximum diameter of the ovulatory follicles was significantly ($p < 0.05$) larger than the maximum diameter of the largest follicles of the other waves. For goats with 3 or 4 waves of follicular growth per cycle, the number of 3 mm follicles emerging per day that subsequently grew to ≥ 5 mm in diameter differed by day ($p<0.05$) (Fig. 3). Follicles growing to ≥ 5 mm in diameter emerged on 0.3 ± 0.5, 6.5 ± 0.2 and 12.1 ± 0.4 days for wave 1, wave 2 and wave 3, respectively in goats with 3 waves of follicular development and on – 0.6 ± 0.3, 4.7 ± 0.2, 9.4 ± 0.5 and 13.4 ± 0.5 days, for wave 1, wave 2, wave 3 and wave 4, respectively in goats with 4 waves of follicular development (Table 3). In goats with 3 follicular waves, the number of 3 mm follicles peaked on days 0, 7 and 11 while in goats with 4 follicular waves, 3 mm follicles peaked on days -1, 5, 11 and 15 (Fig. 3). Individual follicle profile (follicle growing from 3 to ≥ 5mm in diameter) for representative animals and accompanying concentrations of FSH, ir-inhibin, estradiol and progesterone were illustrated in Fig. 4 & 5.

Estradiol 17 β and follicles:

Plasma estradiol concentrations increased from the day of ovulation (1.9 ± 0.1 pg/ml) to around day 4 and then decreased to the basal level. Estradiol17 β levels remained low for the rest of luteal phase, apart from some isolated fluctuations and increased in coincidence with progesterone declination reaching peak (30.9 ± 1.0 pg/ml) 2 days before ovulation. The fluctuation in estradiol level during the luteal phase was associated with the growth of the largest follicles of follicular waves (Fig. 4 & 5).

Progesterone and CL:

The CL could be detected ultrasonically on day 3 post ovulation. The newly forming CL was less echogenic than at later stages. The mature CL was observed as a gray echogenic structure with marked boundaries. CL attained a maximum diameter of 12.1 ± 0.3 mm at day 8 post ovulation. In early luteal phase the mean diameter of corpora lutea increased in parallel with the mean plasma concentration of progesterone, whereas in the late luteal phase, the plasma concentration of progesterone decreased rapidly than the CL regression (Fig. 6). There was a positive correlation ($r = 0.9$; $p < 0.001$) between CL diameter and progesterone concentration during the estrous cycle.

Relationship between follicular wave development, plasma FSH and LH concentrations:

The goats with 3 and 4 waves of follicular emergence per cycle were included in this analysis. The number of emerging follicular waves and the number of identified FSH peak values per goat did not differ (3.6 ± 0.2 and 3.9 ± 0.2). The duration of the interval between adjacent days of wave emergence (interwave intervals) was positively correlated with the duration of the interpeak interval for FSH fluctuations ($r = 0.8$; $p < 0.001$). Association between follicular waves and FSH peaks are shown in Table 4. The length of the interval between emergences of waves did not differ significantly from the intervals between FSH peak values (5.6 ± 0.3 and 5.2 ± 0.2). The number of waves and the number of peaks were positively and significantly correlated ($r = 0.8$; $p < 0.001$). Plasma concentrations of LH did not differ significantly during estrous cycle in goats before the preovulatory surge (Fig. 7).

Relationship between FSH and inhibin:

The FSH concentration was highest at the emergence of each wave and decreased ($p < 0.05$) as the follicle grow to 5 mm in diameter (Fig. 4 & 5),

meanwhile plasma levels of ir-inhibin and inhibin A were low during follicular wave emergence and increased with the growth of follicles at the time of FSH concentrations declining. A negative correlation was found between FSH and both ir-inhibin ($r = -0.79$; $p < 0.001$) and inhibin A ($r = -0.47$; $p < 0.01$). Fig. 8 & 9 show the relationship between FSH and both ir-inhibin and inhibin A during the estrous cycle in goats.

Table 3. Characteristics of follicular waves during the estrous cycle in goats

Parameters	Goats with 4 waves of follicle development (n = 9)				Goats with 3 waves of follicle development (n =5)		
	Wave 1	Wave 2	Wave 3	Wave 4	Wave 1	Wave 2	Wave 3
Mean day of wave emergence	- 0.6 ± 0.3	4.7 ± 0.2	9.4 ± 0.5	13.4 ± 0.5	0.3 ± 0.5	6.5 ± 0.2	12.1 ±0.4
Largest follicle							
Maximum diameter (mm)	6.7±0.1^{b}	6.2±0.2^{c}	6.3±0.1^{cb}	7.8±0.2^{a}	6.6±0.1^{cb}	6.2 ±0.1^{c}	8.0±0.1^{a}
Growth rate (mm / day)	0.9 ±0.1^{a}	1.0 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ±0.1^{a}	0.9 ±0.1^{a}	0.8 ± 0.1^{a}
Regressing rate (mm / day)	0.8 ±0.1^{a}	0.8 ± 0.1^{a}	0.9 ± 0.1^{a}	-	0.9 ±0.1^{a}	0.8 ±0.1^{a}	-
No. follicles / wave	2.6 ±0.2^{a}	2.4 ± 0.2^{a}	2.2 ± 0.2^{a}	2.8 ± 0.2^{a}	3.0 ±0.5^{a}	2.4 ±0.2^{a}	2.6 ± 0.2^{a}

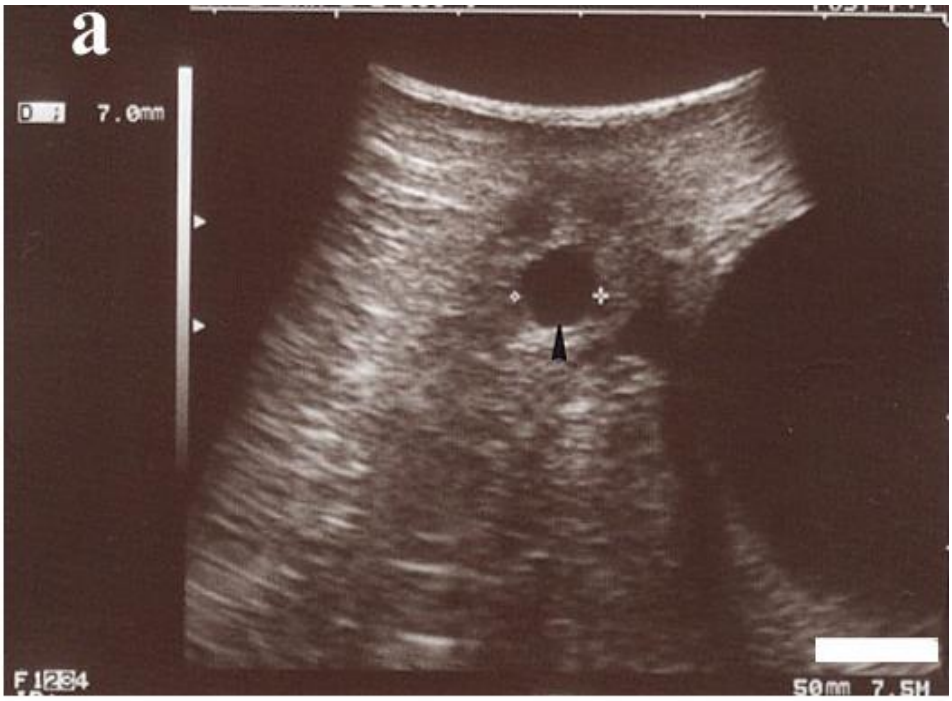
^{abc} Values in the same row with different superscripts differ ($p < 0.05$).

Table 4. Associations between follicular waves and FSH peaks

End point	Mean ± SEM
Interovulatory interval (IOI)	
Number	18
Length (days)	21.3 ± 0.5
Number of follicular wave/IOI[a]	3.6 ± 0.2
Number of FSH peaks/IOI[a]	3.9 ± 0.2
Interwave interval[b] (days)	5.6 ± 0.3
Interpeak interval[b] (days)	5.2 ± 0.2

[a] No significant difference between the number of follicular waves and number of FSH peaks. The number of follicular waves and FSH peaks was positively correlated ($r = 0.8$; $p < 0.001$).

[b] No significant difference between interwave and interpeak intervals. The number of interwave intervals and interpeak intervals were positively correlated ($r = 0.8$; $p < 0.001$).



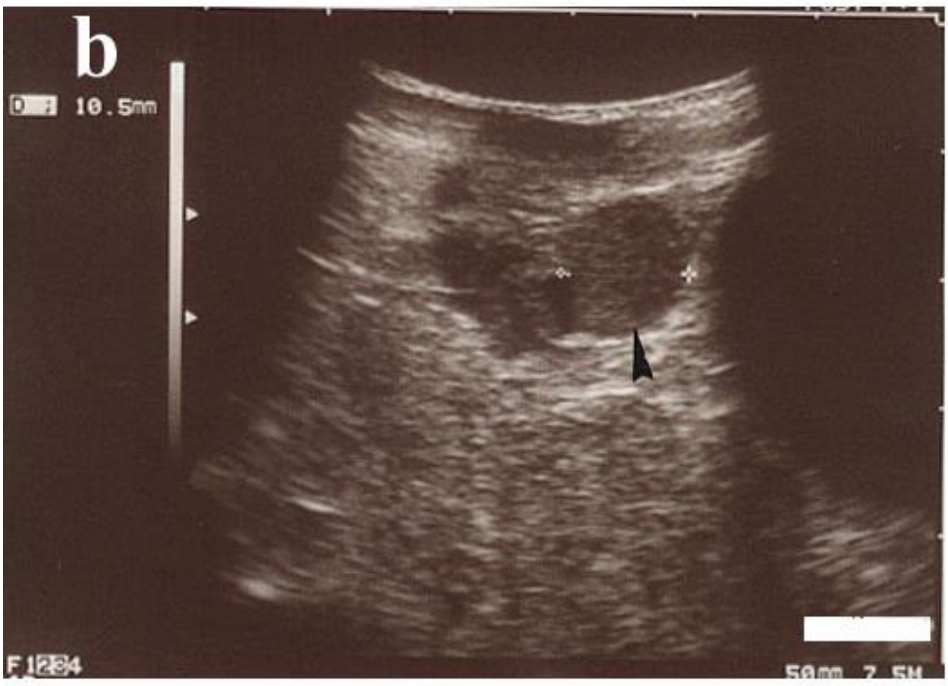


Fig. 2: Ultrasound image of goats' ovaries produced by using a rigid, transrectal 7.5 MHz transducer. (a) the arrow head indicates an antral follicle, (b) the arrow head indicates a functional CL. Scale bar represents 10 mm.

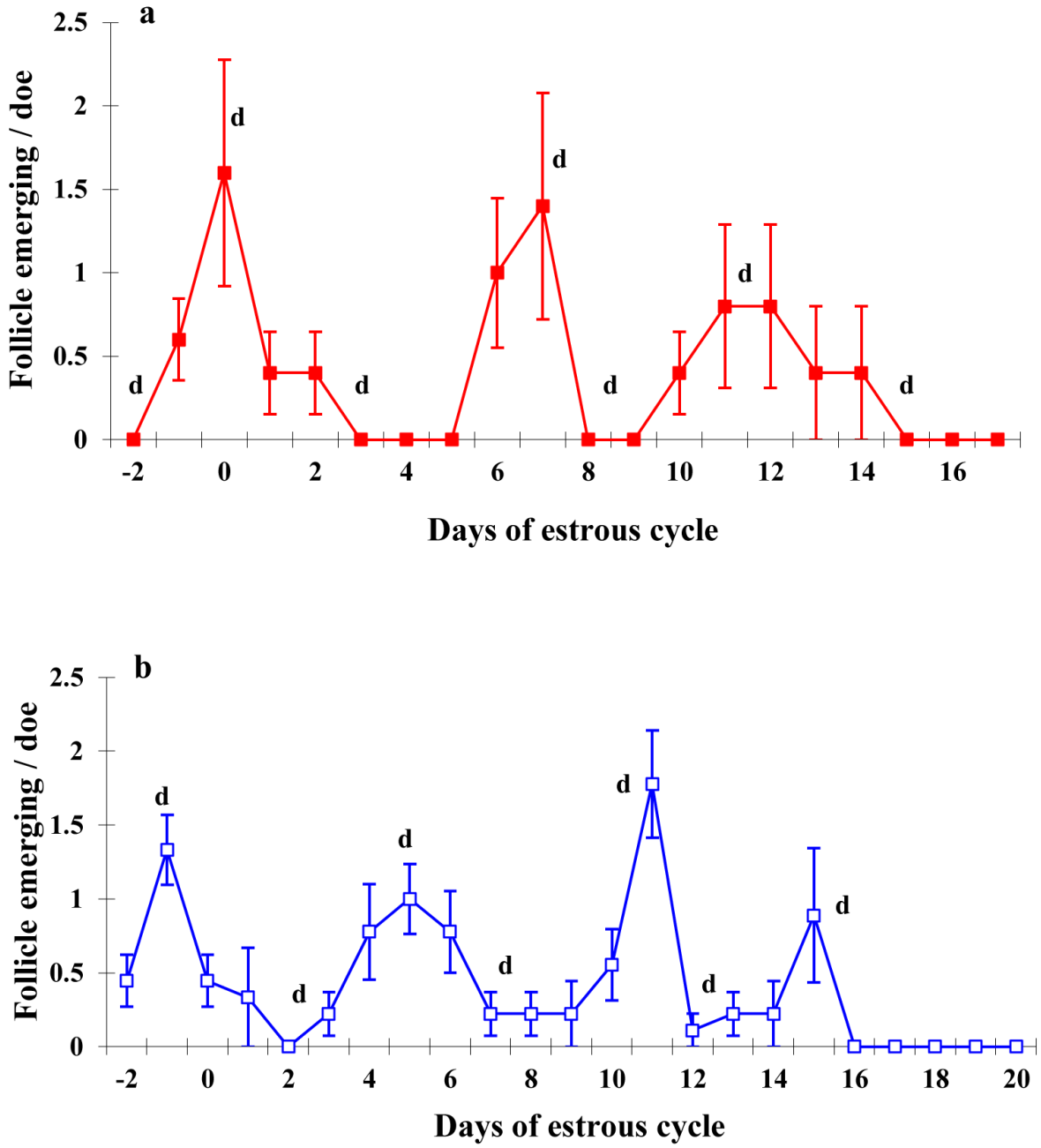


Fig. 3 : Mean (± SEM) number of follicles emerging in goats with 3 waves of follicular development (a; n = 5) and in goats with 4 waves of follicle development (b; n = 9).

within a data set, letters indicate difference ($p < 0.05$) between peaks and nadirs

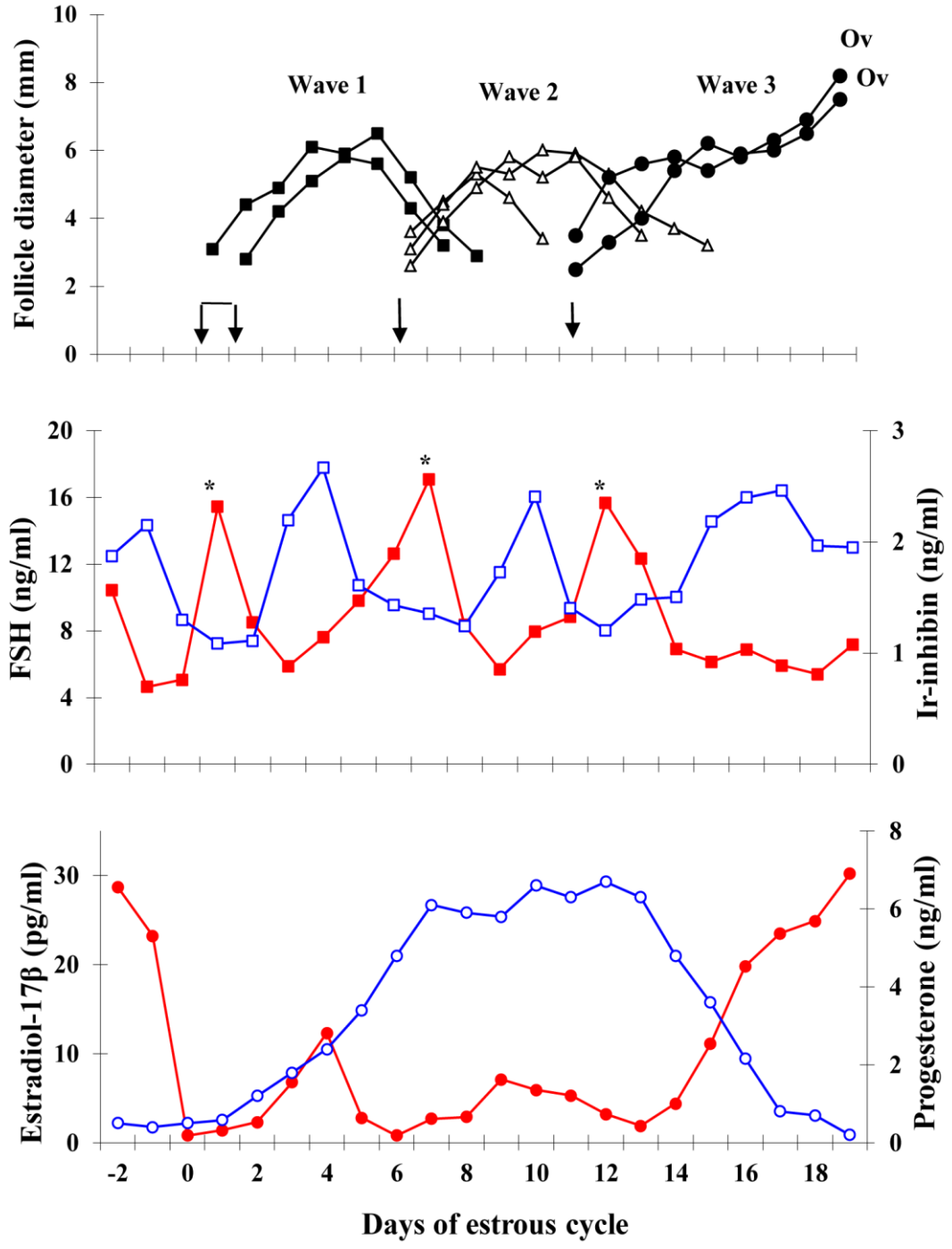


Fig. 4: Representative pattern of growth and regression of individual follicles during estrous cycle in a goat with 3 waves of follicular development and accompanying plasma concentrations of FSH (solid square), ir-inhibin (open square), estradiol (solid circle) and progesterone (open circle). Arrows indicate the emergence of waves and *indicates FSH peaks (Ov = ovulation).

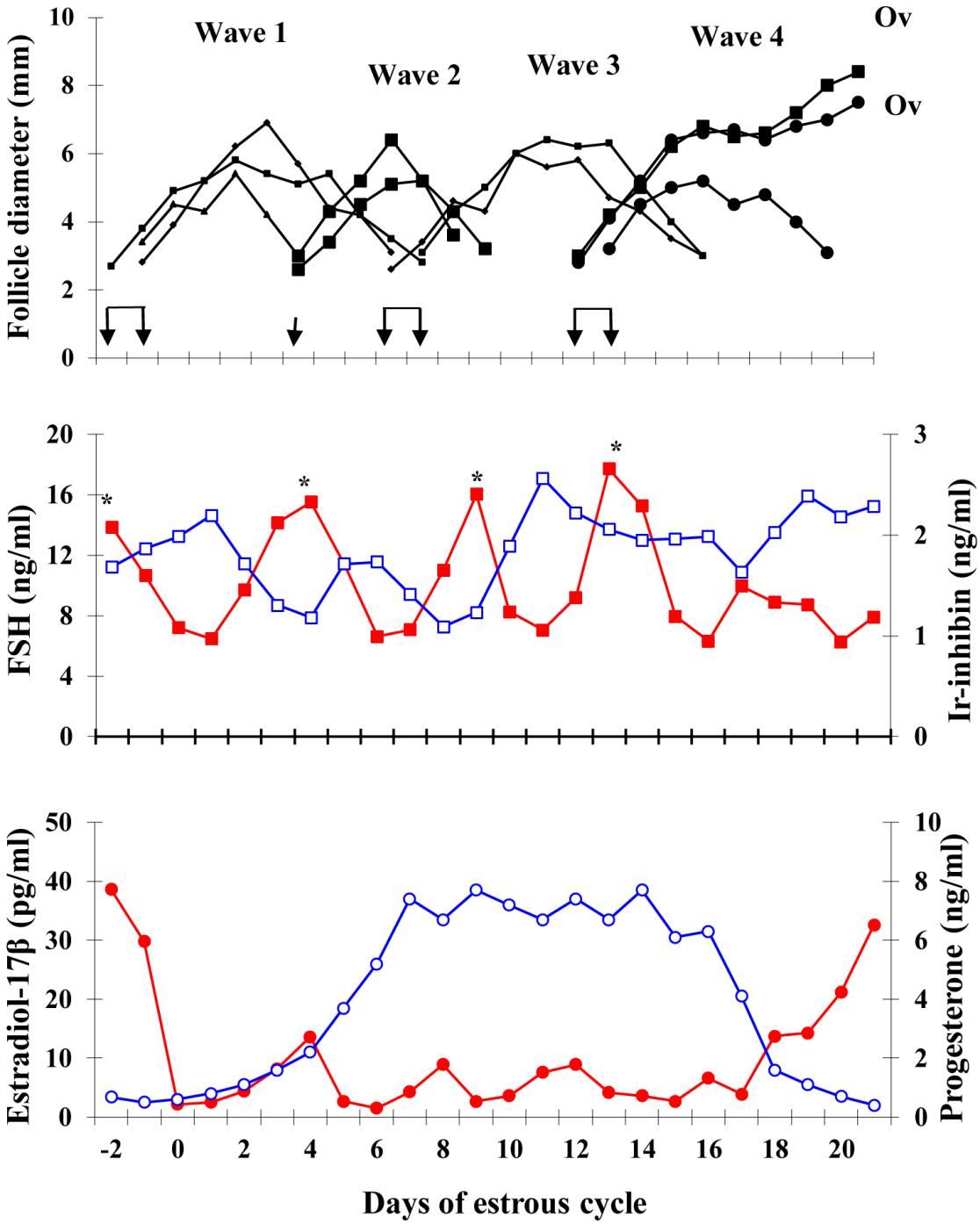


Fig. 5: Representative pattern of growth and regression of individual follicles during estrous cycle in a goat with 4 waves of follicular development and accompanying plasma concentrations of FSH (solid square), ir-inhibin (open square), estradiol (solid circle) and progesterone (open circle). Arrows indicate the emergence of waves and *indicates FSH peaks (Ov = ovulation).

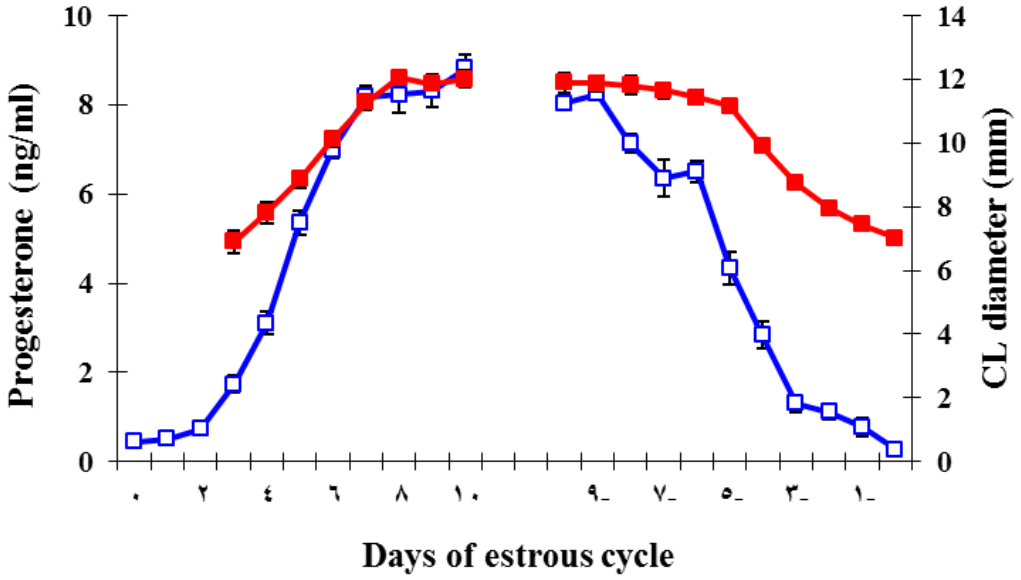


Fig. 6: Mean (± SEM) diameter of CL (solid square) and progesterone concentrations (open square) during the estrous cycle in goats (n = 18)

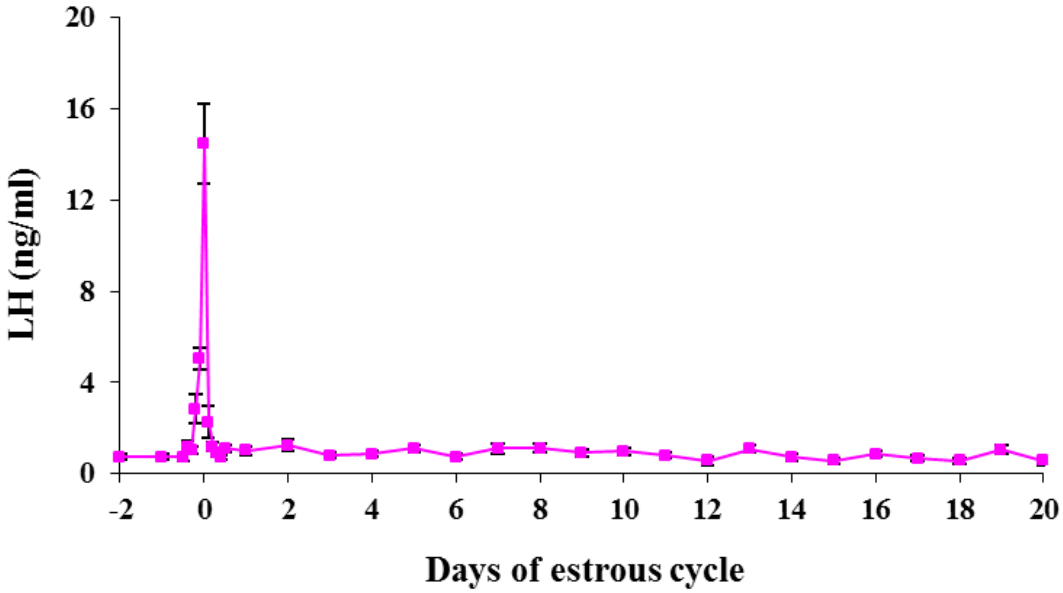


Fig. 7: Mean (± SEM) LH concentrations (ng/ml) during the estrous cycle in goats. All data are normalized to LH surge (time 0). (n = 18).

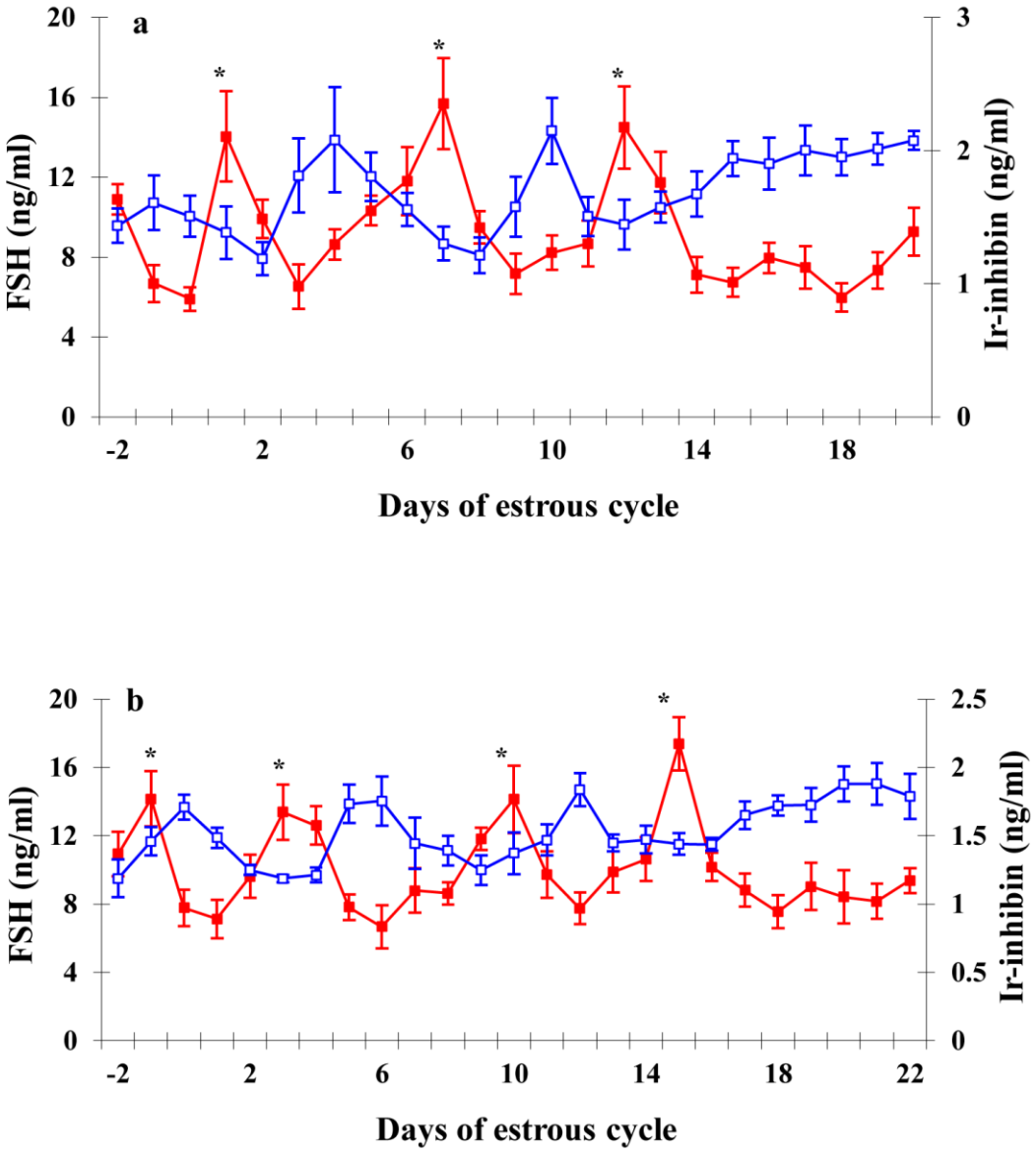


Fig. 8: Mean (± SEM) plasma FSH (solid square) and ir-inhibin (open square) concentrations during the estrous cycle in goats with 3 (a; n = 5) or 4 (b; n = 9) waves of follicular growth.*indicates FSH peaks.

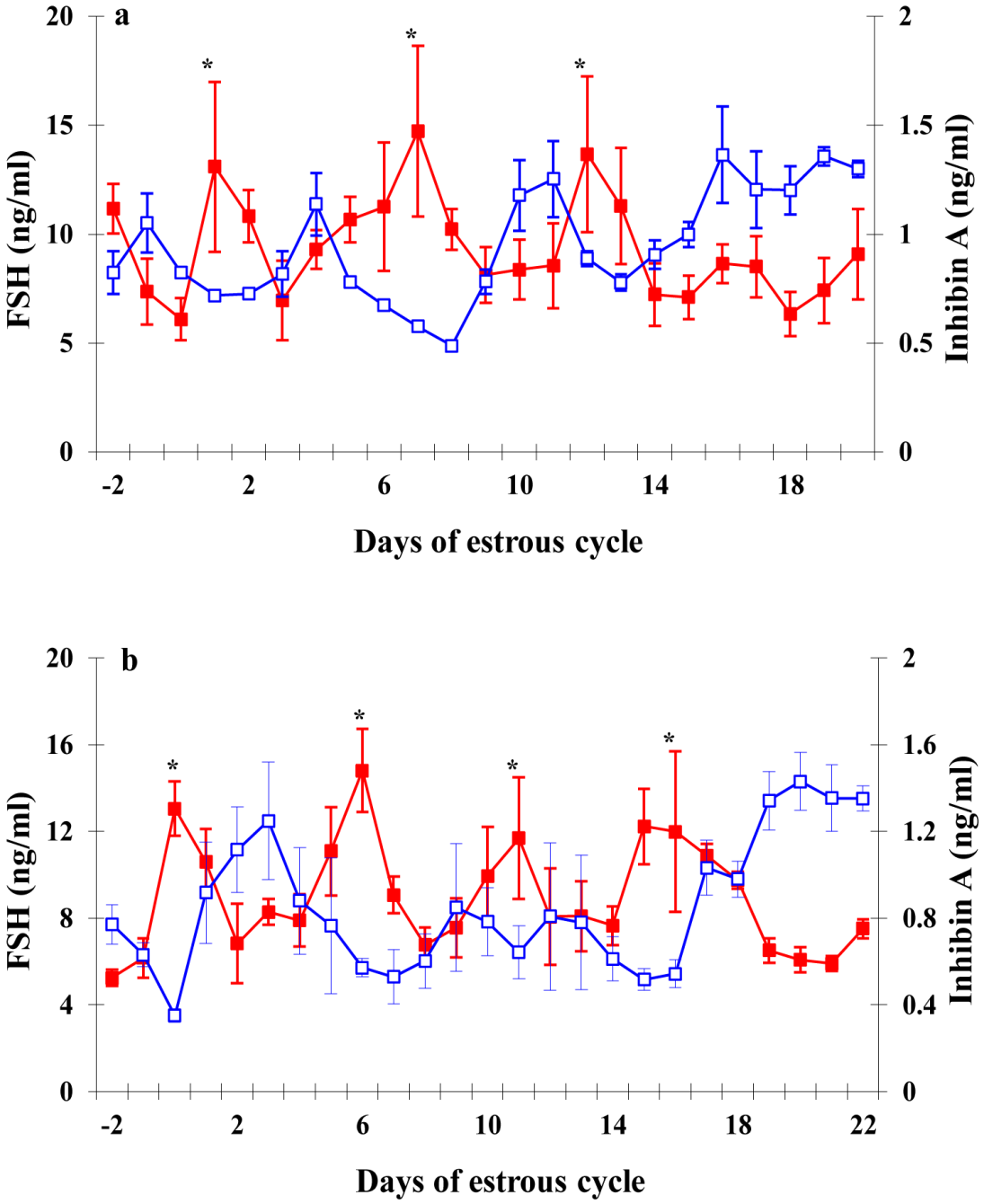


Fig. 9: Mean (± SEM) plasma FSH (solid square) and inhibin A (open square) concentrations during the estrous cycle in goats with 3 (a; n = 3) or 4 (b; n = 3) waves of follicular growth.*indicates FSH peaks.

Experiment 3:

Ovarian response and hormonal profile following injection of inhibin antiserum:

Plasma hormone-binding capacity:

Following immunization, there was a significant increase ($P < 0.01$) in the ability of plasma to bind inhibin in all immunized animals. By 6 h following immunization in the animals immunized against inhibin, plasma inhibin-binding capacity, at a final dilution of 1:10, was 77.7 ± 6.9 % compared with 8.0 ± 0.7 in control group and with a preimmunization non-specific binding of 7.1 ± 1.4 % (Fig. 10) The binding capacity showed a steady decline ($p < 0.01$) over the course of the experiment in immunized group.

Plasma concentrations of FSH and LH:

Following the injection of normal goat serum, plasma concentrations of FSH did not significantly change during the period before FSH surge. In contrast, treatment with the inhibin antiserum resulted in a marked increase ($P < 0.01$) in plasma concentrations of FSH compared with control values. In immunized group, there was four- to fivefold increase ($P < 0.01$) in plasma FSH concentrations within 12 h of treatment (Fig. 11). Thereafter, mean plasma FSH concentrations remained relatively stable for about 24 h and then declined ($P<0.01$). The preovulatory FSH peak in inhibin immunized group occurred before control group and the peak value of the FSH rise in inhibin-immunized was much higher than in control group. There was no significant difference in the basal LH levels between immunized (0.79 ± 0.1 ng/ml) and control (0.86 ± 0.1) goats. However, there was a lower ($P = 0.1$) LH surge displayed by immunized goats compared with controls [(9.4 ± 1.2 ng/ml; n = 6) vs. (13.5 ± 2.2 ng/ml; n = 6)] (Fig. 11).

Plasma concentrations of estradiol-17β and progesterone:

In control animals, plasma estradiol-17β increased significantly ($P < 0.05$) after $PGF_{2\alpha}$ injection and reached a peak (32.4 ± 5.0 pg/ml) around the day of estrus. Treatment with inhibin antiserum induced a marked increase in plasma estradiol-17β concomitant with the growth of a large number of follicles and reached a peak (64.1 ± 5.9 pg/ml) around the day of estrus (Fig. 12).

During the subsequent luteal phase (7 days after ovulation), plasma progesterone values were significantly higher ($P < 0.05$) in immunized animals

compared with control values (Table 5). There was a significant and positive ($r = 0.9$; $P < 0.01$) correlation between progesterone level and the number of corpora lutea.

Estrus and ovarian response:

All goats exhibited estrus and ovulated. Interval from $PGF_{2\alpha}$ injection to onset of estrus was shorter ($P = 0.06$) in passively immunized compared with control goats (Table 5). Following immunization there was a significant ($P < 0.01$) rise in the total number of follicles (≥ 3 mm) in animals immunized against inhibin compared with the control group (Fig. 13). In the group immunized with inhibin antiserum the total number of follicles rose from a mean of 5.0 ± 0.3 before immunization to a mean maximum of 13.5 ± 1.0. Figure 14 shows the number of small (< 3.5 mm), medium (3.5-5 mm) and large (> 5 mm) follicles at each scanning in the two groups. In control group, the mean number of small, medium or large follicles did not vary significantly over the time of the experiment. In goats immunized against inhibin, small follicles increased within 24 h and thereafter the number of medium sized and large follicles increased. Ultrasound monitoring of the ovaries showed that goats administered inhibin antiserum had significantly more preovulatory follicles within the limited ovarian space compared with the control group (Fig. 15). In addition, treatment with inhibin antiserum resulted in significantly more ($P < 0.01$) ovulations than the control group (Table 5).

Table 5. Effect of passive immunization against inhibin on estrus, ovarian response and Plasma progesterone (Day 7 after ovulation) in goats.

Parameters	Control group	Immunized group
Number of treated goats	6	6
Number of goats detected in estrus[a]	6	6
Number of goats ovulating[b]	6	6
Interval to estrus[c] (h)	62.0 ± 4.6	50.0 ± 3.4
Ovulation rate[d]	1.8 ± 0.3	4.2 ± 0.5**
Mean maximum number of follicles ≥ 3 mm in diameter	5.3 ± 0.6	13.5 ± 1.0**
Plasma progesterone (ng/ml) on day 7 after ovulation	4.6 ± 0.5	7.38 ± 0.9*

*: $P<0.05$, significantly different compared with the control value in the same raw.

**: $P<0.01$, significantly different compared with the control value in the same raw.

[a]Detected by mature bucks 6-h intervals within 3 days after Estrumate injection

[b]By using ultrasound scanning (large follicles collapsed and confirmed by formation of corpora lutea at that site)

[c]Hours from $PGF_{2\alpha}$ injection to onset of estrus

[d]The number of collapsed follicles or CL per goat ovulating

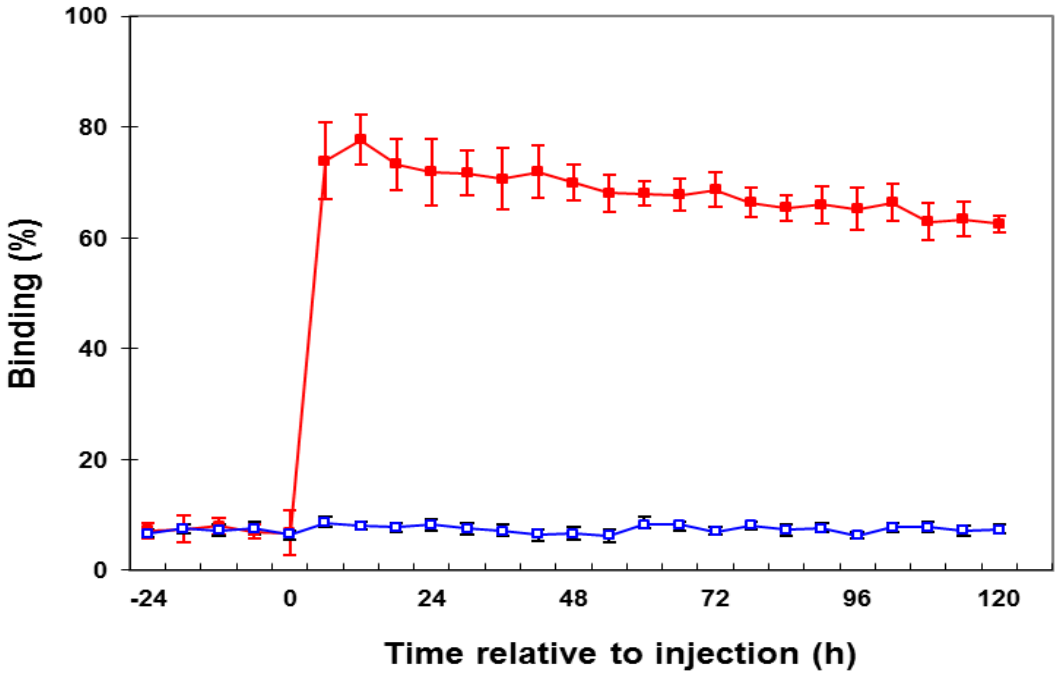


Fig. 10: Inhibin binding capacity (at a dilution of 1:10) in plasma of goats receiving a single i.v. injection at 0 h (arrow) of 10 ml normal goat serum (open square; n = 6) or 10 ml inhibin-AS (solid square; n = 6). Values are means ± SEM.

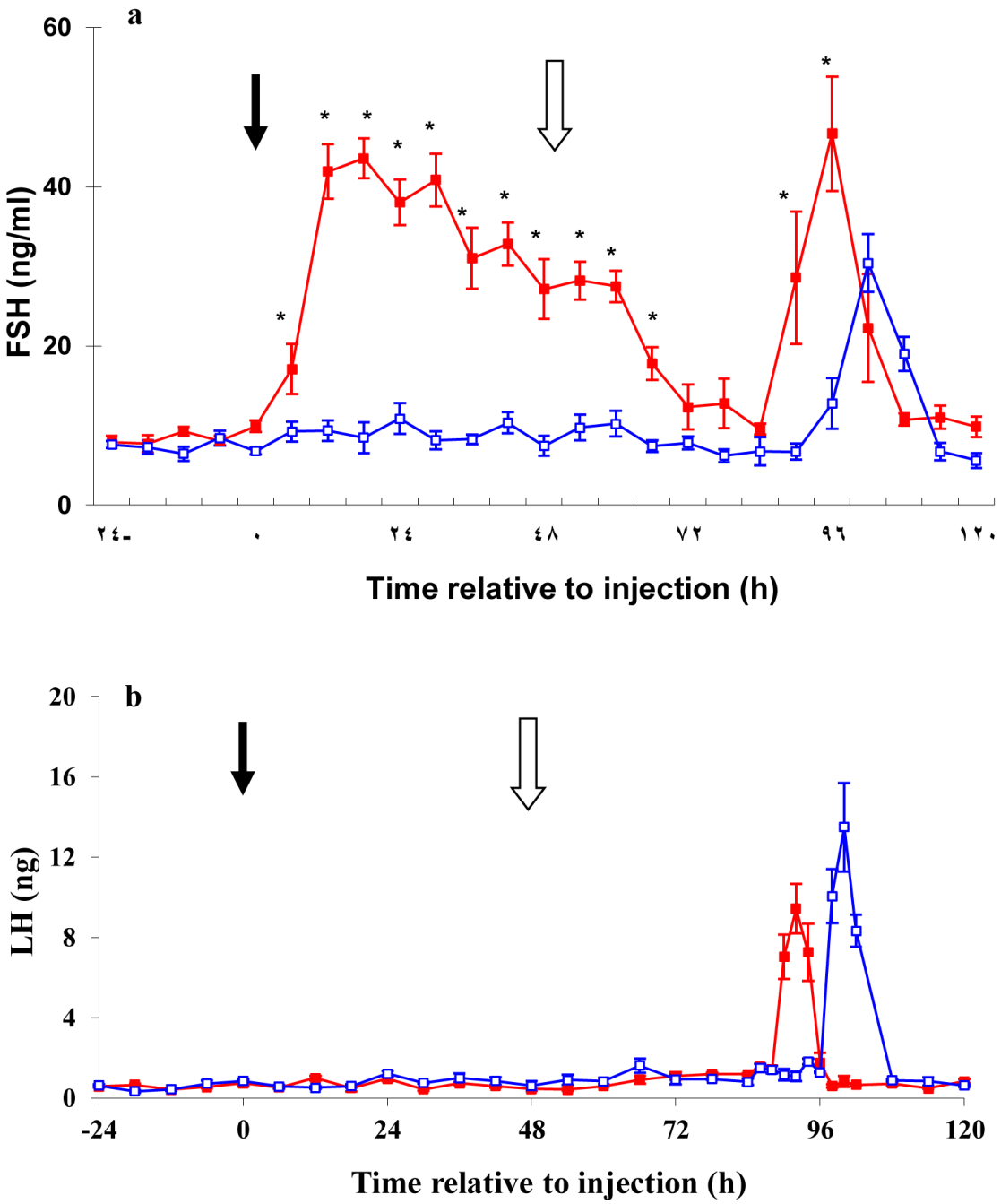


Fig. 11: Mean (± SEM) plasma concentrations of FSH (a) and LH (b) in goats received an i.v. bolus injection of 10 ml normal goat serum (open square; n = 6) or 10 ml inhibin-AS (solid square; n = 6) at 0 h (solid arrow) and 48 h before PGF2α injection (open arrow). * P<0.05 compared with the control values.

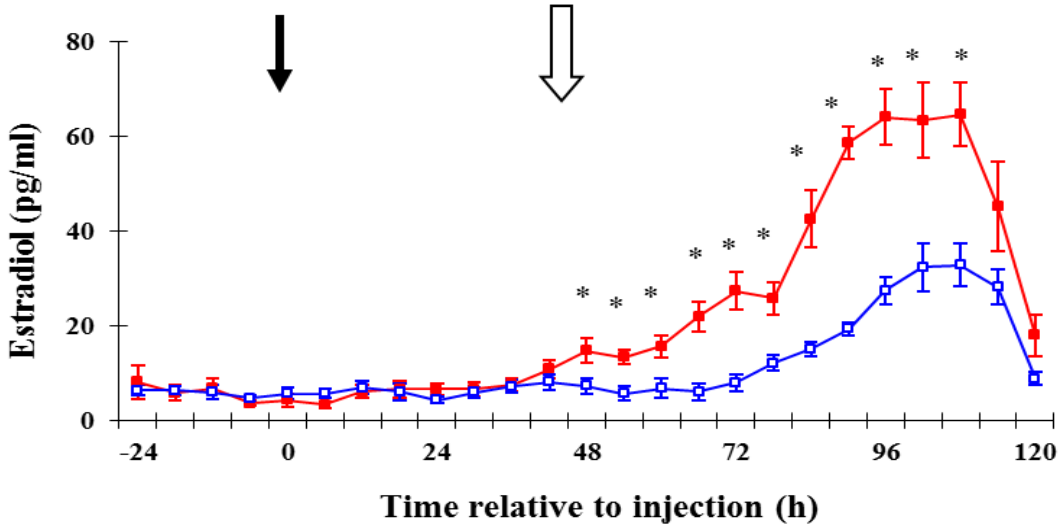


Fig. 12: Mean (± SEM) plasma concentrations of estradiol in goats received an i.v. bolus injection of 10 ml normal goat serum (open square; n = 6) or 10 ml inhibin-AS (solid square; n = 6) at 0 h (solid arrow) and 48 h before estrumate injection (open arrow).* P<0.05 compared with the control values.

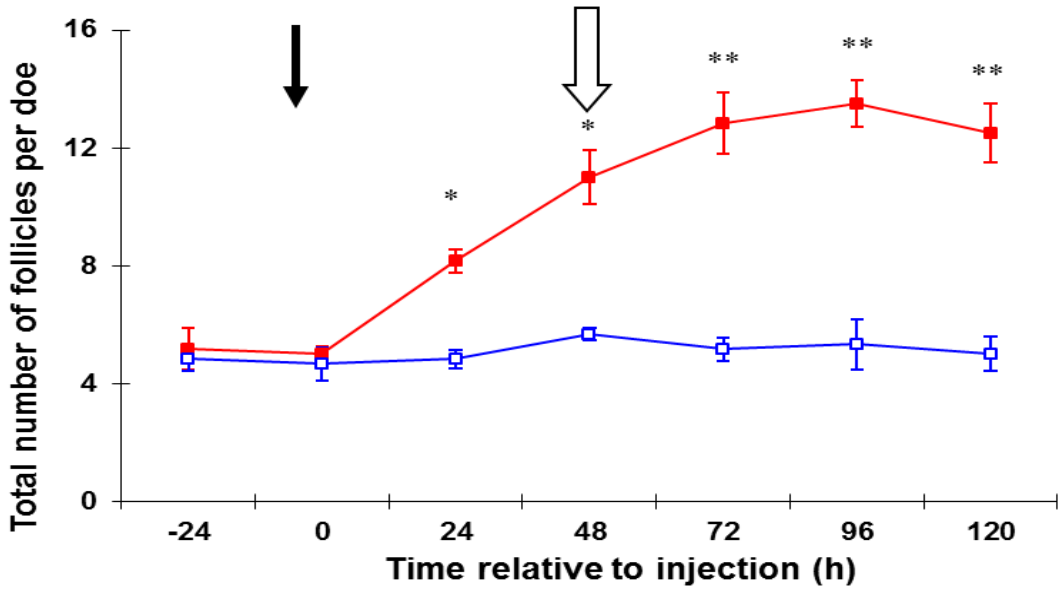


Fig. 13: Mean ± SEM total number of follicles before and after injection of 10 ml normal goat serum (open square; n=6) or 10 ml inhibin-AS (solid square; n=6) to goats on day 10 of the estrous cycle (0; solid arrow) and 48 h before PGF2α injection (open arrow). * P<0.05, **:P<0.01 compared with the control values.

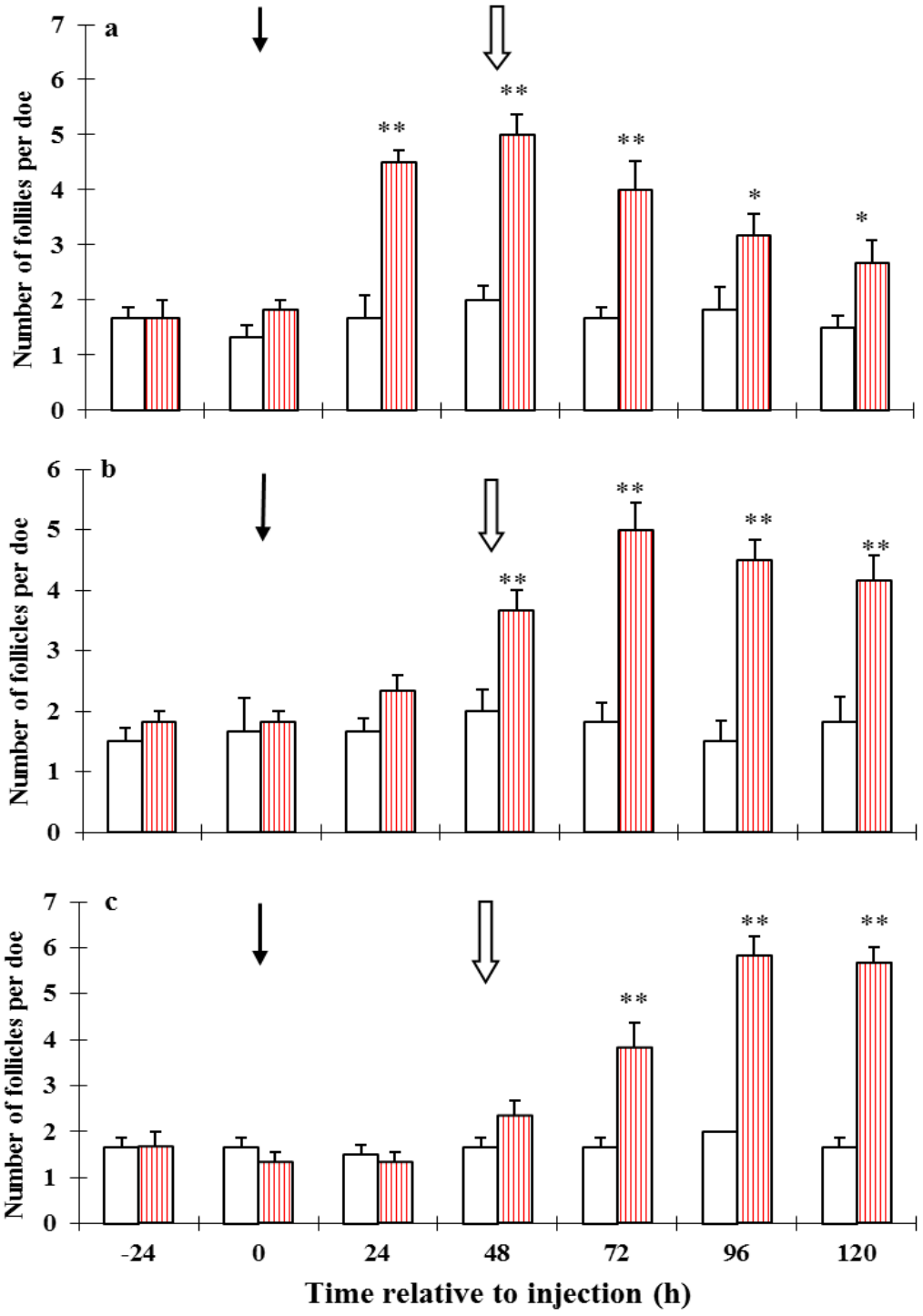


Fig. 14: Mean ± SEM number of small (< 3.5 mm diameter; a), medium sized (4-5 mm diamater; b) and large (> 5 mm diameter; c) follicles in goats treated with i.v.bolus injection of normal goat serum (open bar; n=6) or 10 ml inhibin-AS (shaded bar; n=6) on day 10 of estrous cycle (0 h; solid arrow) and 48 h before induction of luteolysis (open arrow). *:P<0.05, **:P<0.01

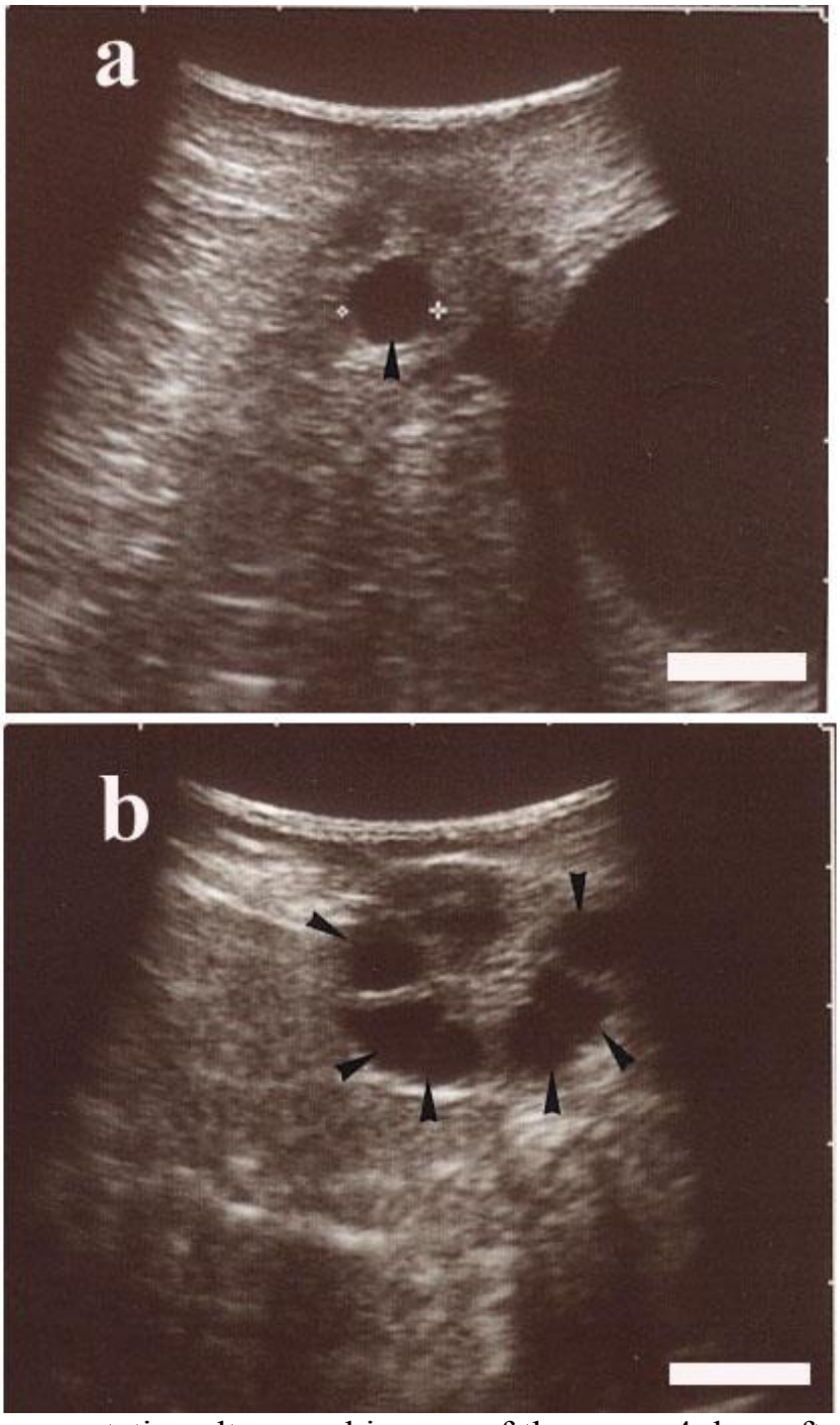


Fig. 15: Representative ultrasound images of the ovary 4 days after treatment with the control serum (a) and inhibin-AS (b) in goats; note that many follicles are located within the limited ovarian space in goat that had been passively immunized with inhibin antiserum (arrows). Scale bar represents 10 mm.

Experiment 4:

Effect of active immunization against inhibin on hormonal concentrations and ovulation rate

Plasma anti-inhibin titers:

The time course of antibody development in immunized animals as determined by binding of the ^{125}I-labelled bovine inhibin; is shown in Fig.16. Antibodies that bound ^{125}I-labelled bovine inhibin were produced by all goats receiving the vaccine. Titres rose after primary immunization reaching 36.2 ± 3.1 %, at 1:10 dilution within 6 weeks. In contrast, antibody titres in the control group remained low (< 3.0%).

Plasma concentrations of FSH and LH:

The overall mean basal FSH concentration for the 30 days period after the 3rd booster immunization ($PGF_{2\alpha}$-shortened cycles) was significantly higher ($P < 0.05$) in inhibin-immunized goats (9.2 ± 0.2 ng/ml, n =132) than in the controls (5.0 ± 0.2 ng/ml, n = 132). In both groups, $PGF_{2\alpha}$ injection was followed by a preovulatory surge in FSH (immunized: 27.1 ± 2.3 vs control: 19.9 ± 0.8 ng/ml; $P < 0.05$) coincident with the LH surge (Fig. 17). Plasma concentrations of LH did not differ significantly between inhibin-immunized and control goats (Fig. 18). Overall mean plasma LH values were 0.9 ± 0.1 ng/ml in immunized group and 1.1 ± 0.1 ng/ml in control group. The mean time interval between $PGF_{2\alpha}$ injection and the occurrence of preovulatory LH surge was significantly ($P < 0.05$) shorter in inhibin-immunized group (53.9 ± 1.5 h) than control group (61.3 ± 1.8 h).

Estradiol and progesterone:

Plasma concentrations of estradiol-17β rose after $PGF_{2\alpha}$-induced luteolysis to reach a peak value which is significantly ($P < 0.01$) higher in immunized (47.8 ± 2.9 pg/ml, n = 15) than controls (24.8 ± 1.7, n = 15). The concentration then fell and remained relatively low until the next $PGF_{2\alpha}$ injection, with the exception of a smaller peak around day 4 after estrus. Three to four days after ovulation (ovulation was determined by ultrasonography as sudden disappearance of large follicles) the expected rise in the plasma concentrations of progesterone were observed in all goats, confirming that ovulation had occurred. Progesterone

values were significantly ($P < 0.05$) higher in immunized compared with perspective values in control (Fig. 19).

Estrus activity and ovulation rate:

After injection of $PGF_{2\alpha}$, all goats exhibited estrus and ovulated. The interval from $PGF_{2\alpha}$ injection to onset of estrus was significantly ($P < 0.05$) shorter in the immunized group (46.8 ± 1.8 h) than the control group (54.4 ± 2.5 h) (Table 6). Ovulation rate was recorded over three $PGF_{2\alpha}$-shortened consecutive estrous cycles after the 3rd booster immunization against inhibin. There was around four-fold increase in ovulation rate in goats actively immunized against inhibin (Fig. 20). The mean ovulation rate was 1.7 ± 0.3 and 7.6 ± 1.1 in control and immunized groups, respectively. There was a positive correlation ($r = 0.9$, $P < 0.001$) between inhibin antibody titre and ovulation rate. Figure 21 shows multiple CLs in an ovarian image.

Table 6. Effect of immunization with a synthetic inhibin on estrus and ovarian response in goats (mean ± SEM).

Parameters	Control	Inhibin-immunized
No. of treated goats	5	5
No. of goats exhibited estrus[a]	5	5
No. of goats ovulated[b]	5	5
Interval from $PGF_{2\alpha}$ injection to estrus[c]	54.4 ± 2.5	46.8 ± 1.8*
Ovulation rate[d]	1.7 ± 0.3	7.6 ± 1.1**

*: $P<0.05$, significantly different compared with the control value in the same raw.

**: $P<0.01$, significantly different compared with the control value in the same raw.

[a]Detected by mature bucks 6-h intervals within 3 days after $PGF_{2\alpha}$ injection

[b]By using ultrasound scanning (large follicles collapsed and confirmed by formation of corpora lutea at that site)

[c]Hours from $PGF_{2\alpha}$ injection to onset of estrus

[d]The number of collapsed follicles or CL per goat ovulating

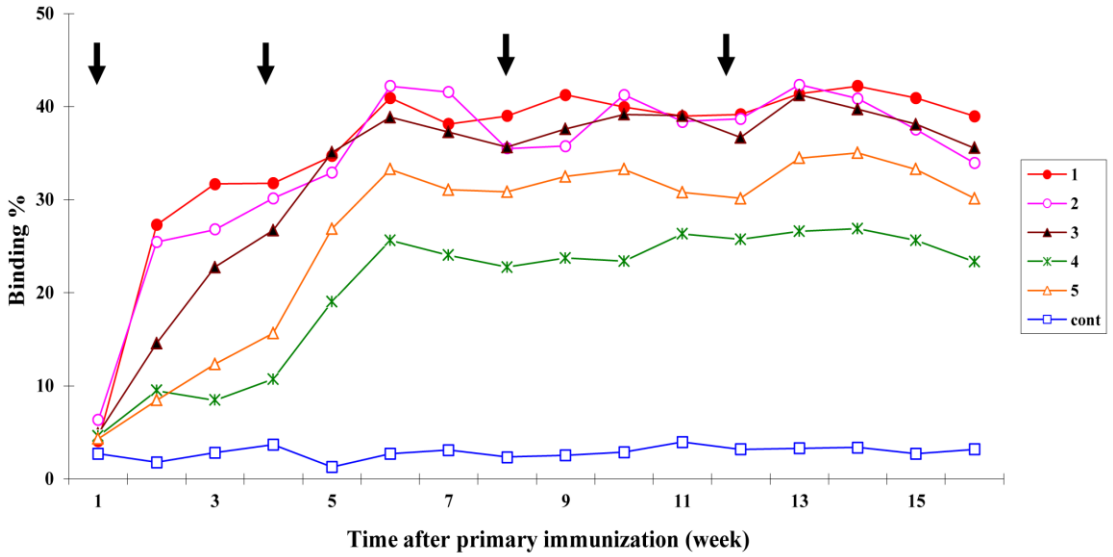


Fig. 16: Time course of inhibin binding in goats immunized against inhibin alpha-subunit (1 to 5) or control goats (open square; n = 5) in plasma diluted 1:10. Arrows indicate time of immunization.

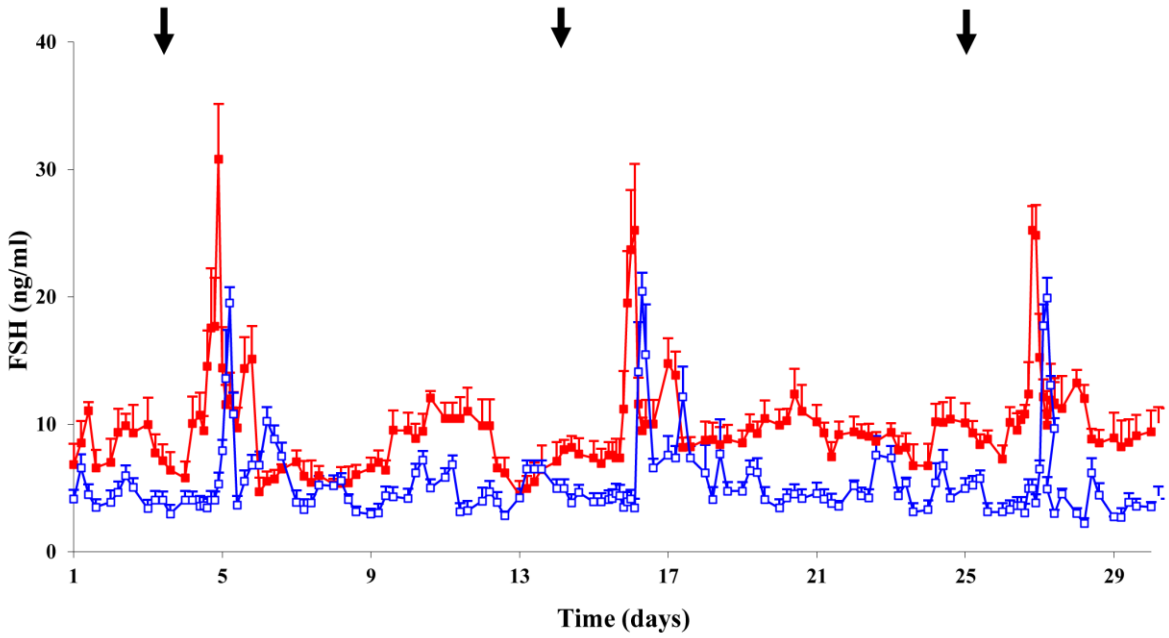


Fig. 17: Mean (± SEM) plasma FSH concentration in inhibin-immunized (solid squres; n = 5) and control goats (open square; n = 5) during a 30-days period encompassing three consecutive estrus using PG 11 days intervals. Arrows indicate PG injection.

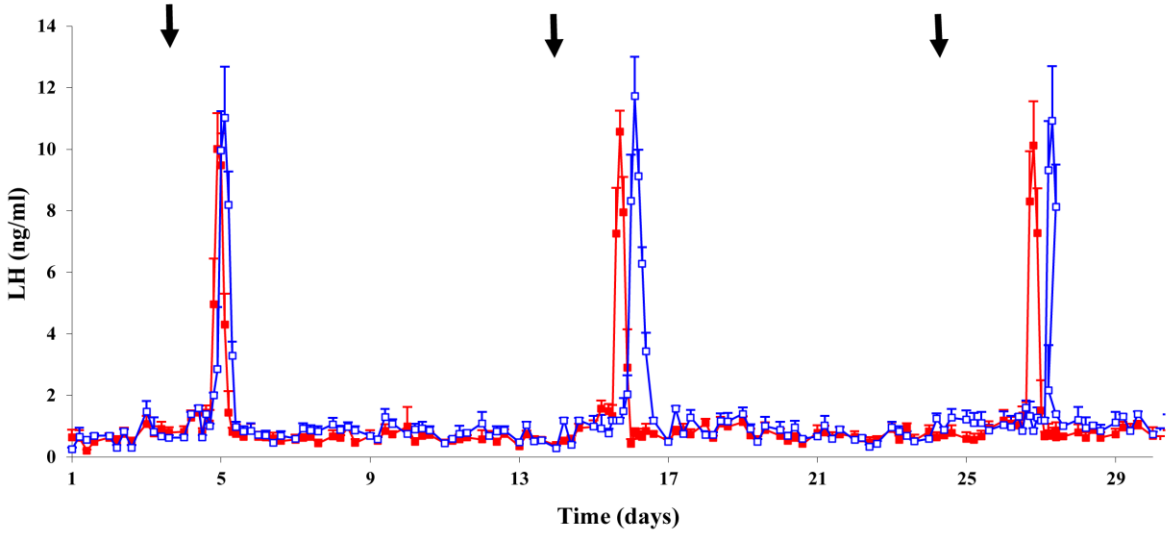


Fig. 18: Mean (± SEM) plasma LH concentration in inhibin-immunized (solid squares; n = 5) and control goats (open square; n = 5) during a 30-days period encompassing three consecutive estrus using PG 11 days intervals. Arrows indicate PG injection.

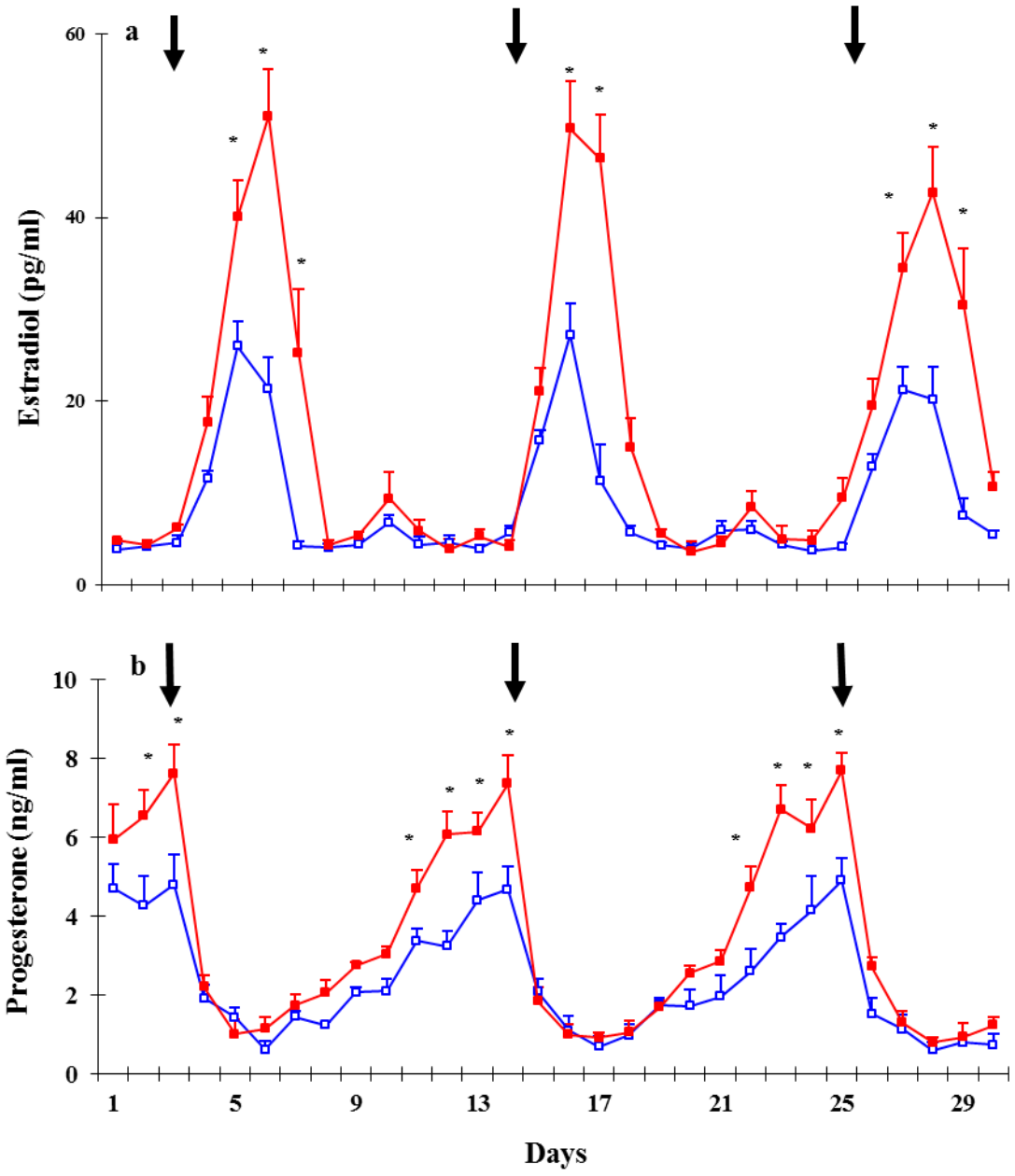


Fig. 19: Mean ± SEM plasma estradiol (a) and progesterone (b) concentrations in inhibin-immunized (solid square; n = 5) and control goats (open square; n = 5) during a 30-days period encompassing three consecutive estrus using PG 11 days intervals. Arrows indicate PG injection. *: $P < 0.05$

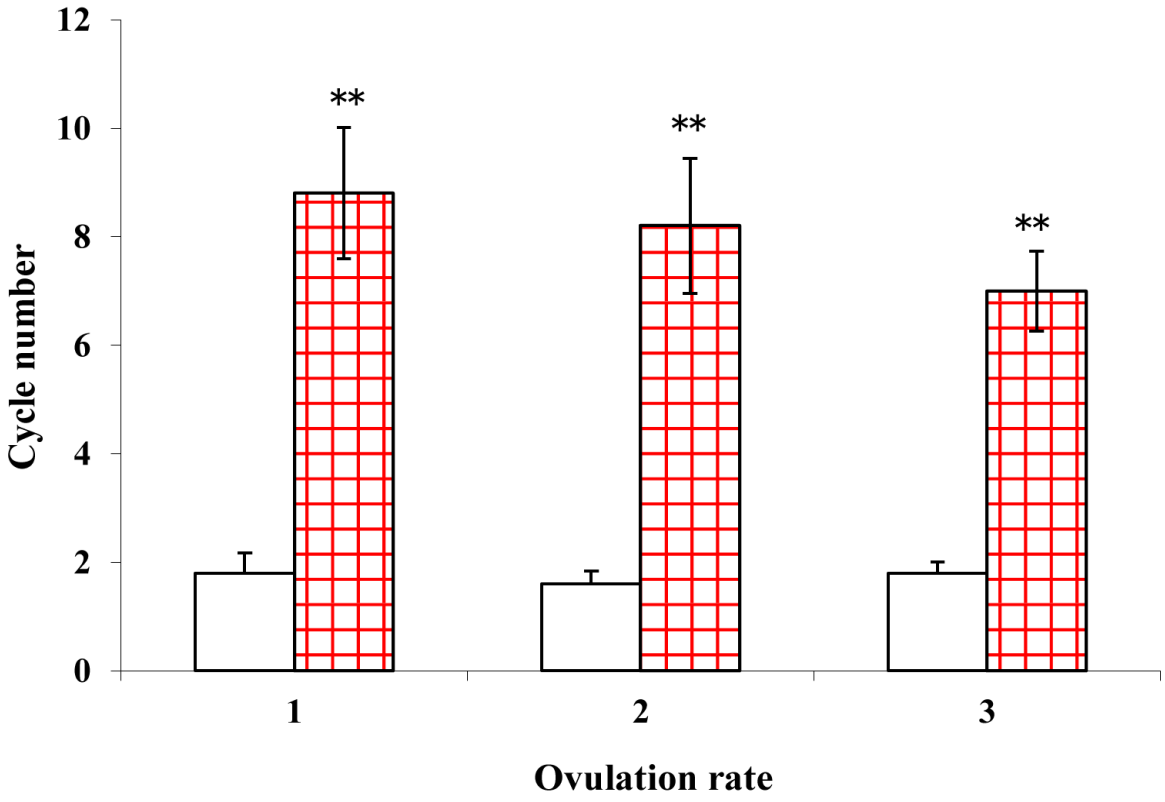


Fig. 20. Ovulation rate in inhibin-immunized (shaded bar; n = 5) and control goats (open bar; n = 5) during 3 consecutive PGF2α-shortened estrous cycles after the 3rd booster immunization. Values are means ± SEM;**: $P < 0.01$.

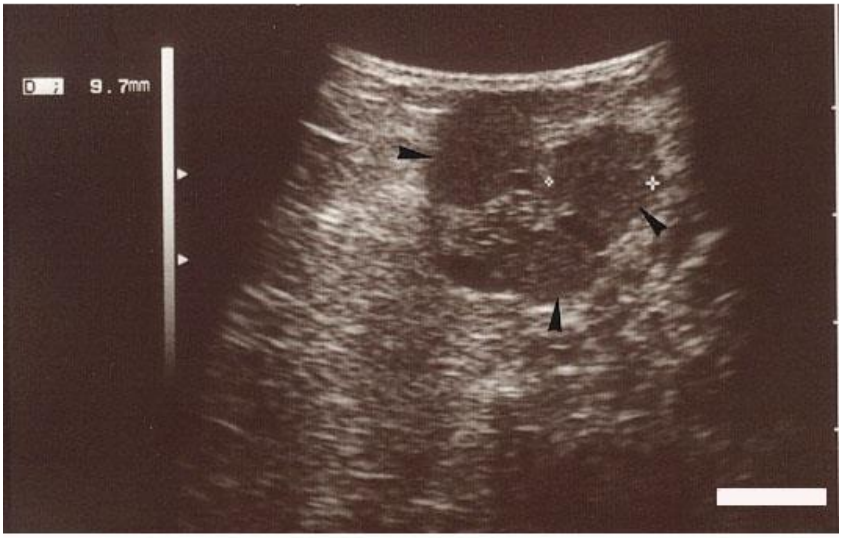


Fig. 21. Ultrasound image of a goat ovary actively immunized against inhibin produced by using a rigid, transrectal 7.5 MHz transducer (note the multiple corpora lutea; arrow heads). Scale bar represents 10 mm.

Experiment 5:

Early pregnancy diagnosis:

This study demonstrated that ultrasound is a reliable and safe method for pregnancy diagnosis in goats. There is no distress or rectal bleeding observed after ultrasound examination. Ultrasound examination of non-pregnant does showed that the uterus may be observed immediately anterior to the bladder. This latter structure is easily recognized as an anechogenic sphere (Fig. 22), appearing immediately after probe introduction into the rectum. The uterus appears as a spherical structure with a medium echogenic density (Fig. 23). No lumen was observed in the uterus before pregnancy.

In pregnant does, the first signs of pregnancy, is characterized by the appearance of a gestational sac which is circular and elongated anechoic structure located in the uterus cranial to the bladder. In this study, gestational sac could be first detected at day 20.2 ± 0.6 of gestation. The size of gestational sac increased with gestational age.

Images consistent with pregnancy include:

1) Multiple fluid-filled uterine luminal sections (Fig. 24) which are dark round fluid filled sacs. These sacs contain the amniotic fluid in which the fetus is developing. The fluid sacs can be round or oblong in shape. It is recommended that learning users use the bladder as a landmark when scanning.

2) Embryo or fetus, at 24.3 ± 0.7 days of gestation, all animals showed an embryo (Fig. 25), typically observed as an area of high echographic density located on the basal zone of gestational sac. Presence of embryos was confirmed by detection of heartbeats.

3) Placentomes, at day 35.4 ± 1.0 of gestation, placentomes were visible as small nodules. As pregnancy progresses, the concave circular shape of the goat placentomes results in C-shaped or O-shaped gray image, depending on the plane

of section against the black uterine fluid (Fig. 26).

Two months pregnancy:

At this stage of pregnancy, skeletal structures are obvious. Bones are the most dense tissue in the body so they will return an image of very bright white. The groups of white dots are ribs. In between ribs you will see an anechoic area which is the heart (Fig. 27). After detection of bone like skull and thorax, you can measure biparietal diameter of the skull (as the greatest head diameter) and similarly thorax height cam be measured as the distance between the ventral and dorsal border of the thoracic cavity, crossing the middle of the heart (Fig. 28).

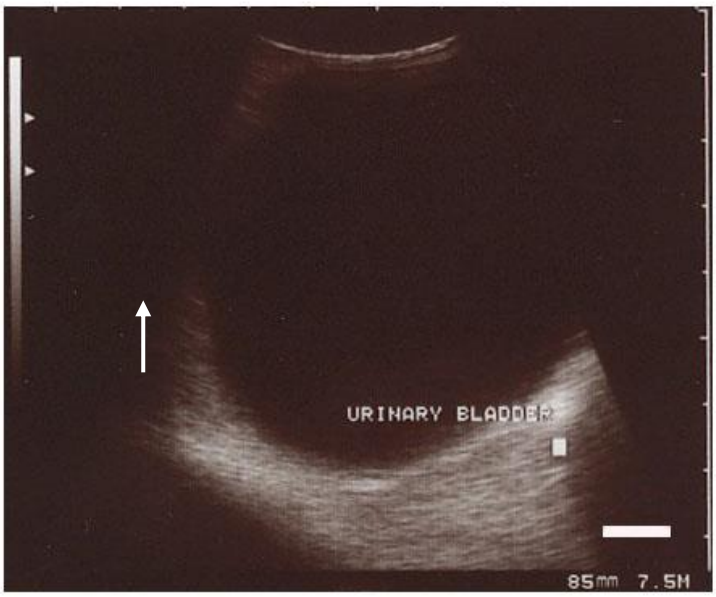


Fig. 22: Ultrasound image of the urinary bladder (black sphere; arrow) in goats. Scale bar represents 10 mm.

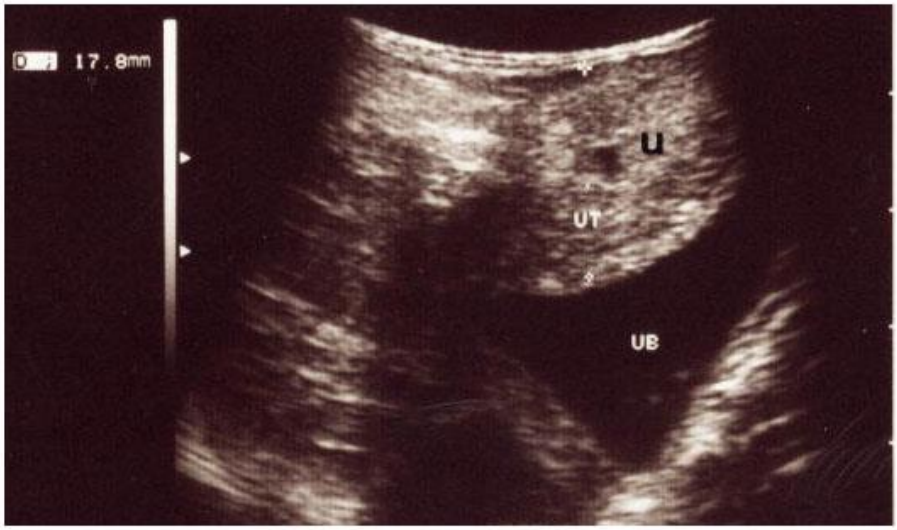


Fig.23: Ultrasound image of non-pregnant uterus (U) in goats. The uterus appears as a spherical structure with a medium echogenic density.

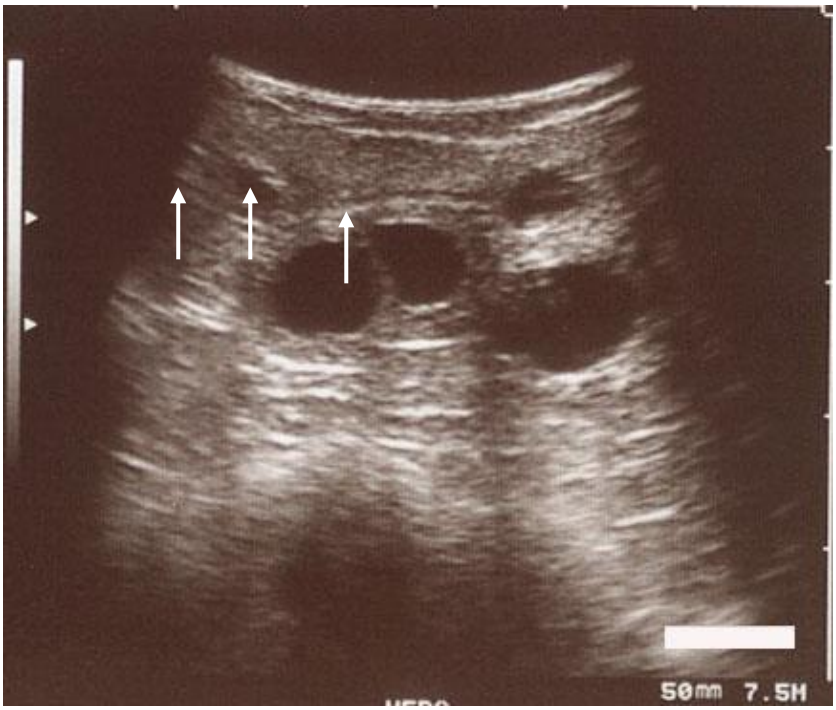


Fig. 24: Ultrasound image of early pregnant uterus (notice the dark, fluid-filled sacs (arrows). These sacs contain the amniotic fluid. The fluid-filled sacs can be round or oblong in shape). Scale bar represents 10 mm.

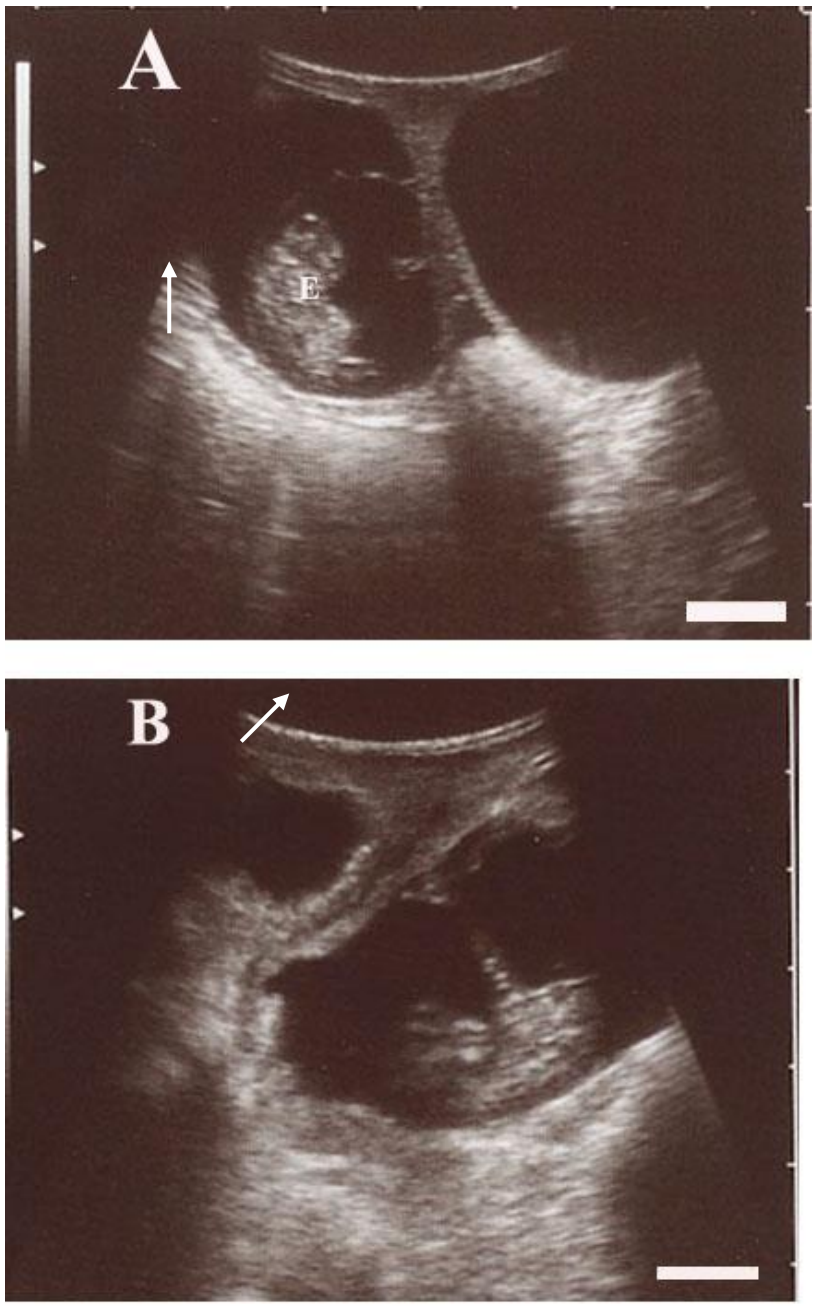


Fig. 25: Ultrasound images of goat embryo at day 30 (A) and 35 (B) of gestation in goats. The embryo (E) observed as an area of high echogenic density. Image B shows the umbilical cord (arrow). Scale bar represents 10 mm.

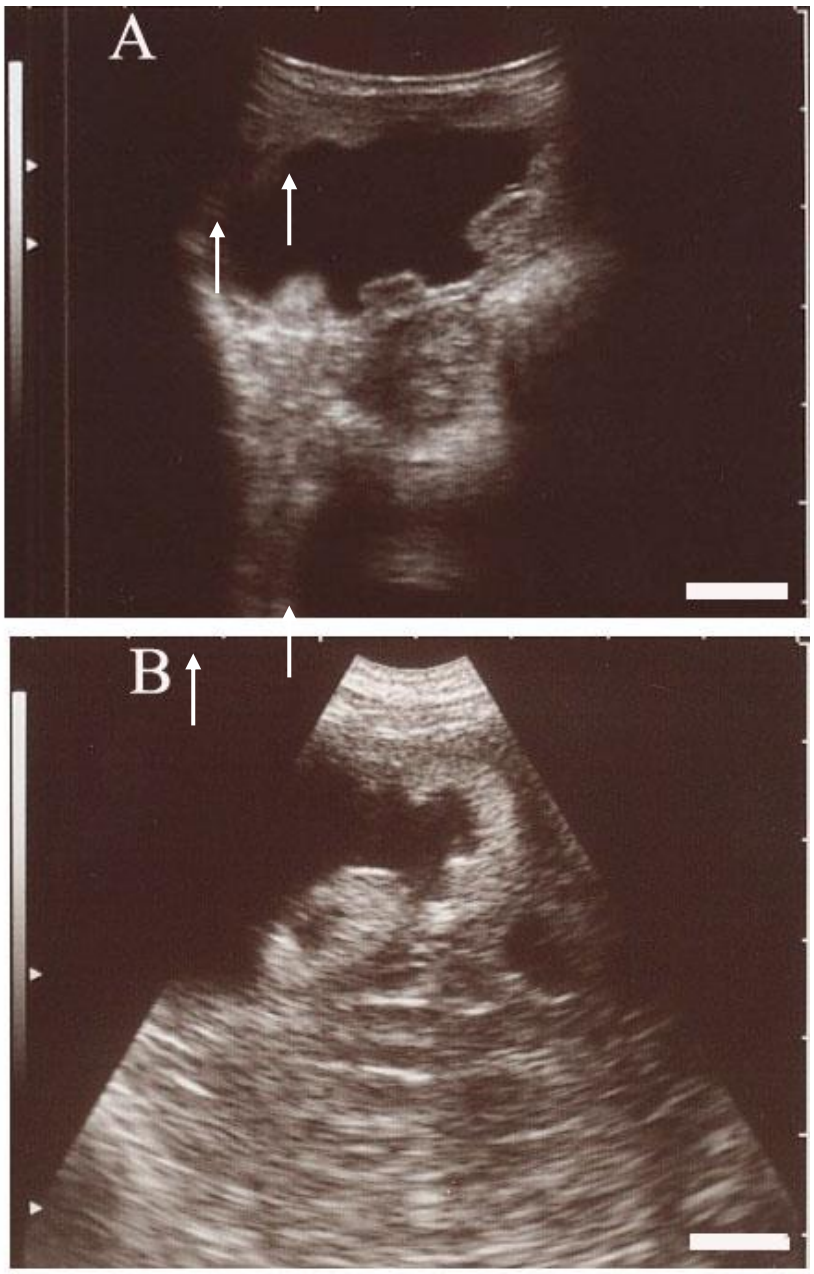


Fig. 26: Ultrasound image of placentomes (arrows) at day 35 (A) and 60 (B) of gestation in goats (note that placentomes are visible as small nodules at day 35 and become C or O shape later and increased in size as in image B). Scale bar represents 10 mm.

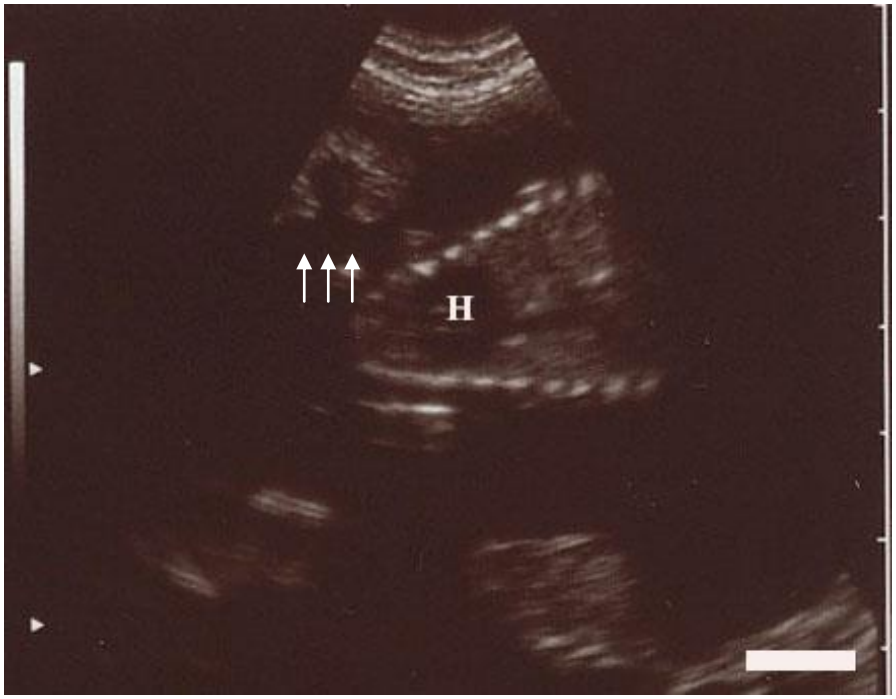


Fig. 27: Ultrasound image of the thorax in goat fetus at 2 months of gestation (note that the heart (H) appears as an anechoic structure between the white dots which represent ribs, arrows). Scale bar represents 10 mm.

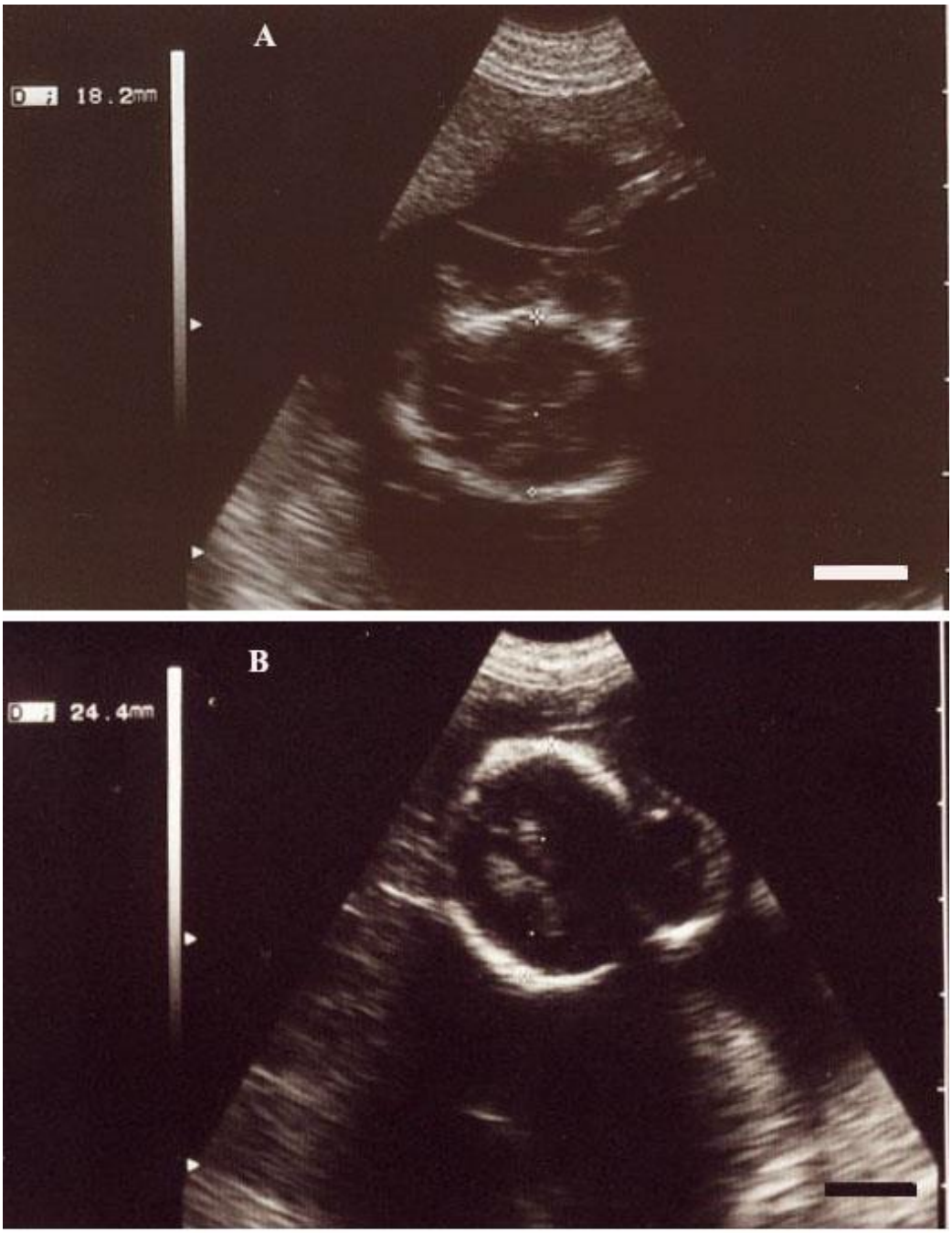


Fig. 28: Ultrasound image of the head of a goat fetus at day 60 (A) and 75 (B) of gestation (note that the diameter of the skull increased from 18.2 mm in image A to 24.4 mm in image B). Scale bar represents 10 mm.

DISCUSSION

Experiment 1:

Induction of estrus during non-breeding season:

Biotechnological research is making it possible for temperate goats known to be seasonal breeders to be bred in other periods of the year and thus to alleviate seasonal limitations in goat production and marketing of their products. The ease of putting in Syncro-Mate-B which is a slow releasing implant, makes it more attractive than using vaginal pessaries impregnated with progesterone.

In the present study, serum progesterone concentrations in samples collected prior to treatment were persistently low (< 0.5 ng/ml), indicating the absence of active corpus luteum at the time of treatment. The use of norgestomet ear implants for 11 days and $PGF_{2\alpha}$ or in combination with injection of GnRH induced estrus and ovulation. The interval from implant removal to estrus was 46.1 ± 1.5, 34.4 ± 1.5 and 60.0 ± 5.9 h in group I, group II and control group, respectively. The shorter interval in group II might be attributed to injection of GnRH. Similarly, Cardwell, Fitch and Geisert (1998) found that ovulation occurred on average 70 to 80 hour after implant removal in ewes treated with norgestomet and equine chorionic gonadotrophin reduced the interval from implant removal to ovulation. The present results are in agreement with those obtained by using other methods of treatment, for example, vaginal pessaries with flurogestone acetate followed by administration of equine chorionic gonadotrophin (Corteel, Gonzalez and Nunes, 1982) or norgestomet plus equine chorionic gonadotrophin (Bretzlaff and Madrid, 1989), suggesting that a single injection of GnRH may be a satisfactory substitute for equine chorionic gonadotrophin because equine chorionic gonadotrophin has a diverse effect on reproduction if used repeatedly. A higher fertility rate (No. of does kidding / No. of does exposed) in group II was indicative of more fertile matings at induced estrus, suggesting that GnRH enhanced ovulation. In cows, injection of GnRH 24 hours after synchro-Mate-B implant removal increased ovulation (Troxel and Kesler, 1984). In a similar study, anestrous ewes treated at different times from February through June with a 3-mg norgestomet implant for 10 days followed by an injection of 750 IU of equine chorionic gonadotrophin at implant removal had an average response on synchronized estrus of 72% compared with 10% for non-treated control ewes (Carpenter and Spitzer, 1981). In conclusion, treatment of goats with norgestomet ear implant induced estrus

during the non-breeding season and the injection of GnRH 24 hours after implant removal improved fertility rate.

Estrus synchronization:

Reliable estrus detection requires several period of intensive observation daily and is therefore time consuming and expensive. Synchronizing the estrous cycle involves the manipulation of ovarian activity so that the time of ovulation can be predicted. In this study, estrus was observed in 77.5, 85.0 and 100.0 % in groups treated with norgestomet plus cloprostenol (A), or norgestomet, cloprostenol and GnRH (B) or cloprostenol only (C), respectively. The 100 % response obtained with treatment C could be attributed to the presence of CL in all does as determined by ultrasonography before treatment. The mean interval from the end of treatments to first signs of estrus was 46.1, 34.4 and 51.2 h in A, B and C treatments, respectively. There was a significant ($P<0.05$) difference between treatment groups in the interval from treatment to estrus. The shorter interval to estrus in treatment B indicated that GnRH treatment stimulate follicular growth and maturation. The interval from cloprostenol injection to onset of estrus (51.2 h) was in line with that (55 h) reported by Greyling and Van Niekerk (1980). However, another study showed a shorter (37-43 h) interval (Akusu and Egbunike, 1990).

Although $PGF_{2\alpha}$ is known to be luteolytic in domestic animals, including goats, for effectiveness, it must be administered at certain times of the estrous cycle to coincide with the time of sensitivity of the corpus luteum. The CL has been found to be receptive to $PGF_{2\alpha}$ or its analogues between Days 5 and 14 of the estrous cycle of the ewe (Acritopoulou and Haresign, 1980). In cattle, the CL is refractory to $PGF_{2\alpha}$ luteolytic signals during the first 5 days of the cycle (Wiltbank, Shiao, Bergfelt and Ginther, 1995). Moreover, between days 5 and 9, $PGF_{2\alpha}$ will cause partial lueolysis with subsequent recovery of CL function. During the estrus cycle of the doe, Ott (1982) reported that the CL was not responsive to luteolytic signals of $PGF_{2\alpha}$ or its analogues between days 4 and 6. As a result, the 2 injections regimen, 11days apart, has been adopted. This increases the chances of coinciding with the stage at which the CL has receptors for $PGF_{2\alpha}$. Thus the need to know the stage of the estrous cycle of the doe is important. In our study, 100% of the does exposed to bucks exhibited overt estrus after the injection of cloprostenol. This high degree of synchrony achieved could be attributed to injection $PGF_{2\alpha}$ at the stage when the CL was responsive. This procedure is consistent with observations reported previously (Molowuku *et al.*, 1980; Ott, 1982).

Experiment 2:

Ovarian dynamics and hormonal profile during estrous cycle:

The present study demonstrates the relationship between follicular dynamics and hormonal profiles during estrous cycle of goats. The results of daily utltrasonography in this study indicate that the interovulatory interval in goats is characterized by a wave-like pattern of follicular development. This is similar to previous findings recorded in other ruminant species as cattle (Sirois and Fortune, 1988; Lucy *et al.*, 1992), sheep (Ginther *et al.*, 1995) and confirms previous observations reported in goats (Ginther and Kot 1994; de Castro *et al.*, 1999). Ginther and Kot (1994) found a predominant wave pattern of 4 waves, emerging, respectively around days –1, 4, 8 and 13 of the interovulatory interval. In this study, the wave pattern found ranged between 2 and 5 follicular waves. The day of emergence of waves for goats that have 4 waves was between the range reported by Ginther and Kot (1994). In goats with 3 follicle waves, the number of 3 mm follicles peaked on days 0, 7 and 11 while in goats with 4 follicular waves, 3 mm follicles peaked on days -1, 5, 11 and 15. This demonstrates that there is a progressive growth of follicles in a wave like fashion.

The estradiol profiles in the present study are characterized by gradual increase from the day of ovulation to day 4 then decreased to basal level and remained low for the rest of luteal phase apart from some isolated fluctuations then increased reaching a peak 2 days before ovulation. Early works in the cow (Badinga, Driancourt, Savio, Wolfenson, Drost, de La Sota and Thatcher, 1992) and sheep (Mann, McNeilly, and Baird, 1992[b]) show that estrogens are mainly produced by the dominant follicle of a wave and subordinate follicles contribute with less than 10 % of the ovarian estradiol production. Our present study showed that during the luteal phase estradiol was produced by the large follicles of each follicular wave. Previous reports showed that the production of estradiol was stopped prior to the time when follicle attained its maximum diameter (Guibault, Bolamba, Desaulnier, and Lussier, 1993). Another study showed that estradiol concentrations were greatest during the growing phase of the first dominant follicle and were significantly reduced when the dominant follicle attained its large diameter (Rhodes, Fitzpatrick, Entwistle and Kinder, 1995). The present study demonstrated that the diameter of the CL was positively correlated with plasma progesterone concentrations. This is in agreement with previous reports (Samartzi, Belibasaki, Vainas and Boscos, 1995; Amiridis, Rekkas, Fthenakis, Vainas, Lymberopoulos, Christodoulou and Belibasaki, 2002). This strong

correlation between CL diameter and progesterone concentration indicates that CL diameter might be used as an index of peripheral progesterone level in goats.

In previous studies using utltrasonography and blood sampling once daily, it was shown that large antral follicles (attaining ≥ 5 mm in diameter) grew in waves across the ewes estrous cycle, and that around the time of wave emergence (growth from 3 mm pool follicles) there was a transient elevation (2 to 3 days) in plasma concentrations of FSH (Ginther *et al.* 1995; Bartlewski *et al.*, 1999). In the present study, we found this pattern of FSH secretion in which plasma FSH concentrations were high coincident with follicular wave emergence, decreased after emergence and remained low during the growing phase of follicles. These results suggest that the fluctuation of the circulating FSH levels is involved in the recruitment and selection of follicles. Within the present study, the number of follicular waves and number of FSH peaks did not differ; also the interwave interval and interpeak interval of FSH did not differ. The positive correlation between the numbers of the 2 events within interovulatory intervals was high and significant. It is well established that the secretion of FSH during the estrous cycle is regulated by both estradiol and inhibin (Mann *et al.*, 1992[a]) and fluctuations in the pattern of secretion of these two hormones would be expected to be responsible for the fluctuations in FSH that are associated with follicular waves. The results of the present study suggest that changes in inhibin secretion control FSH during the estrous cycle in goats. In this study looking at the patterns of plasma FSH concentrations in the alignments to follicular wave strongly suggested a link between FSH secretion and follicle growth patterns. Mean plasma FSH started to increase from around the time of the end of the growth phase/ onset of static phase of the follicular wave, suggesting that secretion of follicular inhibitor of FSH release declined at that time. New follicular waves emerged within 1 or 2 days of the onset of static phase of the previous wave, suggesting that changed secretory activity of follicle in the static phase permits the increase in FSH secretion that heralds the next follicular wave. This would mirror the concepts proposed for cattle (Ginther *et al.*, 1996), except that follicular dominance is less apparent in goats because several follicles can emerge and grow to a similar final stage in a single wave. In contrast to FSH, LH secretion is basal during estrous cycle except the preovulatory LH surge. This may be attributed to the high level of progesterone during the luteal phase.

This experiment showed that the pattern of secretion of dimeric inhibin A is related to the presence of large follicles and is negatively correlated with FSH concentration, suggesting that inhibin A is a product of differentiated follicles and has an important role in controlling FSH secretion. Similarly in rat, Inhibin A is at

its highest concentration during Prooestrus, concomitant with the selection of large follicles. (Woodruff, Besecke, Groome, Draper, Schwartz and Weiss, 1996). Previous studies demonstrated that, while estrogenic large follicles are a major source, both small and large non-estrogenic follicles contribute significantly to the ovarian secretion of immuno-reactive inhibin (Campbell, McNeilly, Mann and Baird, 1991; Mann *et al.*, 1992[b])

Evidence from passive immunoneutralization studies supports a functional role for inhibin in regulating FSH secretion. The relationship between pattern of inhibin release, FSH and follicle growth is similar to that reported by Kaneko *et al.* (1995) in cycling cows. Also inhibin A is inversely correlated with FSH concentration which is similar to that recorded in cattle (Kaneko, Noguchi, Kikuchi, Todoroki and Hasegawa, 2002). This inverse relationship confirms the hypothesis that inhibin A contribute to inhibition of FSH secretion.

In conclusion, Utltrasonography can be reliably used in goats for studying ovarian follicular dynamics and the growth of ovarian follicles exhibits a wave-like pattern. CL diameter has a close relationship with plasma progesterone concentrations and therefore, CL can be used as index of peripheral progesterone levels in goats. The secretion of ir-inhibin and dimeric inhibin A is related to the presence of large antral follicles and is negatively correlated with FSH. The mean FSH secretion peaks at or around antral follicle emergence (follicles growing from 3 mm in diameter to ≥ 5 mm).

Experiment 3:

Ovarian response and hormonal profile following injection of inhibin antiserum:

The present experiment clearly demonstrate that immunoneutralization of endogenous inhibin in cyclic goats caused an increase in plasma concentrations of FSH which, in turn, led to the stimulation of ovarian follicle development and increased ovulation rate.

After injection of inhibin antiserum, plasma FSH levels increased and reached highest concentration within 12 h, after which concentrations decreased to control levels. The preovulatory FSH level in immunized group peaked earlier than the control group. This may be attributed to earlier peak in estradiol-17β in immunized goats which in turn induced GnRH release from the hypothalamus and release of LH and FSH. The decrease in FSH secretion has been attributed to estradiol and inhibin secretion (Wheaton *et al.*, 1992; Mann, Campbell, McNeilly

and Baird, 1993). The magnitude and quantitative nature of FSH secretions induced by passive immunization support an endocrine role for inhibin in regulating FSH secretion. Thus, inhibin, through negative feedback regulation of FSH secretion, appears to be an important factor in regulation of follicular development. An FSH-mediated mechanism of action of inhibin immunization on ovulation rate agreed with that reported by O'Shea, Hillard, Anderson, Bindon, Findlay, Tsonis and Wilkins (1994).

In previous studies, significant increase in plasma concentrations of FSH has been shown by passive immunization against inhibin during the estrous cycle in cows (Kaneko *et al.*, 1995; Takedomi, Kaneko, Aoyagi, Konishi, Kishi, Watanabe and Taya, 1997) sheep (Mann, Campbell, McNeilly and Baird, 1989; Wheaton *et al.*, 1992), rats (Arai, Watanabe, Taya and Sasamoto, 1996) and hamsters (Kishi *et al.*, 1996), indicating the important role of inhibin regulating FSH secretion during the estrous cycle in these species. In goats, a reciprocal relationship between circulating FSH and immunoreactive inhibin levels during the estrous cycle has been reported (Medan *et al.*, 2001), suggesting that inhibin secreted from the ovary plays an important role as a regulator of FSH secretion.

The rise in FSH concentration seen following immunization resulted in a marked stimulation of follicle growth within 24 h. In inhibin-immunized group, there was a rise in the number of small sized follicles 24 h after injection of inhibin-AS. This suggests that the rise in FSH had stimulated a new cohort of small follicles to develop. Similar results were reported in ewes (Mann *et al.*, 1993). The observation that the rise in plasma FSH preceded the emergence of new follicles leads to the conclusion that hypersecretion of FSH stimulates multiple growth of follicles. This action, timed to coincide with $PGF_2\alpha$ injection in the present study, enlarge the pool of recruitable follicles, which gave rise to the greater ovulation rate in inhibin antiserum treated goats. From Day 3 after injection of antiserum, the number of large follicles began to increase associated with declining levels of plasma FSH. The results suggest that selection of large follicles occurred in the recruited follicles as a consequence of the decline in FSH secretion. Previous results obtained from active immunization (Bleach, Muttukrishna, Cunningham, Knight and Glencross, 1996) or repeated injections of inhibin antiserum (Campbell and Scaramuzzi, 1995; Campbell, Gordon, Tsonis and Scaramuzzi, 1995) provided a possibility that ovarian response to inhibin immunization is not only mediated by the rise in circulating FSH levels but also by a direct effect of immunization on follicular growth. Regarding LH levels, there was no marked change in basal LH concentrations between immunized and control goats. However, immunized goats displayed a lower LH surge than

control goats. This might be explained by the increased estradiol level secreted by the large number of developing follicles in immunized goats or due to gonadotropin-surge attenuating factor produced by granulosa cells of follicles (Fowler, Sorsa-Leslie, Cash, Dunbar, Melvin, Wilson, Mason and Harris, 2002).

In goats treated with inhibin antiserum, there was a marked rise in estradiol-17β occurred coincided with the growth of large number of follicles, as compared with control. These results indicate that immunization against inhibin enhances follicular development and in turn secretion of estradiol-17β from the ovarian follicles. Progesterone values were significantly higher on day 7 after ovulation in immunized group than control which is significantly ($r=9$; $p<0.01$) and positively correlated with the number of corpora lutea.

It is well known that repeated use of PMSG to induce superovulation in goats resulted in diverse effects because of the formation of anti-PMSG antibodies (Roy *et al.*, 1999). In addition, a higher incidence of premature corpus luteum regression in eCG-superovulated goats was recorded (Riesenberg *et al.,* 2001). Therefore, using passive immunization against inhibin which selectively increases FSH secretion may be a suitable and simple alternative to PMSG to induce superovulation in goats.

In conclusion, the present experiment demonstrated that inhibin is an important factor as a regulator of FSH secretion in goats. The present results also demonstrated that passive immunization against inhibin 48 h before $PGF_2\alpha$ injection induced a marked increase in FSH, estradiol-17β, ovarian follicle population and ovulation rate. Therefore, the neutralization of inhibin bioactivity may be a potential method for inducing follicular development and increased ovulation rate in goats.

Experiment 4:

Effect of active immunization against inhibin on hormonal levels and ovulation rate:

There is general agreement that vaccination against inhibin causes elevated plasma FSH concentrations in accord with the postulated role of inhibin as a feedback regulator of FSH synthesis.

In the present study we have demonstrated the efficacy of immunization against inhibin α-subunit in increasing ovulation rate and inhibin antibody titers in goats, and we have also shown that superovulation is associated with elevated

FSH plasma levels. In previous studies, immunization against inhibin preparations leads to an increase in ovulation rate in a number of species including rats (Rivier and Vale, 1989), gilts (King, Sesti, Britt, Esbenshade, Flowers and Ireland, 1990), horses (McCue *et al.*, 1992), heifers (Glencross *et al.*, 1994), sheep (Anderson at al., 1998) and goats (Dietrich *et al.*, 1995; Hennies *et al.*; 2001).

Immunization against inhibin stimulated an immune response and all immunized goats generated antibodies that bound ^{125}I-labeled inhibin, but there were marked differences in plasma inhibin binding between individual animals.

Mean plasma concentrations of FSH were significantly higher ($P < 0.05$) in immunized animals compared with control throughout 30 days (period of intensive blood sampling). This provides further evidence to support that immunization against inhibin increased FSH concentrations and then increased ovulation rate. There was no marked change in the LH profile between the immunized and the control goats throughout the experimental period. However, immunized goats at estrus displayed a lower LH surge than control goats. This might be explained by the increased amounts of estrogen secreted by the large number of developing follicles in immunized goats.

Plasma concentrations of estradiol rose immediately after PG injection in all goats. The peak level of estradiol concentrations in inhibin-immunized group was significantly higher than controls. This is similar to previous results recorded in goats (Hennies *et al.*, 2001). The increase in estradiol levels probably due to increased number of estrogenic follicles destined to ovulate. Estradiol induces the gonadotropin release by increasing pituitary sensitivity to GnRH (Clarke, Cummins, Crowder and Nett, 1988), increasing the number of GnRH receptors (Miller, 1993) and by inducing a surge in GnRH release from the hypothalamus (Karsch, Bowen, Caraty, Evans and Moenter, 1997). The progesterone levels in inhibin-immunized goats were significantly higher than controls. This might reflect the increased ovulation rate and increased number of corpora lutea.

This study confirmed that active immunization against inhibin alpha-subunit significantly increased ovulation rate which is associated with a significant raised concentration of circulating FSH. Such an association has been demonstrated in sheep (Mizumachi, Voglmayer, Washington, Chen and Bardin, 1990; Wrathall *et al.*, 1990; Wheaton *et al.*, 1992). An approximately two-fold increase in ovulation rate was observed following the 1st booster immunization and a four-fold increase after the 3rd booster immunization. Similar results were recorded in ewes (Anderson *et al.*, 1998).

In conclusion, the present study demonstrated that active immunization against the inhibin α-subunit increased ovulation rate mainly by attenuation of the suppressive effect of inhibin on FSH secretion.

The use of inhibin vaccination holds promise as a mean to overcome the variability in superovulation for embryo transfer in goats.

Experiment 5:

Early pregnancy diagnosis:

In most flocks of goats, natural service dates are unobserved or unrecorded, making fertile breeding impossible to determine. Accurate pregnancy diagnosis would provide essential information for effective herd management practices such as culling of non-pregnant females and determination of the number of fetuses. Such information would allow producers to group animals based on their nutritional needs so that they are fed appropriate ration during the last trimester of pregnancy and to monitor females near term.

Transrectal ultrasonography is an invaluable tool for early pregnancy diagnosis in goats (Kahn, Fraunholz, Kaspar and Pyczak, 1990). We found that this technique is easily applicable without significant risk for does, which can be examined in standing position in a wooden chute. In fact, no distress, no rectal bleeding or interruption of pregnancies were seen after ultrasonography examinations, in contrast to that reported in ewes by (Schrick and Inskeep, 1993). In addition, the animals became accustomed to this procedure after two or three sessions, facilitating management by the operator.

Results showed that pregnancy diagnosis in does could be carried out from 20.2 ± 0.6 days after mating, with 100% accuracy at day 24.3 ± 0.7. During evaluation of initial stages of pregnancy, the observation of the embryo and its heartbeat in gestational sac is important. In this study the presence of an embryo heartbeat was detected as early as 24.3 ± 0.7 days of gestation. This is in agreement with that reported by Martinez *et al.* (1998). In alpacas and llama, the heartbeat was detected at 23 and 31 days of gestation, respectively while in cows, the embryo image was visible between 26 and 29 days of gestation (White, Russel, Wright and Whyte, 1985). Studies in ewe have reported the detection of an embryo heartbeat from 18 (Schrick and Inskeep, 1993), 21 (Garcia *et al.*, 1993) and 30 days of gestation (Buckrell, Bonnett and Johnson, 1986). Therefore, it appears that the early developmental processes are temporally similar in these ruminant species, independent of the length of gestation.

Early detection of pregnancy would be an aid to develop special care for

pregnant females, including nutritional aspects and minimizing stress. The results presented here indicate that ultrasonography is the most adequate technique for early pregnancy diagnosis. The use of ultrasonography technique will help to improve the reproductive efficiency of goats.

A positive diagnosis of pregnancy is assured by imaging the embryo/fetus or placentome surrounded by fluid. A presumptive diagnosis of pregnancy or hydrometra can be made by imaging multiple anechoic (fluid-filled) sections of uterine lumen cranial to the bladder using a transrectal or transabdominal approach. A false positive pregnancy diagnosis during this period may be caused by hydrometra. This condition occurs commonly enough in goats to advice caution against making a positive diagnosis of pregnancy until the embryo/fetus can be seen. The urinary bladder should not be confused with a fluid-filled uterus. The bladder can be identified transrectally by viewing the characteristic triangular-shaped neck as the transducer is directed caudally. The bladder wall can be seen as an echogenic white line separating the anechoic lumen of the bladder from the anechoic uterine luminal sections. The fetus and fetal heart can be seen at day 24.3 ± 0.7. The fetus appears as an echogenic mass within the uterine lumen. Visualizing fetal movement or beating of fetal heart during real-time imaging can assess fetal viability.

The ability to identify multiple fetuses with real-time ultrasonography is a clear advantage over other techniques. Feeding management can be adjusted for does carrying multiple fetuses or single fetuses. Another advantage of real-time ultrasonography is the ability to distinguish a viable pregnancy from hydrometra, pyometra and fetal mummification.

We found that real-time B-mode ultrasonography can be used in early pregnancy diagnosis in goats. The presence of the embryo (confirmed by heart beats) was essential for an accurate diagnosis (Ishwar, 1995). In addition, ultrasonography may constitute a helpful tool for determination of embryonic mortality in goats based on the presence or absence of heartbeats (Garcia *et al.*, 1993; Schrick and Inskeep, 1993).

SUMMARY AND CONCLUSION

This study was conducted on 100 Baladi goats in Egypt and 40 Shiba goats in Japan in a trial to improve their reproductive efficiency as follow:

Experiment 1:

This experiment was carried out to investigate induction of estrus during the non-breeding season using 100 Egyptian Baladi goats. All animals assigned to treatments had low progesterone concentrations (<0.5 ng/ml) tested 2 times 10 days apart to confirm anestrous condition. Animals were assigned to three groups. A group of animals received subcutaneous norgestomet ear implant for 11 days and a single i.m injection of $PGF_{2\alpha}$ 24 hours before implant removal (group I; n = 40). Second group of animals received subcutaneous norgestomet ear implant for 11 days and a single i.m injection of $PGF_{2\alpha}$ 24 hours before implant removal and gonadotropin releasing hormone 24 hours after implant removal (group II; n = 40). Third group of animals received no treatment (control group; n = 20). The percentage of goats that showed estrous behavior during the first 72-hours after implant removal was 77.5, 85.0 % and 10 % in group I, group II and control group, respectively. The fertility rate was 57.5, 70.0 % and 10.0 % in group I, group II and control group, respectively. In conclusion, estrus can be induced in seasonally anestrous Egyptian Baladi goats using norgestomet and $PGF_{2\alpha}$ and the injection of GnRH 24 hours after implant removal synchronized ovulation in a higher percentage of goats.

Experiment 2:

Ovarian changes determined by daily transrectal ultrasound and its relationship with FSH, LH, estradiol-17β, progesterone and inhibin were investigated in 6 Shiba goats for 3 consecutive interovulatory intervals. Estrous cycles were synchronized using 2 injections of $PGF_{2\alpha}$ analogue 11 days apart. All follicles ≥ 3 mm in diameter and corpora lutea were measured daily. A follicular wave was defined as one or more follicles growing from 3 mm to ≥ 5 mm in diameter; the day at which the follicles growing to 3 mm in diameter defined as the day of wave emergence and the first wave after ovulation defined as wave 1. During the interovulatory interval (21.3 ± 0.5 days), follicular waves emerged on 0.3 ± 0.5, 6.5 ± 0.2 and 12.1 ± 0.4 days for wave 1, wave 2 and wave 3, respectively in

goats with 3 waves of follicular development and on – 0.6 ± 0.3, 4.7 ± 0.2, 9.4 ± 0.5 and 13.4 ± 0.5 days, for wave 1, wave 2, wave 3 and wave 4, respectively in goats with 4 waves of follicular development (day 0 = the day of ovulation). The mean diameter of the largest follicle of the ovulatory wave was significantly larger than the largest follicles of the other waves. Corpora lutea could be identified ultrasonically on day 3 post ovulation and attained 12.1 ± 0.3 mm in diameter at day 8. Transient increases in plasma concentrations of FSH were detected around the day of follicle wave emergence. FSH was negatively correlated with inhibin. These results demonstrated that follicular waves occurred in goats and the predominant follicular wave pattern was 4 waves with ovulation from wave 4. These results also suggested that the follicular waves' emergence was closely associated with increased secretion of FSH.

Experiment 3:

This experiment was conducted to investigate the effect of immunoneutralization against endogenous inhibin on FSH secretions and ovulation rate, aiming to develop a new superovulation method using inhibin antiserum (inhibin-AS) in the goat. Two groups of goats were given an intravenous injection of either 10 ml normal goat serum (control; n = 6) or inhibin-AS (n = 6) 48 h before the treatment with prostaglandin $F_{2\alpha}$. Blood samples were collected at 6h intervals starting 24 h before injection of inhibin antiserum until 120 h after injection. Also, blood samples were collected at 2h intervals from 48 to 72 h after PG injection. Additional blood samples were collected on day 7 after ovulation. The ovaries were examined starting 24 h before injection of inhibin antiserum until the end of the experiment and every 12 h around the time of ovulation using B-mode ultrasound scanner equipped with a 7.5 MHz transducer. Immunization against inhibin resulted in four- to fivefold increase in plasma FSH concentrations. After luteolysis, plasma concentrations of estradiol-17β increased markedly to a preovulatory peak about 2 folds higher than that of controls. The treatment was accompanied by a significant rise in the total number of follicles ≥ 3 mm in diameter 24 h later (8.2 ± 0.4 in inhibin-AS group vs 4.8 ± 0.3 in control group) and 96 h later (13.5 ± 1.0 in inhibin-AS group vs 5.3 ± 0.6 in control group). The ovulation rate was significantly higher in goats treated with inhibin-AS (4.2 ± 0.5; n = 6) than in control goats (1.8 ± 0.3; n = 6). These results indicate that inhibin is an important factor as a regulator of FSH secretion in goats. The present results demonstrate that the passive immunization against inhibin 48 h before $PGF_{2\alpha}$ injection induced the development of an increased number of follicles and increased ovulation rate. Thus, inhibin-AS

treatment may be an alternative to induce superovulation in goats instead of exogenous gonadotropins methods.

Experiment 4:

This experiment was conducted to evaluate the effect of active immunization against inhibin on hormonal levels and ovulation rate in goats. Ten adult Shiba goats in two groups were used in this study. The first group was primary injected with inhibin vaccine (immunized, n = 5) and the second group was injected with saline emulsified in Freund's adjuvant (control, n = 5) followed by three booster injections at 4 weeks intervals. After the 3rd booster injection, three consecutive estrus were induced using prostaglandin $F_{2\alpha}$ at interval of 11 days. Blood samples were collected at 6h intervals starting 3 days before the 2nd injection of $PGF_{2\alpha}$, every 2 h from 48-72 h after the 2nd injection of $PGF_{2\alpha}$ then every 6h. The same regimen of sampling was repeated during the next cycles. The ovaries were monitored using B-mode ultrasonography. All inhibin-immunized goats generated antibodies that bound ^{125}I-labeled bovine inhibin and their FSH concentrations were significantly higher than the corresponding values in the control group. Also, inhibin-immunized goats had significantly higher preovulatory estradiol-17β and higher concentrations of progesterone in the luteal phase. Immunization of goats against inhibin resulted in a significant increase in ovulation rate (control: 1.7 ± 0.3 vs immunized: 7.6 ± 1.1).

These results demonstrate that active immunization against inhibin enhanced ovarian follicular development and ovulation rate by promoting increase in pituitary FSH secretion. Therefore, immunization against inhibin may be a useful alternative to the conventional approach of superovulation in goats.

Experiment 5:

Twelve Shiba goats were used in this experiment. Estrus was synchronized with a single injection of $PGF_{2\alpha}$ 125 μg i.m after the detection of at least one corpus luteum by ultrasonography. Estrous behaviour was evaluated every 6 h by using a teasing buck. The females in estrus were allowed to be mated using mature fertile buck 2 times during estrus. Ultrasonographic examinations were performed transrectally using a real-time B-mode scanner equipped with a 7.5 MHz transducer. Main events observed were recorded by using a thermal-video printer.

This study demonstrated that ultrasound is a reliable and safe method for pregnancy diagnosis in goats. There is no distress or rectal bleeding was observed after ultrasound examination. In pregnant does, the first signs of pregnancy are

characterized by the appearance of a gestational sac which is circular and elongated anechoic structure located in the uterus cranial to the bladder. In this study, gestational sac could be first detected at day 20.2 ± 0.6 of gestation. The size of gestational sac increased with gestational age. In addition, embryo or fetus could be detected at 24.3 ± 0.7 days of gestation. Presence of embryos was confirmed by detection of heartbeats. Placentomes could be detected at day 35.4 ± 1.0 of gestation, placentomes were visible as small nodules. As pregnancy progresses, the concave circular shape of the goat placentomes results in C-shaped or O-shaped gray image. At two months pregnancy, we are looking for skeletal structures. Remember that bones are the densest tissue in the body so they will return an image of very bright white. After detection of bones like skull and thorax, you can measure biparietal diameter of the skull and similarly thorax height can be measured as an indicator of gestational age.

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الملخص العربى

تم اجراء هذه الدراسة على عدد مائة من الماعز البلدى فى مصر وأربعون من ماعز الشيبا فى اليابان بهدف تحسين الكفاءة التناسلية لها وتم تقسيم الدراسة الى عدد من التجارب كالآتى:

التجربة الاولى:
أجريت هذه التجربة بهدف إحداث الشبق فى الماعز خارج موسم التكاثر على عدد مائة من الماعز البلدى و كان مستوى هرمون البروجسترون اقل من ٠,٥ نانو جرام/ملى سيرم فى العينات التى تم فحصها على فترة بينية قدرها عشرة أيام مما يدل على أن جميع الحيوانات المستخدمة خاملة. تم زرع النرجستوميت تحت الجلد لمدة ١١ يوم وحقن البروستاجلاندين ٢٤ ساعة قبل إزالة الإنبلانت (مجموعة ١، العدد = ٤٠). فى المجموعة ٢ (العدد = ٤٠)، تم زرع النرجستوميت تحت الجلد لمدة ١١ يوم وحقن البروستاجلاندين ٢٤ ساعة قبل إزالة الإنبلانت وحقن الهرمون المحفز للجونادوتروبين ٢٤ ساعة بعد إزالة الإنبلانت. المجموعة الضابطة (العدد = ٢٠) لم تتلقى أى علاج.
نسبة الحيوانات التى شاعت فى الاثنين وسبعون ساعة الاولى من إزالة الإنبلانت كانت ٧٧,٥ ، ٨٥,٠ و ١٠,٠ % فى المجموعة ١ ، ٢ و الضابطة على التوالى. وكذلك نسبة الخصوبة كانت ٥٧,٥، ٧٠,٠ و ١٠,٠ % فى المجموعة ١، ٢ و الضابطة على التوالى.
من هذه النتائج نستنتج أنه يمكن إحداث الشياع فى الماعز البلدى خارج موسم التكاثر باستخدام النرجستوميت والبروستاجلاندين وأن استخدام الهرمون المحفز للجونادوتروبين ساعد على إحداث الشياع فى نسبة أكبر من الماعز.

التجربة الثانية:
تم إستبيان التغيرات المبيضية بواسطة الموجات فوق الصوتية وكذلك مستوى الهرمون المحفز للإباضة، الهرمون المحفز للوتين، الاستراديول، البروجستيرون، الإنهيبين لعدد ست من الماعز الشيبا ولمدة ثلاث دورات شياع متتالية. تم توحيد الشياع بواسطة حقن البروستاجلاندين على مرتتين بينهما ١١ يوم و تم قياس الجريبات التى يزيد قطرها عن ٣ مم وكذلك الجسم الاصفر. تم تعريف الموجة الجريبية المبيضية على أنها نمو جريبة مبيضية واحدة او أكثر الى أكثر من ٥ مم فى القطر قبل الإضمحلال و يوم البدايه على أنه اليوم الذى يكون فيه قطر الجريبة ٣مم والموجة الاولى بعد التبويض هى الموجة الاولى. أثناء دورة الشياع (٢١,٣ ± ٠,٥ يوم) بدات الموجات الجريبية المبيضية فى ٠,٣ ± ٠,٥، ٦,٥ ± ٠,٢، ١٢,١ ± ٠,٤ يوم للموجة الاولى والثانية والثالثة على التوالى فى الحيوانات ذات الثلاث موجات وفى ٠,٦- ± ٠,٣، ٤,٧ ± ٠,٢، ٩,٤ ± ٠,٥ ، ١٣,٤ ± ٠,٥ يوم للموجة الاولى والثانية والثالثة والرابعه للحيوانات ذات الاربع موجات(اليوم صفر = يوم التبويض). كان متوسط قطر الجريبات التى فى الموجه التبويضيه أكبر من قطر الجريبات الأخرى. الجسم الأصفر يمكن قياسه بداية من اليوم الثالث بعد التبويض وبلغ قطر الجسم الأصفر ١٢,١ ± ٠,٣ مم فى اليوم الثامن بعد التبويض. كان مستوى الهرمون المحفز للإباضة عالى اثناء بداية الموجات الجريبية المبيضية وكذلك هناك علاقة عكسية بين الهرمون المحفز للإباضة والإنهيبين. من هذه التجربة يتبين ان نمو الجريبات المبيضية فى الماعز يتم فى موجات والأربع موجات هى السائده. كذلك

بداية نمو الموجات الجريبية المبيضية يكون مصاحب لارتفاع مستوى الهرمون المحفز للإباضة.

التجربة الثالثة:

اجريت هذه التجربة لإستبيان تأثير معادلة الإنهيبين على مستوى الهرمون المحفز للإباضة ومعدل التبويض بهدف زيادة التبويض باستخدام الأجسام المضادة للإنهيبين. تم حقن مجموعتين من الماعز عن طريق الوريد ١٠ ملى سيرم ماعز عادى (المجموعة الضابطة، العدد = ٦)) أو ١٠ ملى أجسام مضادة للإنهيبين (العدد = ٦). بعد ٤٨ ساعة من حقن الأجسام المضاده للإنهيبين تم حقن جميع الحيوانات بالبروستاجلاندين. تم تجميع عينات دم كل ٦ ساعات وذلك بداية من ٢٤ ساعة قبل حقن الأجسام المضاده للإنهيبين الى ١٢٠ ساعة بعد حقن هذه الأجسام المضاده. وكذلك تم تجميع عينات دم بداية من ٤٨ الى ٧٢ ساعة بعد حقن البروستاجلاندين ثم تجميع عينات دم أخرى فى اليوم السابع بعد التبويض. تم فحص المبايض بالموجات فوق الصوتية يوميا بداية من ٢٤ ساعة قبل حقن الأجسام المضاده حتى نهاية التجربة وكل ١٢ ساعة مع إقتراب ميعاد التبويض. من النتائج تبين أن معادلة الإنهيبين أدى الى زيادة مستوى الهرمون المحفز للإباضة من أربع الى خمس أضعاف. كذلك ارتفع مستوى هرمون الإستراديول الى حوالى الضعف مقارنة بالمجموعة الضابطة. كانت هناك زياده معنوية فى عدد الجريبات المبيضية التى تزيد فى قطرها عن ٣ مم بعد ٢٤ ساعة من حقن الاجسام المضادة للإنهيبين (٨,٢ ± ٠,٤ فالمجموعة التى تم حقنها بالأجسام المضادة للإنهيبين مقارنة ب ٤,٨ ± ٠,٣ فى المجموعة الضابطة) وكذلك بعد ٩٦ ساعة (١٣,٥ ± ١,٠ فالمجموعة التى تم حقنها بالاجسام المضادة للإنهيبين مقارنة ب ٥,٣ ± ٠,٦ فى المجموعة الضابطة). كان هناك زيادة معنوية فى معدل التبويض فى المجموعة المحقونة بالاجسام المضادة للانهيبين (٤,٢ ± ٠,٥) عن مثيلتها فى المجموعة الضابطة (١,٨ ± ٠,٣).

من هذه الدراسة يتبين ان الإنهيبين عامل مهم للتحكم فى مستوى الهرمون المحفز للإباضة وان معادلة الإنهيبين ٤٨ ساعة قبل حقن البروستاجلاندين يزيد من عدد الجريبات المبيضية وكذلك معدل التبويض ولذلك يمكن استخدام هذه الطريقة لزيادة معدل التبويض فى الماعز.

التجربة الرابعة:

اجريت هذه التجربة لدراسة تاثير التحصين ضد الإنهيبين على مستوى الهرمونات ومعدل التبويض. تم استخدام عشر من ماعز الشيبا فى مجموعتين. تم تحصين المجموعة الاولى (العدد = ٥) ضد الإنهيبين والمجموعة الضابطة (العدد = ٥) بمزيب فرويند وتم حقن ثلاث جرعات منشطة بينهما مدة أربع اسابيع. بعد الجرعة المنشطة الثالثة تم إحداث الشبق ثلاث مرات بواسطة البروستاجلاندين بينهما ١١ يوم. تم تجميع عينات دم كل ٦ ساعات وذلك لمدة ٣ أيام قبل حقن الجرعة الثانية من البروستاجلاندين ثم كل ساعتين من ٤٨-٧٢ ساعة بعد جرعة البروستاجلاندين الثانية ثم بعد ذلك كل ٦ ساعات ويكرر نفس نظام التجميع فى دورتى الشبق التاليتين. تم فحص المبايض بواسطة الموجات فوق الصوتية. من النتائج تبين وجود الاجسام المضادة للإنهيبين فى جميع الحيوانات المحصنة ضد الإنهيبين وكذلك ارتفاع مستوى الهرمون المحفز للإباضة فى هذه الحيوانات، أيضا هناك زيادة معنوية فى مستوى هرمون الإستراديول قبل التبويض وكذلك زيادة معنوية فى مستوى البروجسترون فى فترة الجسم الاصفر مقارنة بالمجموعة الضابطة. كان هناك زيادة معنوية فى معدل التبويض فى المجموعة المحصنة (٧,٦ ± ١,١) عن مثيلتها فى المجموعة الضابطة (١,٧ ± ٠,٣).

من هذه النتائج تبين ان التحصين ضد الإنهيبين أدى الى زيادة معدل نمو الجريبات المبيضية وكذلك معدل التبويض عن طريق زيادة مستوى الهرمون المحفز للإباضة. لذلك يمكن استخدام هذه الطريقة لزيادة معدل

التبويض كطريقة بديلة للجونادوتروبين.

التجربة الخامسة:

تم اجراء هذه الدراسة على عدد ١٢ من ماعز الشيبا. تم احداث الشياع بواسطة حقن جرعة واحدة من البروستاجلاندين بعد التاكد من وجود جسم اصفر واحد على الاقل بواسطة الموجات فوق الصوتية. وتم اكتشاف الشياع كل ٦ ساعات بواسطة الذكر. تم تلقيح الاناث مرتين على الاقل اثناء الشياع باستخدام ذكركامل الخصوبة. تم فحص هذه الحيوانات باستخدام الموجات فوق الصوتية عن طريق المستقيم وتم تسجيل الاحداث بواسطة طابعة فيديو حرارية. هذه الدراسة اثبتت ان الموجات فوق الصوتية طريقة فعالة وآمنة لتشخيص الحمل فى الماعز ولايوجد اى مشاكل او نزيف مستقيمى اثناء الفحص. من اول علامات الحمل وجود اكياس الحمل دائرية او مستطيلة الشكل فى الرحم امام المثانة. تم اكتشاف هذه الاكياس عند يوم ٢٠,٢ ± ٠,٦ من الحمل ويزداد حجمها مع ازدياد مدة الحمل. اضافة الى ذلك تم رؤية الجنين عند اليوم ٢٤,٣ ± ٠,٧ من الحمل وتم تاكيد وجود الجنين بواسطة وجود نبضات القلب. ايضا تم رؤية البلاسنتوم عند اليوم ٣٥,٤ ± ١,٠ من الحمل ومع تقدم الحمل فان الشكل المقعر للبلاسنتوم ادى الى رؤيتها على شكل C او O . عند شهرين من الحمل يتم البحث عن الهيكل العظمى مع العلم ان العظام هى اعلى الانسجة كثافة وتظهر بيضاء بواسطة الموجات فوق الصوتية و يمكن قياس قطر الجمجمة وكذلك ارتفاع الصدر كمقياس لمدة الحمل.